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Gece Publishing
ABD Adres/ USA Address: 387 Park Avenue South, 5th Floor, New York, 10016, USA
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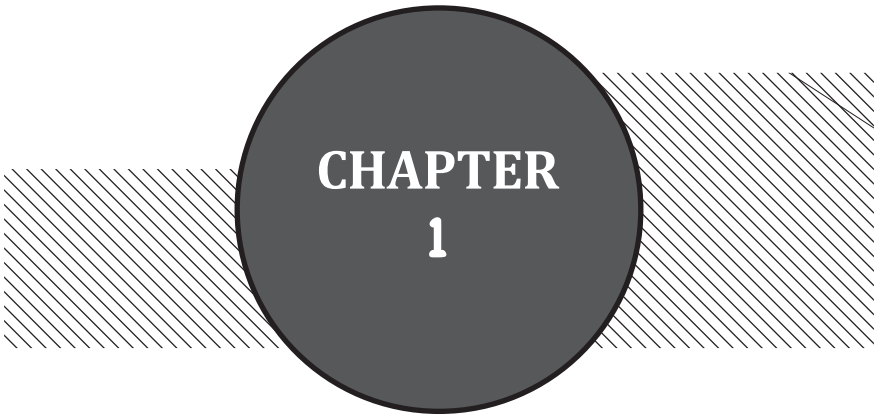
To Understand The Physiopathology of Traumatic Brain Injury and to Follow Therapeutic Developments: New Hopes in Treatment

Öznur ULUDAĞ[1]



[1] Anesthesiology and Reanimation Department, Adiyaman University Medical School, Adiyaman, Turkey

INTRODUCTION

Brain is the most delicate and the most complex organ in our body. An event that continues for seconds in our brain can change our lives completely. Traumatic brain injury (TBI) may happen anywhere, at any moment, and in everyone. TBI affects cognitive functions, leads to physical problems and behavioral changes. TBI is a global emergency with high morbidity and mortality (1). Although there are different rates in developing and developed countries, the majority of TBI is caused by falls and vehicle accidents. Most of the patients admitted to the hospital are diagnosed with mild brain injury but may cause long-term permanent damage. The TBI increases the financial burden of the society with the loss of the young population, which constitutes the productive workforce for the society and the prolonged treatment process, as well as the decrease in productivity (2,3).

TBI has become a large research subject due to its frequent occurrence and long-term negative results. The biggest problem in the treatment of TBI is that the damage is not standard and the treatment is not specific. From the first moment after trauma; hypo / hypertension, hypo/hyperglycemia, hypoxaemia and increased intracranial pressure in patients will directly affect the prognosis of the patient. These patients require a strict control of hemodynamic stability, glycemic control, normoosmolarity, thermoregulation, infection control and seizure in all stage when necessary. Clinically treatments will prevent or reduce secondary damage caused by primary damage (4). The treatment should be planned by targeting the pathophysiological changes of the trauma in the brain and the treatment of these changes.

Physiopathological changes in Traumatic Brain Injury

Primary brain injury is defined by the direct mechanical forces which occur at the time of the traumatic impact to the brain tissue. These forces and the injury they cause to the brain tissue trigger secondary brain injury over time. The impact of secondary brain injury caused by dysautoregulation of brain vessels and blood–brain barrier (BBB) disruption may be magnified by these processes, leading to the development of brain edema, increased intracranial pressure (ICP), and finally, decreased cerebral perfusion pressure (5). The clinically critical aspect to manage patients with TBI is the minimization of secondary cerebral damage.

The first period of head trauma is characterized by direct tissue damage caused by trauma, brain blood flow and damage to the brain metabolism. Cerebrovascular autoregulation and CO2 reactivity are important mechanisms that provide brain blood flow. After head trauma, the brain blood flow autoregulation is completely or partially impaired, and the oxygen consumption of the brain increases (6). Therefore, when talking about hypoperfusion or hyperperfusion of the brain, brain oxygen consumption should be evaluated with brain blood flow. An imbalance between the oxygen supply and the brain's oxygen consumption occurs after head trauma, and hypoxia occurs in the brain tissue. Infarcts develop in the brain when the partial pressure of oxygen in the brain tissue is under 10-15 mmHg (7). The hypoxia of brain tissue can be seen even if the intracranial pressure (ICP) and brain perfusion pressure (BPB) are normal. Therefore, the balance between oxygen delivery and consumption should be taken into consideration in the treatment of head trauma cases in addition to the CBT and BPB. Vasospasm occurs as a result of chronic depolarization of vascular smooth muscle cells (8). Hypoperfusion is seen in most patients who develop vasospasm. In this period, the increase in membrane permeability, edema and lactic acid accumulation due to anaerobic glycolysis occurs. When sufficient energy cannot be supplied by anaerobic glycolysis, ion pumps that are dependent on ATP are damaged. Excitatory neurotransmitters such as aspartate and glutamate are released. Increased glutamate stimulates receptors in neurons and astrocytes. Terminal membrane activation occurs by activation of voltage-dependent

sodium and calcium channels via N-methyl-D aspartate. Continuous intracellular flow of sodium and calcium initiates intracellular processes that cause death of cells (9). This triggers catabolic processes and causes cell damage, and disrupts the blood-brain barrier (BBB). BBB is formed by the endothelial cells of the brain. Microglial cells and astrocytes are in tight contact with these endothelial cells. This area is free of immune cells and factors produced by cells. With trauma as well as disruption trauma destroys BBB which is not permeable to proteins and bioactive substances in the blood and then glial cells and astrocytes are involved in the process. Dysfunction in BBB is suggested to cause neuronal loss, affect brain function, change response to treatment and delay neuronal repair. Increase of Matrix Metallo Proteinase-9 (MMP-9) protein is the most common mechanism of BBB dysfunction (10). In recent studies, it has been reported that microglial cells in the brain are not affected by peripheral perfusion until the cerebral perfusion pressure is very low, the function of the lost functions is restored when perfusion is given, and the main role of neuromuscular disorders in the neuroinflammatory events is the role of macrophages from the peripheral blood to the brain (11). Pericytes have a critical role in the maturation and continuity of BBB. It has also been shown in studies that the brain contains specialized pericytes or that the vessels in the brain respond to pericyte signals differently from other vessels (12). Intracellular calcium enhances the concentration of intracellular free fatty acids and free radicals by activating lipid peroxidase, protease and phospholipases. In addition, activation of substances such as intracellular translocase and endonuclease causes damage to the cell membrane and DNA, resulting in necrosis or apoptosis. Overexcitation of glutamate receptors also leads to the release of nitrous oxide and free radicals. Oxidative stress is characterized by abnormal accumulation of reactive oxygen species (ROS) and reactive nitrogen species (RON). When the amount of ROS increases, the antioxidant level decreases and this results in the dysfunction of the oxidizing protein (13). After head trauma, cytotoxic edema caused by vasogenic edema and intracellular water accumulation due to impaired BBB may occur. Edema may cause secondary damage by increasing intracranial pressure. Cytotoxic edema is characterized by an increase in water content within the intracellular compartment in response to an osmotic gradient. It is generally associated with a failure of the ATP-dependent Na+/K+-pumps under conditions of energy failure typically observed in severe TBI. This leads to an increase in cellular ionic content, an overall increase in cell osmolality and the influx of water into the cells. It is substantially a compartment shift of water in the skull, with water shifting from the extracellular to the intracellular compartment. Aquaporins, especially AQP4, are thought to play a regulatory role in brain water transport in edema. AQPs are integral membrane proteins belonging to a family that form pores in the membranes of mammalian cells. AQP1, AQP4 and AQP9 are highly expressed in brain. AQP4 is predominately expressed in the astrocytic end foot processes in close proximity to intracerebral vessels and at the ventricular interface. Preliminary studies proposed that upregulation of AQPs after brain injury promoted edema formation and it was in accordance with postulated that therapeutic inhibition of AQP4 would be beneficial in edema control (14). Nevertheless, it later became evident that the alterations in AQP4 expression are regionally distinct and dependent on the type of edema (15).

Rat cerebral ischemia model results in cytotoxic edema. In a model of rat cerebral ischemia, inhibition of AQP4 expression was associated with reduction in edema, infarct area and an improvement in functional outcome (16). Unlike, vasogenic edema induced by cold lesion injury was exacerbated in AQP4 knockout animals, suggesting that AQP4 is essential for clearance of vasogenic edema (17). It is understood that AQP4 activators have the potential to facilitate the clearance of the vasogenesis component of edema, but AQP4 inhibitors have the potential to protect the brain from cytotoxic edema.

Serum biomarkers have recently been used to support clinical and radiological variables

in the diagnosis and prognosis of TBI. Measurements are objective and independent of user comments. The measurement of proteins that cannot be passed from BBB to peripheral circulation but which can be detected in the periphery when BBB integrity is deteriorated has become increasingly accepted in the present day S100-B protein, NSE, GFAP, MBP and CTP are used serum biomakers. Especially the S100-B protein synthesized by astrocytes is a significant development of blood in cases where the BBB is damaged. However, the short half-life of this protein in the peripheral blood and the amount of release due to the activation of direct astrocytes have the disadvantages (18).

Therapeutic Developments for TBI

Efforts are underway to find an effective drug for the treatment of TBI. In the light of new studies today; Therapeutic strategies targeting the BBB and treatment strategies targeting immuno-modulation have gained importance.

Therapeutic strategies targeting the blood-brain barrier: In spite of the predominance of brain edema in the first week after traumatic brain injury, brain swelling can only occur with addition of water to the cranial vault from the vasculature. Therefore, regulation of blood–brain barrier permeability has become a focus of recent research seeking to manage brain edema.

1. Matrix metallo proteins and vascular endothelial growth factor antagonists: Matrix metallo proteins (MMPs) are zinc-dependent endopeptidases involved in the process of tissue remodeling following various pathologic conditions. Extreme permeability of post-traumatic BBB is important in terms of edema and immunological response. The result of excitation MMPs and vascular endothelial growth factor (VEGF) are released, enhancing the permeability of endothelium that acts as a barrier. Therefore; MMP and VEGF antagonists are in the experimental phase of anti-edema treatment. The up regulation of MMP-9 in particular has been temporally and spatially associated with BBB disruption and edema formation. In accordance with, mice lacking the MMP-9 gene have been shown to be protected in both focal and global ischemia as well as TBI (19). MMP inhibitors, such as TIMP-1, have also been shown to block BBB injury, cerebral edema and cell death in a number of experimental animal models (20). MMPs reported to play an important role in neurogenesis, neurovascular remodeling and matrix-trophic signaling in the later stages of recovery from TBI and stroke. Inhibition at these delayed time points may in fact worsen recovery (21).
2. Dexamethasone: Dexamethasone act on the glucocorticoid receptor and are highly effective in reducing BBB permeability. Helps stabilize the barrier by reducing the inflammatory response. Dexamethasone appears to reduce infarct volume when administered following cerebral ischemia in rats (22). But steroids have recently been shown to worsen cerebral edema under acidotic conditions in the rat. Corticosterone treatment has been shown to exacerbate kainic acid induced neurotoxicity in the CA3 region of the hippocampus and concomitantly increase the number of immunoreactive cells.
3. Endothelin receptor antagonists (bosentan, ambrisentan, clazosentan) approved for pulmonary hypertension and scleroderma but yet use in cerebral vasogenic edema. Endothelin 1 (ET-1) is a powerful vasoconstrictor that has been localized to brain endothelial cells, glia and neurons. The datas support a role for endothelin receptors A (ETrA), but not endothelin receptors B (ETrB), in mediating the dysfunctional microcirculation after TBI and ensuing cell injury following TBI. The findings further provide a rationale for developing targeted therapy using selective ETrA antagonists to improve cerebral blood flow (CBF) autoregulation after TBI. In 1995, it was published one of the first reviews which characterized endothelin antagonists as potential clinical therapeutics for vascular disorders (23). In 1999, Benigni and Remuzzi published a

follow-up which summarized data from pre-clinical and clinical studies which showed promise for specific endothelin receptor A antagonists in controlling hypertension. Bosentan, a mixed antagonist was discussed and clinical trial suggested that the potential opposing effects of ETrA and ETrB may render bosentan less effective (24). In 2003, it was reported that, after thorough investigation of ongoing clinical trial, bosentan had some success in control of pulmonary arterial hypertension; however, it was not more effective than other, non-endothelial specific drugs (25). It was suggested that clazosentan, may be useful for traumatic brain injury, ischemia, and subarachnoid hemorrhage. Clazosentan, given at 30 minutes post-traumatic brain injury, was suggested effective in improving outcome following injury (26,27).

4. Platelet-derived growth factor (PDGF) Inhibitors: PDGF-CC subunit induces PDGF alpha receptors in astrocytes in the neurovascular system and causes neurogenic delivery by increasing BBB permeability. Imatinib is an agent used as an inhibitor of PDGF as a protein kinase inhibitor and has received FDA approval in the treatment of chronic myeloid leukemia in 2001. The place of treatment for TBI remains experimental (28). As imatinib is known to reduce brain edema and hemorrhage after stroke by inhibiting the PDGF-CC pathway and thus improving BBB integrity. Neuroinflammation is a self-sustaining process with the BBB acting as a central regulator and imatinib can be used for modulating the mechanisms that control the function and integrity of the BBB under pathological conditions (29).

Therapeutic strategies targeting the Immuno-Modulation

1. Agents regulating glutamate amount (NMD Receptor antagonists): After trauma, AMPA-NMDA receptors are stimulated as a result of excessive glutamate release from the damaged nerve cell to the synaptic space. Increased calcium concentration in the cytosol and the process of fragmentation of DNA develops. The neuroprotective effect of NMDA receptor antagonists is investigated in this process. Studies on the efficacy of NMDA receptor blockers, such as ketamine, amantadine, memantine, are available (30,31). But are still controversial. CRMP-2 (Collapsin Response mediator protein 2); a group of proteins involved in neurodegeneration. It has been shown in a study that the interaction of NMDA receptors with CRMP-2 blocked calcium exchange with sodium (32). The place of treatment for TBI remains controversial.
2. Magnesium sulfate: It plays a role in the energy metabolism and protein synthesis of the central nervous system. It is an important intracellular cation. Magnesium causes relaxation of the vascular smooth muscle and thus potentially increases cerebral blood flow. Hypomagnesemia after TBI is associated with decreased ATP production and increased calcium levels in the intracellular space (33). There are studies reporting that the onset of magnesium sulfate after traumatic brain injuries is neuroprotective (34,35,36,37,38).
3. Oxidative Stress Reducing / Glutathione Enhancing Agents: N-acetyl cysteine, melatonin and progesterone are the agents used in this group. After TBI, the amount of glutathione decreases in intracellular and mitochondria. Tissue damage increases. Agents that increase the amount of glutathione required to dissolve the increased amount of ROS in the traumatic cell are becoming increasingly important. Melatonin (N-acetyl-5-methoxypyrraminamine) is a hormone that is synthesized overnight through the pineal gland, which has a strong antioxidant and free radical reductive properties. It has been shown to have neuroprotective effect when applied to patients with TBI, to decrease edema, to prevent neuronal apoptosis, to regulate BBB permeability and to increase survival (39). However, it has not yet entered into routine clinical practice. Progesterone works with many areas and mechanisms to improve repair of damage to nerve tissue caused by trauma or ischemic injury. At lower doses,

progesterone increases cell proliferation and provides anti-apoptotic effect. Loss of oxygen and glucose as a result of disruption of blood flow to the local site of injury leads to energy failure and ultimately cell death. Progesterone protects neurons from damage-related ischemia and reduces the width of the necrotic area and secondary degeneration (40). It has also been shown to reduce the synthesis of pro-inflammatory cytokines such as TNF-α and IL-1. Combination therapy including progesterone and vitamin D hormone has been reported to be more effective than monotherapy for nervous system damage and disease (41).

4. Anti-inflammatory Agents: Minocycline, cyclosporine, statins are agents used in this group. Minocycline is a tetracycline derivative having strong anti-inflammatory, anti-apoptotic and antioxidant properties, independent of its antibacterial activity. Because of its high lipid solubility, the peripherally administered minocycline easily passes the blood-brain barrier. Along with other inflammatory mediators, it reduces the production of proinflammatory cytokines and chemokines, reduces nitric oxide, and inhibits the proliferation of microglial cells. Evidence from in vivo and in vitro neurological disease models has shown that minocycline inhibits IFN-α stimulated microglial activation, reducing IL-1β and TNF-α production and showing neuroprotective effects (42). In a randomized study examined the effects of the minocycline on chronic microglial activation (CMA) and neurodegeneration in 15 patients who had sustained a TBI at least 6 months earlier. The investigators found that minocycline treatment reduced CMA. These findings raise the prospect that CMA has a beneficial role after TBI, which might be harnessed to improve patient outcomes. In this experimental medicine study, minocycline after traumatic brain injury reduced chronic microglial activation while increasing a marker of neurodegeneration. These findings suggest that microglial activation has a reparative effect in the chronic phase of traumatic brain injury (43,44)

Cyclosporin is a cyclic peptide that is isolated from the fungus and is immunosupressive. Mitochondrial dysfunction plays an important role in the mechanisms of secondary cell death after TBI. Some reports have shown that inhibition of mitochondrial permeability transition pores with the immunosuppressant drug cyclosporin A is effective. Cyclosporin A is recommended to reduce neuronal damage in traumatic mitochondrial dysfunction. After studies supporting the effectiveness of brain preservation in severe TBI, FDA approval for the treatment of TBI was received in 1999 and progressing to advanced clinical trials for the treatment of TBI (45). The proposed mechanism of the neuroprotective effect of cyclosporin A has been shown to involve the inhibition of mitochondrial insufficiency in the brain tissue by binding to the cyclophylline D. The binding of cyclosporine A to cyclophylline D prevents the formation of mPTP, a catastrophic event in terms of neuronal cell survival. mPTP causes a sudden collapse of the mitochondrial membrane potential that separates the electron transport system from ATP production. The release of pro-apoptotic molecules (eg, cytochrome C, apoptosis inducing factor) from mitochondria is partly regulated by mPTP formation and leads to activation of cell death pathways. An additional consequence of mPTP formation is the generation of ROS, which contributes to cellular damage by oxidizing cellular proteins and lipids (46).

Statins have wide availability, therefore statins are an ideal candidate therapy for acute brain injury. Statins influence multiple mechanisms of acute and secondary neuronal injury; they have endothelial and vasoactive properties, anti-oxidant, anti-inflammatory, anti-excitotoxicity, and anti-thrombotic effects. Statins have been shown to promote angiogenesis in both TBI and ischemic stroke models. Preclinical studies have shown significant benefit of statins in models of TBI and related disease processes, including cerebral ischemia, intracerebral hemorrhage, and subarachnoid hemorrhage. It has also been shown to have neuroprotective effects, along with antiinflammatory

effects in a rat model with subarachnoid hemorrhage, while normally used as lowering cholesterol (47). Hypercholesterolemia is an ischemic and inflammatory risk factor in some neurodegenerative diseases (48). Statins can alleviate oxidative stress, stabilize the atherosclerotic plaque, improve endothelial function, reduce blood vessel inflammation, and promote expression of neuroprotective genes in the brain (49). A study reported that treatment of TBI with statins improved spatial learning on days 31-35 after onset of TBI; in the non-neurogenic region of the hippocampal CA3 region, statin treatment reduced the neuronal loss after TBI, demonstrated the neuroprotective effect of statins; in the neurogenic region of the dentate gyrus, treatment of TBI with statins enhanced neurogenesis; statin treatment increased TBI-induced angiogenesis; and treatment with simvastatin at the same dose provided a therapeutic effect superior to treatment with atorvastatin (50).

CONCLUSION

TBI is a systemic disease. It is not just a simple, localized event that points to only a small amount of neural tissue. Traditional research of pharmacological agents to treat TBI or stroke should typically not focus on finding a single receptor mechanism to take into account beneficial effects. It is reasonable to consider how different research designs might be used to identify which treatments work best, for whom, and under what circumstances. The candidate therapeutics, clinically relevant biomarkers, outcome measures, and standardization would greatly facilitate the development of successful combination therapies for TBI. Apparent progress in diagnosis and treatment will improve management strategies in the future.

REFERENCES:

1. Peeters W, van den Brande R, Polinder S, Brazinova A, Steyerberg EW, Lingsma HF, et al. Epidemiology of traumatic brain injury in Europe. Acta Neurochir (Wien) 2015; 157: 1683-1696.
2. Sloan S, Winkler D, Anson K. Long term outcome following TBI. Brain Impairment 2007; 8: 251-261.
3. Thornhill S, Teasdale GM, Murray GD, McEwen J, Roy CW, Penny KI. Disability in young people and adults one year after head injury: prospective cohort study. BMJ (Clinical research ed.) 2000; 320: 1631- 1635.
4. Stover JF, Stocker R. Intensive care treatment options of elevated intracranial pressure following severe traumatic brain injury. In: Oestern H J, Trentz O, Uranues S, editors. Head, thoracic, abdominal, and vascular, injuries. 1st ed. Berlin: Springer-Verlag; 2011. pp. 93–152.
5. Kinoshita K. Traumatic brain injury: pathophysiology for neurocritical care. J Intensive Care 2016; 4: 29.
6. Stiefel MF, Udaetuk JD, Spiotta AM, Gracias VH, Goldberg A, Maloney-Wilensky E, et al. Conventional neurocritical care and cerebral oxygenation after traumatic brain injury. J Neurosurg 2006; 105: 568-575.
7. Jaeger M, Schuhmann MU, Soehle M, Meixensberger J. Continuous assessment of cerebrovascular autoregulation after traumatic brain injury using brain tissue oxygen pressure reactivity. Crit Care Med 2006; 34: 1783-1788.
8. Evans TA, Barkauskas DS, Myers JT, Hare EG, You JQ, Ransohoff RM, et al. High-resolution intravital imaging reveals that blood-derived macrophages but not

resident microglia facilitate secondary axonal dieback in traumatic spinal cord injury. Exp Neurol 2014; 254:109-120.

9. Tran, N, Kim S, Vincent H, Rodriguez A, Hinton D, Bullock, M, Young H. Aquaporin-1-mediated cerebral edema following traumatic brain injury: effects of acidosis and corticosteroid administration. J. Neurosurg 2010; 112: 1095-1104.

10. Suehiro E, Fujisawa H, Akimura T, Ishihara H, Kajiwara K, Kato S, et al. Increased matrix metalloproteinase-9 in blood in association with activation of interleukin-6 after traumatic brain injury: influence of hypothermic therapy. J Neurotrauma 2004; 21: 1706-1711.

11. Marmarou A, Signoretti S, Fatouros PP, Portella G, Aygok GA, Bullock MR. Predominance of cellular edema in traumatic brain swelling in patients with severe head injuries. J Neurosurg 2006; 104: 720-730.

12. Castejón OJ. Ultrastructural pathology of cortical capillary pericytes in human traumatic brain oedema. Folia Neuropathol 2011; 49: 162-173.

13. Lozano D, Gonzales-Portillo GS, Acosta S, Pena I, Tajiri N, Kaneko Y, et al. Neuoinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatric Disease and Treatment 2015;11: 107-106.

14. Taya K, Gulsen S, Okuno K, et al. Modulation of AQP4 expression by the selective V1a receptor antagonist, SR49059, decreases trauma-induced brain edema. Acta Neurochir Suppl 2008; 102:425–429.

15. Ghabriel MN, Thomas A, Vink R. Magnesium restores altered aquaporin-4 immunoreactivity following traumatic brain injury to a preinjury state. Acta Neurochir Suppl 2006; 96: 402–406.

16. Kikuchi K, Tancharoen S, Matsuda F, et al. Edaravone attenuates cerebral ischemic injury by suppressing aquaporin-4. Biochem Biophys Res Commun 2009; 390:1121–1125.

17. Papadopoulos MC, Manley GT, Krishna S, Verkman AS. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. Faseb J 2004; 18: 1291–1293.

18. Berger RP. The Use of Serum Biomarkers to Predict Outcome After Traumatic Brain Injury in Adults and Children. J Head Trauma Rehabil 2006; 21: 315-333.

19. Gidday JM, Gasche YG, Copin JC, et al. Leukocyte-derived matrix metalloproteinase-9 mediates blood–brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. Am J Physiol Heart Circ Physiol 2005; 289: 558–568.

20. Tejima E, Guo S, Murata Y, et al. Neuroprotective effects of over-expressing tissue inhibitor of metalloproteinase TIMP-1. J Neurotrauma 2009; 26: 1935–1941.

21. Zhao B-Q, Wang S, Kim H-Y, et al. Role of matrix metalloproteinases in delayed cortical responses after stroke. Nat Med 2006; 12: 441–445.

22. Bertorellia R, Adamia M, Santob ED, Ghezzib P. MK 801 and dexamethasone reduce both tumor necrosis factor levels and infarct volume after focal cerebral ischemia in the rat brain. Neurosci. 1998; 246: 41-44

23. Lu¨scher TF, Wenzel RR. Endothelin and endothelin antagonists: pharmacology and clinical implications. Agents Actions Suppl 1995;45: 237–253.

24. Benigni A, Remuzzi G. Endothelin antagonists. Lancet 1999;353:133–138.

25. Krum H, Liew D. Current status of endothelin blockade for the treatment of cardiovascular and pulmonary vascular disease. Curr Opin Investig Drugs 2003;4: 298–302.

26. Kreipke CW, Rafols JA, Reynolds CA, Schafer S, Marinica A, Bedford C, Fronczak M, Kuhn D, Armstead WM. Clazosentan, a novel endothelin A antagonist, improves cerebral blood flow and behavior after traumatic brain injury. Neurol Res. 2011;33: 208-213.

27. Davenport AP, Hyndman KA, Dhaun N, Southan C, Kohan DE, Pollock JS, et al. Endothelin. Pharmacol Rev 2016; 68: 357-418.

28. Su EJ, Fredriksson L, Kanzawa M, Moore S, Folestad E, Stevenson TK, et al. Imatinib treatment reduces brain injury in a murine model of traumatic brain injury. Front Cell Neurosci 2015; 9: 385.

29. Adzemovic MV, Zeitelhofer M, Eriksson U, Olsson, Nilsson I Imatinib ameliorates neuroinflammation in a rat model of multiple sclerosis by enhancing blood-brain barrier integrity and by modulating the peripheral immune response. PLoS One 2013;8:e56586.

30. Aksu NM, Şenlikçi H, Akkaş M, Özmen MM The Neurological Improvement of a Patient after Amantadine Infusion. JAEMCR 2013; 4: 161-3.

31. Giacino JT, Whyte J, Bagiella E, Kalmar K, Childs N, Khademi A, et al. Placebo-controlled trial of amantadine for severe traumatic brain injury. N Engl J Med 2012; 366: 819-26.

32. Sawyer E, Mauro LS, Ohlinger MJ. Amantadine enchancement of arousal and cognition after traumatic brain injury. Ann Pharmacother 2008; 42: 247-52.

33. İmer M, Uzunkol A, Omay BS, Sabancı PA, Sencer A, Erdem T, Karasu A Kaya M. The Effect of Magnesium, Memantine and Combination of Magnesium and Memantine on Blood Brain Barrier Permeability and Brain Oedema After Experimental Traumatic Diffuse Brain Injury. J Nervous Sys Surgery 2008; 1: 153-160.

34. Petratos S, Ozturk E, Azari MF, Kenny R, Lee JY, Magee KA, et al. Limiting multiple sclerosis related axonopathy by blocking Nogo receptor and CRMP-2 phosphorylation. Brain 2012; 135: 1794-1818.

35. Temkin N, Anderson G, Winn H, Ellenbogen R, Britz G, Schuster J, et al. Magnesium sulfate for neuroprotection after traumatic brain injury: a randomised controlled trial. Lancet Neurol 2007; 6: 29-38.

36. Özdöl Ç Investigation of the Effects of Magnesium Sulfate Therapy on Recovery in Traumatic Brain Injury. Med J SDU 2018; 25: 293-297.

37. Li W, Bai Y, Li Y, Liu K, Wang M, Xu G, et al. Magnesium sulfate for acute traumatic brain injury. J Craniofac Surg 2015; 26: 393-398.

38. Salehpour F, Shakeri M, Ahmadvand A, Vafaee R, Jafari R, Safaiyan A. Magnesium sulfate effect on the clinical course and GCS of patients with a severe diffuse axonal injury. J Paramed Sci 2012; 3: 2-6.

39. Alluri H, Wilson RL, Anasooya Shaji C, Wiggins-Dohlvik K, Patel S, Liu Y, et al. Melatonin preserves blood-brain barrier integrity and permeability via matrix metalloproteinase-9 inhibition. PLOS One 2016;11: e0154427.

40. Stein DG. Progesterone exerts neuroprotective effects after brain injury. Brain Res Rev 2008; 57: 386-397.

41. Cekic M, Sayeed I, Stein DG. Combination treatment with progesterone and vitamin D hormone may be more effective than monotherapy for nervous system injury and disease. Front Neuroendocrinol 2009; 30: 158-172.

42. Wang N, Mi X, Gao B, Gu J, Wang W, Zhang Y, Wang X. Minocycline inhibits brain inflammation and attenuates spontaneous recurrent seizures following pilocarpine-induced status epilepticus. Neuroscience 2015; 287: 144–156.

43. Bi Q, Shi L, Yang P, Wang J, Qin L. Minocycline attenuates interferon-α-induced impairments in rat fear extinction. J Neuroinflammation 2016; 30: 172.

44. Scott G, Zetterberg H, Jolly A, Cole J, De Simoni S, Jenkins PO, Feeney C, Owen D, Lingford-Hughes A, Howes O, Patel M, Goldstone A, Gunn R, Blennow K, Matthews P, Sharp D. Minocycline reduces chronic microglial activation after brain trauma but increases neurodegeneration. Brain 2018:141;459-471.

45. Sullivan PG, Sebastian AH, Hall ED. Therapeutic window analysis of the neuroprotective effects of cyclosporine a after traumatic brain injury. J Neurotrauma 2011; 28: 311-318.

46. Mazzeo A.T. Beat A. Singh A. Bullock M.R. The role of mitochondrial transition pore, and its modulation, in traumatic brain injury and delayed neurodegeneration after TBI. Exp. Neurol. 2009; 218: 363–370.

47. Fernández-Navarro J, Aldea P, de Hoz R, Salazar JJ, Ramírez AI, Rojas B, et al. Neuroprotective effects of low-dose statins in the retinal ultrastructure of hypercholesterolemic rabbits. PLoS One 2016; 4;11(5):e0154800.

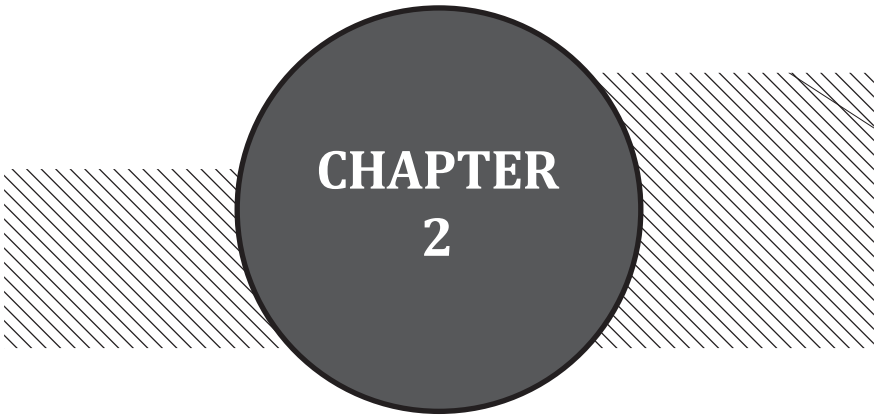
48. Fourgeux C, Martine L, Gambert-Nicot S, Bron A, Creuzot-Garcher C, Bretillon L. Cholesterol and ocular pathologies: focus on the role of cholesterol-24S-hydroxylase in cholesterol homeostasis. OCL 2015; 22: D204.

49. Johnson-Anuna LN, Eckert GP, Keller JH, Igbavboa U, Franke C, Fechner T, et al. Chronic administration of statins alters multiple gene expression patterns in mouse cerebral cortex. J Pharmacol Exp Ther. 2005; 312: 786–793.

50. Lu D, Qu C, Goussev A, et al. Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. J Neurotrauma 2007;24: 1132–1146

History Of Liver Transplantation Surgery

Hüseyin Kerem TOLAN[1]



[1] Dr., Sağlık Bilimleri Üniversitesi Ümraniye Eğitim ve Araştırma Hastanesi Genel Cerrahi AD.

The modern transplantation era started after the French surgeon Alexis Carrel first described the surgical technique of the vascular anastomosis (1). After this improvement a lot of transplantation attempts were done in skin graft and kidney transplantation but they unfortunately failed due to the misunderstanding of the immunologic reactions (2) (3). Then the first successful kidney transplant was reported at 1954 by Joseph Murray in Boston, Massachusetts. It was performed among the two identical twins and the recipient patient lived 8 more years after the transplantation and died not because of the transplantation process (4). All these led people think about the causes of rejections and the immunological processes were encountered. In 1963 by the help of the introduction of the immunosuppressive regimen which was a combination of the corticosteroids with a less toxic derivative of 6-mercaptopurine; the azathioprine, made a breakthrough in the transplantation era (5) (6). This improvement made people work more eagerly on the other solid organ transplantations.

The first report on the liver transplantation was reported at the fifty-fourth Congress of the Italian Society of Surgery; even before the kidney transplantation by Vittorio Staudacher from University of Milan in 1952 (7).In his report he described the five steps of experimental orthotropic liver transplantation in dogs which was resembling the modern transplantation process in humans. His work was unnoticed for over 60 years (8). Published in 1955; C. Stuart Welch of Albany Medical College's report was known as the first reported liver transplant until the recent discovery of Staudacher's published work (9). It was an auxiliary liver transplant in which the native liver of the recipient dog was not removed different than the Staudacher's work and the a hepatic allograft was implanted into the right paravertebral gutter of the recipient dog. In his work it was seen that after 3-4 days the implanted graft gone to atrophy which was first thought to be due to blood supply but after a decade it was only and better understood that the immunologic factors were the cause of this shrinkage, by other means the rejection of the liver graft. Not knowing the work of Staudacher; Jack Cannon of University of California, Los Angeles (UCLA)'s work was considered the first experimental description of the orthotropic liver transplantation (10). In late 1950s and at the beginning of 1960 more and more centers became interested in the liver transplantation such as the; Brigham Hospital at 1958 under the direction of Francis D. Moore (11), Northwestern University in Chicago also in 1958 with Thomas E. Starzl (12), Michael Francis Addison Woodruff in 1960 from the University of Otago Dunedin School of Medicine in New Zealand (13). During these investigations both in the technical and the immunologic aspect of the liver transplantation; the Northwestern University group with Thomas E. Starzl first described the currently well-known and widely used the piggy-back technique in the dog transplantation models (14) and also applied this retro-hepatic vena cava preserving method successfully in human liver transplantations (15).

These both groups; Northwestern University and the Brigham Hospital conducted these researches without being aware of each other until the meeting of the American Surgical Association in 1960 and by that time they have already performed 80 (12) and 31 (11) total liver replacement procedures in dogs respectively.

It was in 1963 when the first attempts were started to be made; after the experience gathered in kidney transplantation, by Thomas E. Starzl at the University of Colorado. The first liver transplantation surgery in humans was performed by this team on March 1, 1963. This patient was a 3-year-old boy named Bennie Solis. He had been operated for several times up to this age due to biliary atresia and at that time he became unconscious and was put on a ventilator which made him a candidate for a liver transplant (16). Because of the previous operations and the adhesions it took several hours just to enter the surgical field. Even though the tem had an enormous number of experimental liver transplantation experience; Bennie was lost during the transplantation surgery due to the

uncontrollable coagulation defect and bleeding. After this unfortunate attempt two more liver transplantations were performed; one in May 5, 1963 for a hepatoma (HCC) and the other one was in June 3, 1963, for a cholangiocarcinoma patient. Even the surgeries went well and as the immunosuppression protocol used for the Kidney transplantation in Colorado was applied (azathioprine administered both before and after transplantation and also adding a high-dose prednisone if there is a rejection) both of these 2nd and the 3rd recipients died at 22 and 7.5 post-operative days, respectively. The 2nd patient died due to pulmonary embolism and sepsis; the 3rd patient was lost due to pulmonary embolism according to the autopsies performed. Also the autopsies showed that both patients had already extra-hepatic metastases of their disease but also showed that there were no graft rejections in both explanted livers (17). The cause of the pulmonary embolism was due to the passive venovenous bypass used. This was commonly used in the canine models but in 2nd and the 3rd cases; because of a lesson learned from Bennie, coagulation-promoting therapy was given to help the coagulation in the surgery. But this also led to the clot formation in the plastic bypass tubes that passed to the lungs of the patients causing pulmonary emboli, abscess and infections as a result. This mechanism was thought to be the reason of the death of the two cases. In 1980 Pittsburg group introduced a venovenous system tht uses an external pump; working without a systemic anticoagulation that could be used not in all but in some selective cases of liver transplantation (18).

After all these failed attempts the Starzl's group at the University of Colorado performed two more liver transplants; 4th case was a 52 years old another Hepatocellular carcinoma (HCC) patient that was operated on 7th of October 1963 but also was lost 6 days after the operation due to a pulmonary emboli and liver failure. The 5th case was a 29 years old HCC patent operated on 10th of April 1963; who had the longest survival days of all the previous cases with 23 days, but died due to again a pulmonary emboli and concomitant sepsis (19). In the US the Brigham hospital Boston group directed by F.D. Moore in the same year at 1963 performed a liver transplantation in their center to a patent 58 years of age due to the colon cancer metastasis. The patient liver 11 days after the surgery and was lost due to pneumonitis, liver abscess and liver failure (20). Also in 1964 Europe made the first liver transplantation. This was performed in the Hospital St Antoine in Paris by Jean Demirleau; to a 75 years old patient with colon metastasis. Surgery took 4 hours but he patient died 3 hours after the operation due to bleeding (21). The transplanted liver was from a 71 years old donor so literally this was the first "marginal donor transplantation" performed in the history (22). After these patient losses in the world this made the surgeons stop and retrospectively work on the lost patients in order to make the liver transplantation feasible from 1964 to 1967. During this standby period; the organ preservation policies, immunosuppression protocols and the surgical techniques for liver transplantation were all studied by all groups.

In the immunosuppression protocols used in the kidney transplantation the azathioprine-prednisone protocol was successful in 70% of cases but the 30% mortality rates could not be improved (23). In the standby period between 1963 and 1966; the antilymphocyte globulin (ALG) was studied on which prepared from antilymphocyte serum obtained from horses immunized against dog for preclinical canine studies in kidney transplantation. After the success of ALG in experimental studies; the human-specific ALG was used in human kidney recipients in a trial conducted at the University of Colorado in 1966 (24) (25). After the success of this trial; 1- to 4-month course of ALG was being added to the azathioprine and prednisone protocol, and a "triple-drug cocktail" was founded thus decreasing the mortality in kidney recipients down to 10% (23).

The liver program at the University of Colorado was reopened in July 1967. Carl Gustav Groth joined the team in Colorado and the clotting problems during the previous surgeries leading to the patients loss were being solved by his knowledge on the blood

and the blood clotting mechanisms (26). As in the previous transplantations the problem was mainly emboli and bleeding by overcoming these problems; Starzl performed the first successful liver transplant in 1967 on an 18-month-old child with hepatoblastoma named Julie Rodriguez. With the triple-drug cocktail and the well knowledge of the coagulation system; the girl survived the operation and lived until the metastatic recurrence of her disease 400 days after her transplant surgery (16).

In Europe at the same period in the University of Cambridge, England; Roy Calne started a new program and by the joining of Moore acting as the first assistant performed their first liver transplant on May 2, 1968. Also by the collaboration of a hepatologist; Roger Williams at King's College Hospital in London the program was so called the Cambridge-King's Program, several successful transplants were performed (27). After the Cambridge-King's program; the University of Hannover led by Rudolf Pichlmayr in 1972; Hôpital Paul Brousse in Villejuif, led by Henri Bismuth in 1974; and the group in Groningen led by Ruud Krom in 1979 performed their first liver transplantations in Europe. All these centers; even if they were working in different continents made great attributions to the liver transplantation era. Also some technical improvements in biliary, arterial and venous anastomosis; experience in surgical dissection techniques, cold preservation, better understanding of coagulation process were made.

In the immunosuppression there was a new drug introduced. It was from a company named Sandoz in Basel, Switzerland; the cyclosporine. It was first used as an immunosuppressant by Jean-Francois Borel and was presented in 1976 in British Society for Immunology meeting in London (28). At that time Calne of the Cambridge-King's program and his immunologist colleague David White worked on this drug and showed it to be a powerful immunosuppressive drug that can be taken orally. Also they have a great effort in the development and the continuous production of this drug personally (29).The use of cyclosporine by the Cambridge-King's group as a monotherapy starting at 1979 and the successful results of this group passing the 50% one year survival in liver transplant results were also taken by the Starzl at the University of Colorado. Colorado group used this drug not as a monotherapy but they added it to their protocol as a double drug regimen with prednisone in kidney transplantations (30). Then they used it in the liver transplantation patients and made a 92% 1 year survival rate with this drug combination in 1980 (31).

After the improvements in coagulation, surgery and the immunosuppression in transplantation another major advancement also was made in organ preservation. First used in kidneys; in 1969 by Geoffrey Collins working in laboratory of Paul Terasaki in UCLA; made a perfusion fluid to preserve kidneys and then was also modified and called as Euro-Collins solution by which the cadaveric harvested kidneys were preserved until implantation (32). After this improvement in kidney preservation which could not be used for livers Folkert Belzer with James H. Southard in University of Wisconsin created the initial University of Wisconsin (UW) solution in 1979 which was then modified and started to being used successfully in the liver transplantation era from 1987 (33).

The improvements in these steps giving the courage to make more successful liver transplantations also took the attention of other companies working in this field. Such as in immunosuppression the emerging and ongoing success of Sandoz company with the cyclosporine other pharmaceutical companies also started investing more on the researches. A company from Japan; Fujisawa Pharmaceutical company found a fungi in a soil sample taken from an island and started working on it. They finally identified a macrolide as an immunosuppressant named as FK506 in 1984. The University of Pittsburgh group made a lot of experimental researches with this FK506 and reported it to be a very potent immunosuppressant with a low side effect potential (34). The first

trial conducted by the Pittsburg group showed a remarkable result in salvage treatment of 70% chronically rejected liver transplant cases and in patients used as a new drug showed a 93% one year survival rate (35). After getting the same results in liver transplant cases in multicenter trials FK506 was shown to be superior then the cyclosporine and became the preferred choice of immunosuppressant. This was also approved by the FDA in 1993 (36).

After all these successful improvements in increasing the graft and the patient life there was another new obstacle. This time; the transplantations were successful, the immunosuppressants were much more effective but there was a shortage of organs to be transplanted. Also as the success rates increased the demand to get a liver transplantation also increased gradually. So another important question was who should get the organ in first place. The Marginal Donor usage first appeared to attempt to solve the organ shortage problem. In the Hospital St Antoine in Paris in 1964 Jean Demirleau performed a liver transplant to a 75 years old patient from a 71 years old donor; even if the patient died 3 hours after the operation due to bleeding, literally this was the first "marginal donor transplantation" performed in the history (21) (22). Later in time; Makowka and friends from Pittsburgh popularized much more this organ usage in time (37). Thus a lot of cases are then published favoring the use of marginal donors in life saving situations (38). Even after these improvements and pushing the limits of marginal donor usage there was still a need for more livers to be transplanted to more and more patients. So the idea of splitting one whole liver in to anatomically two lobes and using one liver in two patients came in mind. Pichlmayr's group in 1988, from the University of Hannover reported the first ex situ split-liver transplant (39). Following them Bismuth's team from Paris reported a reduced size liver graft transplantation in children (40) and Christopher Broelsch from University of Chicago in 1990 also published their experience in split and reduced graft transplantations (41). After these ex-situ splits the first in-situ split was then performed by Xavier Rogiers in 1995 from the University of Hamburg in order to overcome the limitations of the donor pool (42). These remarkable improvements were seen but still there were not enough organs to match the needs of the patients. The in-situ split operations, size reduction methods and the technical innovations being performed in the liver surgery in deceased donor patients; made the surgeons think about performing the same techniques in the volunteer adult patients. As a result in 1989; the first adult to pediatric patient living donor liver transplantation was done by Russell W. Strong and Stephen V. Lynch of the University of Queensland in Brisbane, Australia (43). Following them; Broelsch and colleagues from the University of Chicago also reported their living donor results in pediatric cases at the American Surgical Association conference took place in 1990 (44). All these publications and performed surgeries the attention was then on performing the adult to adult living donor liver transplantation. The first living related liver transplantation was performed by the Kyoto group in Japan using a right lobe in 1994. Also in United States; following the Kyoto group, Colorado group in 1997 by the team of Igal Kam performed the first right lobe living donor liver transplantation (45). Several centers had been in to Living Donor Liver Transplantation since. The most influencing and the most successful of all these programs is from South Korea, Asan Medical center directed by Sung-Gyu Lee and his team (46). Currently they just celebrated their 6000 Liver transplantation cases; of which 5000 of them are Living Donor Liver transplantations, in November 30 2018. Starting their LDLT program in 1994 Sung-Gyu Lee and his team get to this astonishing number and success in 24 years of time. Also they are currently performing annually; the number of LDLT cases performed in all of United States, alone with great success and low morbidity rates. They get these numbers of LDLTs by the help of utilizing the successful transplantations in the Blood type incompatible cases and also by using dual grafts to overcome the need of the recipients (47) (48) (49).

There is still an enormous need for liver grafts in order to treat these patients and this led to the search for different sources of organs such as using organs from the other species which is called the Xenotransplantation. First the chimpanzee livers (50) and then the baboon livers (51) were used as the xenografts; but they all failed to succeed and the patients were all lost due to mainly infectious complications. After that the first porcine to human liver transplantation was performed in October 1992 by Makowka at the Cedars-Sinai program in Los Angeles; to gain some time to the transplantation as a bridging, but the patient could not make it to the operation. After these failures with the implantation of the xenograft livers to the humans; the porcine liver was this time was used as an extra-corporal filter. Marlon Levy from Dallas in the Baylor University Medical Center; reported the first successful ex vivo porcine liver used in perfusion as a bridging treatment to the liver allotransplantation (52). There are still researches going on in this xeno-transplantation field as it could be an important source of grafts for people in need of transplantation.

REFERENCES:

1. Carrel A. The surgery of blood vessels, etc. B Johns Hopkins Hosp 1907;18:18-28.
2. Medawar PB. Immunity to homologous grafted skin; the suppression of cell division in grafts transplanted to immunized animals. Br J Exp Pathol. 1946;27:9-14.
3. Hamilton D, Reid, WA. Yu Yo Voronoy and the first human kidney allograft. Surg Gynecol Obstet. 1984;159(3):289-294.
4. Merrill JP Murray J, Harrison JH. Successful homotransplantation of the human kidney between identical twins. JAMA. 1956;160(4):277-282.
5. Murray JE, Merrill J, Harrison JH, et al. Prolonged survival of human-kidney homografts by immunosuppressive drug therapy. N Engl J Med. 1963;268:1315-1323.
6. Starzl TE, Waddell WR, Marchioro TL. Reversal of rejection in human renal homografts with subsequent development of homograft tolerance. Surg Gynecol Obstet. 1963;117(4):385.
7. Brent LA. History of Transplantation Immunology. London: Academic Press; 1997. 1-482.
8. Staudacher V. Trapianti di organi con anostomosi vascolari. La Riforma Medica. 1952;66:1060.
9. Welch CS. A note on transplantation of the whole liver in dogs.Transplant Bull. 1955;2:54-55.
10. Cannon JA. Brief report. Transplant Bull. 1956;3:7.
11. Moore FD, Wheeler HB, Demissianos HV, et al. Experimental whole organ transplantation of the liver and of the spleen. Ann Surg. 1960;152:374-387.
12. Starzl TE, Kaupp HA Jr, Brock DR, et al. Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet. 1960;111:733-743.
13. Woodruff MFA. The Transplantation of Tissues and Organs. Springfield, Illinois: Charles C Thomas; 1960. 1-777.
14. Starzl TE, Bernhard VM, Benvenuto R, et al. A new method for one-stage hepatectomy in dogs. Surgery. 1959;46:880-886.
15. Tzakis A, Todo S, Starzl TE. Orthotopic liver transplantation with preservation of the inferior vena cava. Ann Surg. 1989;210:649-652.

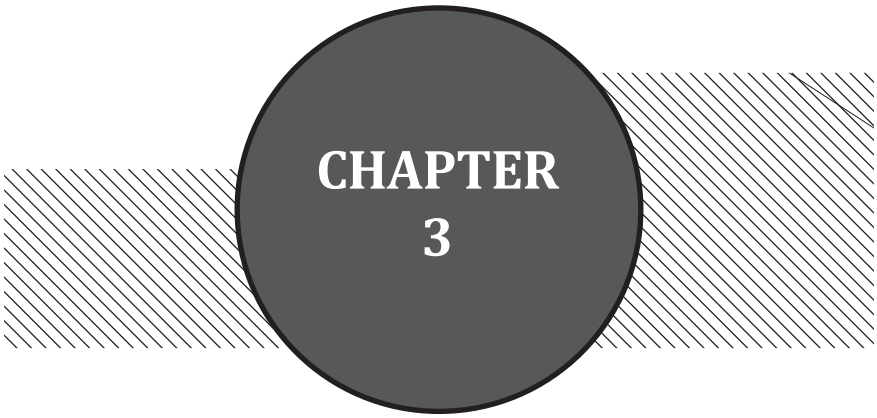
16. Starzl TE. The Puzzle People: Memoirs of a Transplant Surgeon. Pittsburgh: University of Pittsburgh Press; 1992.
17. Starzl TE, Marchioro TL, Von Kaulla KN, et al. Homotransplantation of the liver in humans. Surg Gynecol Obstet. 1963;117:659-676.
18. Denmark SW, Shaw BW Jr, Starzl TE, et al. Veno-venous bypass without systemic anticoagulation in canine and human liver transplantation. Surg Forum. 1983;34:380-382.
19. Starzl TE, Marchioro TL, Rowlands DT Jr, et al. Immunosuppression after experimental and clinical homotransplantation of the liver. Ann Surg. 1964;160:411-439.
20. Moore FD, Birtch AG, Dagher F, et al. Immunosuppression and vascular insufficiency in liver transplantation. NY Ann Acad Sci. 1964;120:729-738.
21. Demirleau J, Noureddine M, Vignes p. Tentative d'homogreffe hepatique [Attempted hepatic homograft]. Mem Acad Chir (Paris). 1964;90:177.
22. Maggi U, Azoulay D. Further details from the first human liver transplantation in Europe. Transplantation. 2013;96:47-48.
23. Starzl TE, Brettschneider Penn I, et al. A trial with heterologous antilymphocyte globulin in man. Transplant Proc. 1969;1:448-454.
24. Starzl TE, Marchiora TL, Porter KA, et al. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation, and in human renal homotransplantations. Surg Gynecol Obstet. 1967;124:301-308.
25. Starzl TE, Groth CG, Terasaki PI, et al. Heterologous antilymphocyte globulin, histocompatibility matching, and human renal homotransplantation. Surg Gynecol Obstet. 1968;126:1023-1035.
26. Groth CG, Pechet L, Starzl TE. Coagulation during and after orthotopic transplantation of the human liver. Arch Surg.1969;98:31-34.
27. Calne RY, Williams R, Dawson JL, et al. Liver transplantation in man. II A report of two orthotopic liver transplants in adult recipients. Br Med J. 1968;4:541-546.
28. Heusler K, Pletscher A. The controversial early history of cyclosporine. Swiss Med Wkly. 2001;131:299-302.
29. Hamilton DA. History of Organ Transplantation: Ancient Legends to Modern Practice. Pittsburgh: University of Pittsburgh Press; 2012. xii.
30. Starzl TE, Weil III R, Iwatsuki S, et al. The use of cyclosporin A and prednisone in cadaver kidney transplantation. Surg Gynecol Obstet. 1980;151:17-26.
31. Starzl TE, Klintmalm GBG, Porter KA, et al. Liver transplantation with the use of cyclosporine A and prednisone. N Engl J Med. 1981;305:266-269.
32. Collins GM, Halasz NA. Clinical comparison of methods for cadaveric kidney preservation. J Surg Res. 1978;24:396-400.
33. Todo S, Nery J, Yanaga K, et al. Extended preservation of human liver grafts with UW solution. JAMA. 1989;261:711-714.
34. Wallemacq PE, Redling R. FK506 (tacrolimus), a novel immunosuppressant in organ transplantation: clinical, biomedical and analytical aspects. Clin Chem. 1993;39:2219-2228.
35. Todo S, Fung JJ, Tzakis A, et al. One hundred ten consecutive primary orthotopic liver transplants under FK506 in adults. Transplant Proc. 1991;23:1397-1402.
36. Starzl TE, Todo S, Demetris AJ, et al. Tacrolimus (FK506) and the Pharmaceutical/Academic/Regulatory Gauntlet. Am J Kidney Dis. 1998;31:S7-S14.

37. Makowka L, Gordon RD, Todo S, et al. Analysis of donor criteria for the prediction of outcome in clinical liver transplantation. Transplant Proc. 1987;19:2378-2382.
38. Tolan K, Kayaalp C, Ispir M, Kirmizi S, Yilmaz S. Having a Healthy Birth With a 100-Year-Old Liver. Prog Transplant. 2016 Dec;26(4):392-393.
39. Pichlmayr R, Ringe B, Gubernatis G, et al. Transplantation einer spenderleber auf Zwis Empfanger (Split liver transplantation) Eine neue Methode in der Weitzentwicklung der Lebesegment transplantation. Langenbecks Arch Surg. 1989;373:127-130.
40. Bismuth H, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. Surgery. 1984;95:367-370.
41. Broelsch CE, Emond JC, Whitington PF, et al. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. Ann Surg. 1990;212:368-375.
42. Rogiers X, Malago M, Gawad K, et al. In situ splitting of cadaveric livers. The ultimate expansion of a limited donor pool. Ann Surg. 1996;224:331-339.
43. Strong RW, Lynch SV, Ong TH, et al. Successful liver transplantation from a living donor to her son. N Engl J Med. 1990;322:1505-1507.
44. Emond JC, Whitington PF, Thistlethwaite JR, et al. Transplantation of two patients with one liver. Analysis of a preliminary experience with 'split-liver' grafting. Ann Surg. 1990;212:14-22.
45. Yamaoka Y, Washida M, Honda K, et al. Liver transplantation using a right lobe graft from a living related donor. Transplantation. 1994;57:1127-1130.
46. Lee SG. A complete treatment of adult living donor liver transplantation: a review of surgical technique and current challenges to expand indication of patients. Am J Transplant. 2015 Jan;15(1):17-38.
47. Moon DB, Lee SG, Hwang S, et al. Toward more than 400 liver transplantations a year at a single center. Transplant Proc 2013; 45: 1937–1941.
48. Lee S, Hwang S, Park K, et al. An adult-to-adult living donor liver transplant using dual left lobe grafts. Surgery 2001; 129: 647–650.
49. Hwang S, Lee SG, Lee YJ, et al. Lessons learned from 1,000 living donor liver transplantations in a single center: How to make living donations safe. Liver Transplant 2006; 12: 920–927.
50. Starzl TE. Orthotopic heterotransplantation. In: Starzl TE, ed. Experience in Hepatic Transplantation. Philadelphia: WB Saunders; 1969:408-421.
51. Starzl TE, Fung JJ, Tzakis A, et al. Baboon-to-human liver transplantation. Lancet. 1993;341:65-71.
52. Levy MF, Crippin J, Sutton S, et al. Liver allotransplantation after extracorporeal hepatic support with transgenic (hCD55/ hCD59) porcine livers. Transplantation. 2000;69:272-280.

Advances In Red Blood Cell Transfusion For Cardiac Surgery

Ayşe LAFÇI[1]

Derya GÖKÇINAR[2]



[1] M.D., Department of Anesthesiology and Reanimation, Ankara Numune Training and Research Hospital, University of Health Sciences, Ankara, Turkey

[2] M.D., Assoc. Prof., Department of Anesthesiology and Reanimation, Ankara Numune Training and Research Hospital, University of Health Sciences, Ankara, Turkey

INTRODUCTION

In patients undergoing cardiac surgery, red blood cell (RBC) transfusion is needed for a variety of reasons. These include anemia in the preoperative period, hemodilution occurring during cardiopulmonary bypass (CPBP), and decreased hemoglobin due to surgical bleeding, where it is performed to restore tissue hypoxia and provide adequate tissue oxygenation (Carson et al., 1996). However, reducing the amount of RBC transfusion has become a current issue due to concerns about the side effects and costs. High numbers of allogeneic RBC transfusion to overcome inadequate tissue oxygenation by diminished hemoglobin levels were found to be associated with mortality and morbidity (Lilly et al., 2018; Koch et al., 2006). Main strategies to limit the numbers of RBC transfusion were to restore preoperative anemia with other pharmacological modalities except RBC transfusion, to avoid intraoperative hemodilution, and to decrease the target threshold of hemoglobin levels (Hajjar et al., 2010). Some studies and meta-analyses have been published to determine optimal RBC transfusion limits and improve patient outcomes in cardiac surgery. New strategies developed to reduce blood transfusion in open heart surgery include preoperative iron treatment, determination of a lower target level of auto-transfused perioperative hemoglobin, avoiding of intraoperative hemodilution, administration of tranexamic acid to reduce bleeding, and use of surgicel, cell saver, and patient blood management program trainings. In this article, we will review evidence based data on RBC transfusion thresholds and novel approaches in cardiac surgery.

HISTORY

The first successful human-to-human blood transfusion was performed by James Blundell in London in 1818 (Dzik, 2007). Research on transfusion reactions achieved an important milestone when Karl Landsteiner discovered blood groups in 1900. In 1932, cadaver blood was started to be used for transfusion (Schmidt & Huestis, 2007). Since fibrinolysis occurs after death, use of anticoagulants were not needed in cadaveric blood. In 1967, seven patients received successful cadaveric blood transfusion (Giangrande, 2000).

Successful clinical use of blood transfusions greatly contributed cardiac surgery. In addition, discovery of heparin and introduction of extracorporeal circulation made a breakthrough in heart surgery. Gibbon, in 1953, performed a successful atrial septal defect closure surgery in a young girl (Gibbon, 1953). Beside technological advances, contemporary cardiac surgery builds on the improvements in hypothermia, cardioplegic solutions, and myocardial protection.

Adverse events associated with blood transfusion

Hemorrhage and hemodynamic instability are the most important indications for RBC transfusion. However, RBC transfusion can also be performed in some anemic patients though they are hemodynamically stable. While being critically important for a number of patients, RBC transfusion may also lead to fatal reactions in some patients. These adverse reactions may develop via immunological or non-immunological mechanisms. The former includes febrile non-hemolytic and hemolytic reactions, allergic and anaphylactic reactions, transfusion-related acute lung injury, transfusion-related graft versus host disease, post-transfusion purpura, and immune-mediated mechanisms. On the other hand, infectious contamination, transfusion-associated volume overload, hypothermia, various electrolyte imbalances, iron overloading due to high number of transfusion constitute non-immune adverse reactions. All these reactions may also be classified as acute when develop within six hours and delayed when occur after 24 to 48 hours or even over months or years. The National Healthcare Safety Network Hemovigilance Module reported the adverse

reactions after RBC transfusion during 2010-2012 as one case of transfusion-associated fluid overload, transfusion-related allergic reaction, and transfusion-related acute lung injury in every 100, 250, and 12,000 patients; respectively (Harvey et al., 2015).

Immune hemolytic reaction usually occurs due to incompatibilities of blood groups such as ABO, Kidd, Kell, and Duffy. It may develop either within first minutes (early) or five to seven days (late) of transfusion. Risk-groups include those who receive increased numbers of transfusion. It could cause fever, pain in the chest or back or at infusion site, hypotension symptoms, acute renal failure, shock, disseminated intravascular coagulation, and even progress to death (Aubron et al., 2018). This reaction may manifest as hematuria, severe bleeding at surgical field, hypotension, or shock in patients under anesthesia. The treatment consists of cessation of transfusion and reversing the shock. Very severe cases require exchange transfusion.

Transfusion-related allergic and anaphylactic reaction is IgA, IgG, and IgE reactions that develop against plasma proteins. It is the most common of blood transfusion reactions. Clinical manifestations include acute urticaria, pruritus, angioneurotic edema, nausea, bronchospasm, hypotension, arrhythmia, and cardiac arrest. In patients with a history of transfusion-related allergy, the decision favoring transfusion should be considered comprehensively, and the patient should be monitored during transfusion. Antihistaminic medication may be beneficial in patients with allergic reactions. Transfusion-related acute lung injury (TRALI) originates from anti-leukocyte antibodies in the blood where the inflammatory process causes pulmonary edema. As anti-leukocyte antibodies may be more common in the plasma of some donors, a careful selection of donors is critical. Use of leukocyte-reduced blood products may decrease the risk of TRALI. It typically occurs during second to sixth hours of transfusion. Protective mechanical ventilation strategies are important in the management of these patients. It is a self-limiting condition after 48-96 hours when treated quickly (Harvey et al., 2015; Aubron et al., 2018).

Post-transfusion purpura is associated with a rapid decline in platelet counts after around one week of the transfusion and bleeding diathesis. Patients with reduced platelets may feature gastrointestinal and urinary tract bleeding. Though hemorrhagic episodes are usually self-limiting, intracranial bleeding may be fatal. In transfusion-related graft versus host disease, T-lymphocytes mediate an immunological attack in the immunocompromised transfusion recipient. It is characterized by fever, maculopapular rash, hemorrhagic bullae, watery diarrhea, enterocolitis, elevated serum transaminases, and pancytopenia. This reaction usually begins eight to ten days after transfusion; and the mortality is around 90%. The irradiation of the blood product before transfusion prevents from this reaction, especially in high-risk patients. Infectious contamination may complicate transfusions via bacterial, viral, or parasitic pathogens or prions. Severe clinical deterioration up to consumption coagulopathy and renal failure may develop in Gram (-) bacterial contamination. It presents with chills, fever, abdominal pain, and hypotension. The presence of bacteremia in the donor and the storage conditions of the blood product could be important etiological factors. Beside viruses, prion diseases such as Creutzfeldt-Jakob disease or kuru can also be transmitted by transfusion. Care should be taken for hypothermia after transfusion in neonates or in patients who receive massive transfusion; as it may cause cardiac arrhythmias and metabolic disorders. The blood product to be transfused can be heated. Nevertheless, as the blood cells may undergo thermal damage, heating should be carried out under appropriate conditions. Patients with advanced age, left ventricular dysfunction, renal failure, atrial fibrillation, or hypertension constitute the risk groups for transfusion-related volume overload. Transfusion rate may be adjusted and prophylactic diuretic medication may be administered. Electrolyte imbalances, especially hyperkalemia, may occur in patients undergoing multiple RBC transfusions. In particular, patients with renal dysfunction may experience poor outcomes, including cardiac arrest.

In patients undergoing transfusion, cessation of transfusion should be prioritized among other measures as soon as any acute reaction occurs. Afterwards, supportive treatment should be initiated for affected vital organs to preserve cardiac, respiratory, or renal functions (Harvey et al., 2015; Aubron et al., 2018).

Restrictive RBC transfusion strategy in cardiac surgery

Although evidence-based findings from studies on RBC transfusion should ideally be translated into successful outcomes in clinical practice, no consensus has been still achieved on RBC use in cardiac surgery in preoperative, intraoperative, or postoperative setting.

In terms of target hemoglobin levels, restrictive or liberal transfusion strategies are implemented in RBC transfusion. Mazer et al., in their study, reported no difference of length of stay at the hospital or in the intensive care unit between the patients undergoing restrictive or liberal RBC transfusion strategy (Mazer et al., 2017). Several studies reported lower rate of mortality associated with restrictive strategy compared to that in liberal strategy. The study by Murphy et al. reported that patients who were administered RBC transfusion had six-fold higher mortality than did those without RBC (Murphy et al., 2007; Paone et al., 2012). On the other hand, some studies comparing these two strategies reported no difference in terms of mortality (Bracey et al., 1999; Slight et al., 2007; Hajjar et al., 2010).

Target hemoglobin levels differ even among those studies advocating restrictive RBC transfusion strategy. Similarly, intraoperative and postoperative target hemoglobin levels also vary among studies. In their study, Mazer et al. reported to determine intraoperative target hemoglobin level as 7.5 g/dL in the restrictive transfusion group (Mazer et al., 2017). Shehata et al. suggested intraoperative hemoglobin threshold as 7 g/dL and postoperative hemoglobin as 7.5 g/dL (Shehata et al., 2012). While Lilly et al. reported both intraoperative and postoperative hemoglobin target as 7 g/dL, Bracey et al. suggested a postoperative hemoglobin level of 8 g/dL (Lilly et al., 2018; Bracey et al., 1999). These studies might imply that a definite target for the hemoglobin level is still controversial in the restrictive transfusion strategy.

The determination of hemoglobin level before major surgery is very important, as preoperative anemia was found to be associated with an increased need for transfusion and postoperative morbidity (Jans et al., 2014). Anemia is common in patients with coronary artery disease (Sabatine et al., 2005). In open heart surgery, RBC transfusion causes increased long-term mortality. Perioperative RBC transfusion has been suggested to cause localized inflammation and coagulation abnormalities in the vessels anastomosed during coronary artery bypass grafting surgery (CABG), which in turn, may lead to thrombosis. Engoren et al. reported that perioperative RBC transfusion was associated with graft occlusion after CABG (Engoren et al., 2015). Preoperative iron treatment is recommended in patients who undergo major surgery (Elhenawy et al., 2015). Limited definitive evidence exist about the optimal administration method of iron treatment before elective cardiac surgery. Iron can be given by oral or intravenous route in such patients. A study comparing oral and intravenous iron therapy before elective cardiac surgery reported no significant difference between the two modalities in terms of the need for RBC transfusion (Padmanabhan et al., 2018). Studies on the administration of efficacious iron therapy are still ongoing.

Reducing intravenous fluid replacement during open heart surgery and reducing the volume of prime solution during CPBP may diminish the need for RBC transfusion by preventing hemodilution. Apart from the use of topical hemostatic agents and hemostatic sealants in the intraoperative period, the use of anti-fibrinolytic tranexamic

acid also decrease the amount of postoperative bleeding. This can reduce the numbers of RBC transfusion, an important step towards many complications (Carless et al., 2003; Abrishami et al., 2009).

CONCLUSION

In conclusion, studies regarding the use of various RBC transfusion protocols in open heart surgery are still ongoing. The criteria for perioperative RBC use are tried to be determined in these interventions. It is important for physicians and other healthcare providers to practice RBC transfusions in accordance with evidence-based up-to-date recommendations. This might protect patients against transfusion reactions and complications in perioperative period.

REFERENCES

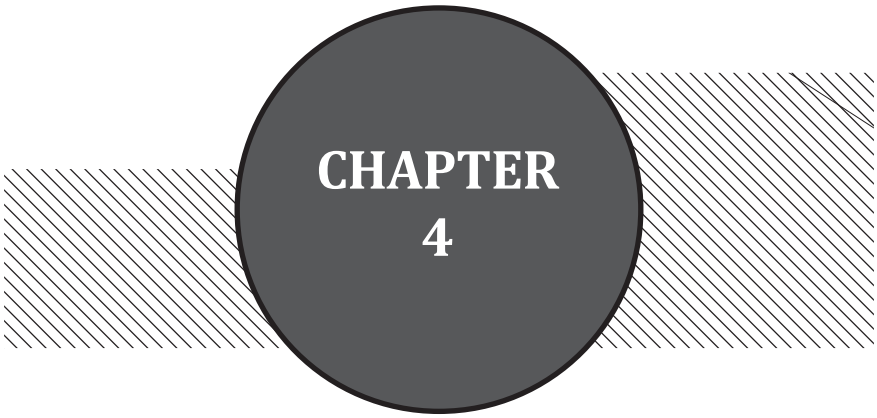
1. Elhenawy AM, Meyer SR, Bagshaw SM, MacArthur RG & Carroll LJ. (2015). Role of preoperative intravenous iron therapy to correct anemia before major surgery: study protocol for systematic review and meta-analysis. Syst Rev 4: 29.
2. Abrishami A, Chung F & Wong J. (2009). Topical application of antifibrinolytic drugs for on-pump cardiac surgery: a systematic review and meta-analysis. Can J Anaesth 56:202–12.
3. Aubron C, Aries P, C. Le Niger C, Sparrow RL & Ozier Y. (2018). How clinicians can minimize transfusion-related adverse events? Transfus Clin Biol 25(4), 257-261.
4. Bracey AW, Radovancevic R, Riggs SA, Bracey AW, Radovancevic R, Riggs SA, Houston S, Cozart H, Vaughn WK, Radovancevic B, McAllister HA Jr & Cooley DA. (1999). Lowering the hemoglobin threshold for transfusion in coronary artery bypass procedures: Effect on patient outcome. Transfusion 39:1070–1077.
5. Carless PA, Henry DA & Anthony DM. (2003). Fibrin sealant use for minimising peri-operative allogeneic blood transfusion. Cochrane Database Syst Rev;2:CD004171.
6. Carson JL, Duff A, Poses RM, Berlin JA, Spence RK, Trout R, Noveck H & Strom BL. (1996). Effect of anaemia and cardiovascular disease on surgical mortality and morbidity. Lancet. 19;348(9034):1055-60.
7. Dzik WH. (2007). The James Blundell Award Lecture 2006: transfusion and the treatment of haemorrhage: past, present and future. Transfusion Medicine 17, 367–374.
8. Engoren M, Schwann TA, Jewell E, Neill S, Benedict P, Likosky DS & Habib RH. (2015). Is transfusion associated with graft occlusion after cardiac operations? Ann Thorac Surg. **99(2):502-8.** doi: 10.1016/j.athoracsur.2014.09.028.
9. Giangrande PLF. (2000). The history of blood transfusion. Br J Haem 110; 758-767.
10. Gibbon JH Jr. (1953). Application of a Mechanical Heart and Lung Apparatus to Cardiac Surgery, Recent Advances in Cardiovascular Physiology and Surgery. A Symposium Presented by the Minnesota Heart Association and the University of Minnesota, Minneapolis, University of Minnesota, Sept. 14–16.
11. Hajjar LA, Vincent JL, Galas FR, Nakamura RE, Silva CM, Santos MH, Fukushima J, Kalil Filho R, Sierra DB, Lopes NH, Mauad T, Roquim AC, Sundin MR, Leão WC, Almeida JP, Pomerantzeff PM, Dallan LO, Jatene FB, Stolf NA & Auler JO Jr. (2010). Transfusion requirements after cardiac surgery: The TRACS randomized controlled trial. JAMA 2010;304:1559–67.

12. Harvey AR, Basavaraju SV, Chung KW & Kuehnert MJ. (2015). Transfusion-related adverse reactions reported to the National Healthcare Safety Network Hemovigilance Module, United States, 2010 to 2012. Transfusion 55(4):709-18.
13. Jans O, Jorgensen C, Kehlet H & Johansson PI. (2014). Lundbeck Foundation Centre for Fast-track Hip and Knee Replacement Collaborative Group Role of preoperative anemia for risk of transfusion and postoperative morbidity in fast-track hip and knee arthroplasty. Transfusion 54(3):717–26.
14. Koch CG, Li L, Duncan AI, Mihaljevic T, Cosgrove DM, Loop FD, Starr NJ & Blackstone EH. (2006). Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. Crit Care Med 34(6):1608-16.
15. Lilly CM, Badawi O, Liu X, Christine SG & Harris I. (2018). Red Blood Cell Product Transfusion Thresholds and Clinical Outcomes. J Intensive Care Med 1:885066618762746.
16. Mazer CD, Whitlock RP, Fergusson DA, Hall J, Belley-Cote E, Connolly K, Khanykin B, Gregory AJ, de Médicis É, McGuinness S, Royse A, Carrier FM, Young PJ, Villar JC, Grocott HP, Seeberger MD, Fremes S, Lellouche F, Syed S, Byrne K, Bagshaw SM, Hwang NC, Mehta C, Painter TW, Royse C, Verma S, Hare GMT, Cohen A, Thorpe KE, Juni P & Shehata N; TRICS. (2017). Investigators and Perioperative Anesthesia Clinical Trials Group. Restrictive or Liberal Red-Cell Transfusion for Cardiac Surgery. N Engl J Med 30;377(22):2133-2144. doi: 10.1056/NEJMoa1711818.
17. Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L & Angelini GD. (2007). Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation 116:2544–2552.
18. Paone G, Brewer R, Theurer PF, Bell GF, Cogan CM & Prager RL. (2012). Michigan Society of Thoracic and Cardiovascular Surgeons: Preoperative predicted risk does not fully explain the association between red blood cell transfusion and mortality in coronary artery bypass grafting. J Thorac Cardiovasc Surg 143:178–185.
19. Padmanabhan H, Siau K, Nevill AM, Morgan I, Cotton J, Ng A, Brookes MJ & Luckraz H. (2018). Intravenous iron does not effectively correct preoperative anaemia in cardiac surgery: a pilot randomized controlled trial. Interact Cardiovasc Thorac Surg Aug 9:1-8 doi: 10.1093/icvts/ivy226.
20. Sabatine MS, Morrow DA, Giugliano RP, Burton PB, Murphy SA, McCabe CH, Gibson CM & Braunwald E. (2005). Association of hemoglobin levels with clinical outcomes in acute coronary syndromes. Circulation 111(16):2042–9.
21. Schmidt PJ & Huestis DW. (2007). Blood from cadavers: the final recycling. Transfusion 47:555-556.
22. Shehata N, Burns LA, Nathan H, Hebert P, Hare GM, Fergusson D & Mazer CD. (2012). A randomized controlled pilot study of adherence to transfusion strategies in cardiac surgery. Transfusion 52:91–99.
23. Slight RD, Fung AKY, Alonzi C, McClelland DB & Mankad PS. (2007). Rationalizing blood transfusion in cardiac surgery: Preliminary findings with a red cell volume-based model. Vox Sanguinis 92:154–156.

Reduced Serum Bilirubin Levels İn Patients With Normal Pressure Glaucoma

Sadık Altan ÖZAL[1]

Ece ÖZAL[2]



[1] Trakya University, School of Medicine, Department of Ophthalmology, Edirne TURKEY

[2] Edirne State Hospital, Department of Ophthalmology, Edirne TURKEY

INTRODUCTION

Glaucoma is the main cause of irreversible vision loss worldwide, leading to detriment of peripapillary retinal nerve fiber layer (RNFL) and optic nerve head. It is estimated that more than 60 million people in the world are affected by this disease. [1] The greatest risk factor for developing optic nerve damage and visual field deterioration is intraocular pressure (IOP) elevation. [2] However, there is also a clinical entity known as normal pressure glaucoma (NPG), that is characterised by optic nerve damage, progressive RNFL thinning and visual field defects, although the IOP is within normal limits.[3,4] Normal pressure glaucoma is a subtype of primary open-angle glaucoma, and it is the most widespread form of glaucoma in Asian populations.[5,6]

Although the IOP is normal in NPG patients, the main reason of the associated optic nerve detriment has not been precisely elucidated. It has been suggested that the deterioration of the optic nerve head blood stream by reduced arterial blood pressure or vasospasms leads to optic nerve detriment in NPG patients.[7] The association between NPG and vasospastic disorders such as migraine, Raynaud's phenomenon, and nocturnal hypotension have been shown in various studies.[8,9] The effect of oxidative stress on the development of NPG has also been shown.[10,11] It has been suggested that increased oxidative stress may result in impaired optic nerve head blood stream and ganglion cell death without IOP elevation. [12]

Bilirubin is a final product of haeme metabolism pathway, and it has been accepted as a waste matter for a long time. Haemoglobin is released from senescent erythrocytes. Haemoglobin is divided into haeme pigment and globin chains. After the metabolism of the haeme pigment to biliverdin by the enzyme of haeme oxygenase, biliverdin is reduced to indirect bilirubin by the biliverdin reductase enzyme. After the uptake of hepatocytes, the indirect bilirubin is turned into direct bilirubin by the uridine-diphosphoglucurate glucuronosyltransferase enzyme.[13] However, the idea that bilirubin is only a waste product is changing with the clinical studies that have been conducted in recent years showing that bilirubin has useful properties for the human body, such as anti-oxidant, anti-inflammatory and cytoprotective effects.[14-16] Decreased serum bilirubin concentrations have been reported to be related with various diseases such as, coronary artery disease, migraine, arterial hypertension, increased arterial stiffness and multiple sclerosis.[17-21]

Since both NPG and reduced serum bilirubin concentrations are related with systemic vascular disorders, we targeted to analyse the relationship between NPG and serum bilirubin concentrations.

METHODS

This is a cross-sectional study of cases with previously diagnosed NTG who were admitted to our clinic between January 2010 and February 2017. This study included 38 patients with NPG and 38 healthy controls with similar age and sex distribution, for a total of 76 cases. Written informed consent was taken from all of the cases. The study was approved by the institutional review board of the hospital and adhered to the tenets of the Declaration of Helsinki.

A detailed ocular examination containing best-corrected visual acuity, Goldmann applanation tonometry (AT 900, Haag-Streit Diagnostics, Koeniz, CH), slit-lamp biomicroscopy, corneal thickness evaluation (Pachymeter SP-3000, Tomey Corporation, Nagoya, JP), gonioscopy and ophthalmoscopy was performed in all NTG cases. Additionally, optical coherence tomography (OCT RS-3000 Lite, NIDEK Corporation, Tokyo, JP) measurement of the peripapillary RNFL, and visual field test (Carl-Zeiss Meditec, Dublin, CA) were also performed.

Normal pressure glaucoma was diagnosed based on a clinical examination showing a normal IOP (corrected for corneal thickness), thinning of the peripapillary RNFL, optic nerve deterioration, and characteristic glaucoma-related visual field defects. Patients with a follow-up period of at least one year were included in the study. The exclusion criteria included other forms of glaucoma such as, primary closed-angle glaucoma, primary open-angle glaucoma, pseudoexfoliative glaucoma and pigmentary glaucoma. Patients with congenital optic nerve anomalies (coloboma, pit), steroid-induced glaucoma, history of trauma or previous surgery, ischemic optic neuropathy, and optic nerve compression were not included in the study. Patients who had active systemic diseases such as affecting the liver, biliary system, pancreas, kidney and infectious diseases or malignant tumours that could influence the serum bilirubin concentrations were also excluded.

Fasting blood samples were taken from each case to measure direct bilirubin, indirect bilirubin, and the total serum bilirubin concentrations. The direct bilirubin and total bilirubin concentrations were evaluated with an Architect C16000 Clinical Chemistry Analyzer (Abbott Laboratories, Abbott Park, IL, USA) device. Then, the indirect bilirubin concentrations were calculated by the following formula: indirect bilirubin = total bilirubin – direct bilirubin.

STATISTICAL ANALYSIS

All the data were compiled into a computer file, and Statistical Package for Social Sciences for Windows 22.0 was utilized to perform the statistical analysis. Student's t test or the Mann-Whitney U test were used to compare the mean values between the two groups, after the evaluation of the data for a normal distribution using the Kolmogorov-Smirnov test. A p value less than 0.05 was considered as significant statistically.

RESULTS

In patients with NPG, the mean serum concentrations of direct bilirubin, indirect bilirubin and the total bilirubin were 0.19 mg/dL, 0.36 mg/dL, and 0.60 mg/dL, respectively. The direct bilirubin and indirect bilirubin levels were significantly lower in the patients with NPG than the control group, as displayed in Table 1. In addition, the total bilirubin concentrations were lower in patients with NPG than the control group, but the disparity between the two groups was not significant statistically.

DISCUSSION

Although the IOP is in normal limits in NPG patients, the cause of their optic nerve damage has not been completely elucidated. One of the primary theories used to explain the NPG pathogenesis is vascular theory. Systemic and ocular vascular disorders such as migraine Raynaud's phenomenon, peripheral arterial stiffness, nocturnal hypotension and reduced ocular perfusion pressure have been demonstrated to be associated with NPG.[22]

Reduced serum bilirubin levels have been reported to be associated with various diseases such as migraine, coronary artery disease, multiple sclerosis and hypertension.[17-20] The decrease in serum bilirubin levels in migraine is attributed to the strong anti-oxidant property of bilirubin and the reducing effect of neuroinflammation.[23] The high incidence of migraine in patients with NPG may have led to differences in the bilirubin levels between the NPG group and the control group. The similarity on the etiopathogenesis of migraines and NPG may also be related to the effect of the reduction in the serum bilirubin levels in both diseases.

Furthermore, reduced serum bilirubin levels have been reported to be associated with increased arterial stiffness.[21] A decrease in serum bilirubin levels leads to an increase in vascular disorders, which is attributed to the bilirubin causing increased nitric oxide release, leading to vasodilatation and reducing platelet aggregation.[13] We consider that decreased serum bilirubin levels may lead to the deterioration of the optic nerve blood stream and an increase in NPG development through the same mechanisms.

It is known that oxidative stress is occured by the imbalance between the production of reactive oxygen species and the elimination of these products by anti-oxidants. The role of oxidative stress on the etiopathogenesis of NPG has been also demonstrated in some publications.[10,11] It is suggested that increased oxidative stress may result in impaired optic nerve head blood flow and retinal ganglion cell demise without IOP elevation. The fact that bilirubin is a strong anti-oxidant that binds reactive oxygen species means that a reduced serum bilirubin concentration can lead to increased oxidative stress and the death of retinal ganglion cells. On the other hand, reduced serum bilirubin levels may be a indicator of the reduced activity of haeme oxygenase enzyme (a potent anti-oxidant), or it may be a indicator of high oxidative stress that leads to the depletion of natural anti-oxidants, including bilirubin, in NPG patients. High oxidative stress in NPG patients may lead to a reduced serum bilirubin concentration.

In addition, bilirubin has anti-inflammatory features and prevents oxidant-induced microvascular leucocyte adhesion. The anticomplement effect of bilirubin has been shown in animal models. [24,25] Furthermore, total bilirubin levels have been shown to have an inverse relationship with indicators of inflammation, such as red cell distribution width, neutrophil-to-lymphocyte ratio and, C-reactive protein (CRP).[13] Previous studies have demonstrated elevated serum CRP levels and increased inflammation in NPG patients. [26,27] In our study, low serum bilirubin levels may also have led to increased inflammation and NPG development.

Our study is the first clinical trial in the literature investigating the serum bilirubin concentrations in NPG patients. The outcomes of our study indicate that lower bilirubin levels may be associated with NPG. Yet, there are some limitations to this study; the small number of patients limited our comparisons. Although the total bilirubin levels were lower in the NPG group, the fact that a statistically significant difference could not be detected between the two groups may be due to our small sample size. Consequently, our findings show that there may be other ways to fully elucidate the pathogenesis of the disease. This observation may contribute to the development of alternative treatment modalities for NPG. This research findings should be verified in studies with larger numbers of subjects and in prospective studies.

REFERENCES

1. Tham Y-C, Li X, Wong TY, Quigley HA, Aung T, Cheng C-Y. Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. Ophthalmology 2014;121(11):2081-90.
2. Flammer J, Mozaffarief M. What is the present pathogenetic concept of glaucomatous optic neuropathy? Surv Ophthalmol 2007;52(2):162-73.
3. Werner EB. Normal-tension glaucoma. In: Ritch R, Shields MB, Krupin T, editors. The Glaucomas. 2nd . St. Louis: Mosby-Year Book;1996. p.769-97.
4. Mallick J, Devi L, Malik PK, Mallick J. Update on normal tension glaucoma (review). J Ophthalmic Vis Res 2016;11(2):204-8.
5. Cho HK, Kee C. Population-based glaucoma prevalence studies in Asians. Surv Ophthalmol. 2014;59(4):434-47.

6. Iwase A, Suzuki Y, Araie M, Yamamoto T, Abe H, Shirato S, et al. The prevalence of primary open-angle glaucoma in Japanese: the Tajimi study. Ophthalmology 2004;111(9):1641-48.
7. Yamamoto T, Kitazawa Y. Vascular pathogenesis of normal tension glaucoma: a possible pathogenic factor, other than intraocular pressure, of glaucomatous optic neuropathy (review). Prog Retin Eye Res. 1998;17(1):127-43.
8. Cursiefen C, Wisse M, Cursiefen S, Jünemann A, Martus P, Korth M. Migraine and tension headache in high-pressure and normal tension glaucoma. Am J Ophthalmol. 2000;129(1):102-4.
9. Henry E, Newby DE, Webb DJ, O'Brien C. Peripheral endothelial dysfunction in normal pressure glaucoma. Invest Ophthalmol Vis Sci 1999;40(8):1710-14.
10. Himoro N, Kunikata H, Shiga Y,Omadaka K, Maruyama K, Takahashi H, et al. The association between systemic oxidative stress and ocular blood flow in patients with normal-tension glaucoma. Graefes Arch Clin Exp Ophthalmol 2016;254(2):333-41.
11. Yuki K, Dogru M, Kimura I, Ohtake Y, Tsubota K. Reduced-serum vitamin C and increased uric acid levels in normal-tension glaucoma. Graefes Arch Clin Exp Ophthalmol 2010;248(2):243-48.
12. Harada T, Harada C, Nakamura K, Quah HM, Okumura A, Namekata K, et al. The potential role of glutamate transporters in the pathogenesis of normal tension glaucoma. J Clin Invest 2007;117(7):1763-70.
13. Gupta N, Singh T, Chaudhary R, Garg SK, Sandhu GS, Mittal V, et al. Bilirubin in coronary artery disease: cytotoxic or protective? (review) World J Gastrointest Pharmaco Ther 2016;7(4):469-76.
14. Kaputulnik J. Bilirubin: an endogenous product of haeme degradation with both cytotoxic and cytoprotective properties. Mol Pharmacol 2004;66(4):773-9.
15. Liao SL. The role of bilirubin and phototherapy in the oxidative/antioxidant balance. Pediatr Neonatol 2015;56(2):77-78.
16. Wu TW, Fung KP, Wu J, Yang CC, Weisel RD. Antioxidation of human low density lipoprotein by unconjugated and conjugated bilirubins. Biochem Pharmacol 1996;51(6):859-62.
17. Cao L, Xue L, Luo D-M. Lower serum bilirubin concentration in patients with migraine. Int J Clin Exp Med 2015;8(8):13398-402.
18. Vitek L, Jirsa M, Bradonova M, Kalab M, Marecek Z, Danzig V, et al. Gilbert syndrome and ischemic heart disease: a protective effect of elevated bilirubin levels. Atherosclerosis 2002;160(2):449-56.
19. Peng F, Deng X, Yu Y, Chen X, Shen L, Zhong X, et al. Serum bilirubin concentrations and multiple sclerosis. J Clin Neurosci 2011;18(10):1355-9.
20. Demir M, Demir C, Keçeoğlu S. Relationship between serum bilirubin concentration and nondipper hypertension. Int J Clin Exp Med 2014;7(5):1454-8.
21. Tanındı A, Erkan AF, Alhan A, Töre HF. Arterial stiffness and central arterial wave reflection are associated with serum uric acid, total bilirubin, and neutrophil-to-lymphocyte ratio in patients with coronary artery disease. Anatol J Cardiol 2015;15(5):396-403.
22. Kim KE, Park K-H. Update on the prevalence, etiology, diagnosis, and monitoring of normal-tension glaucoma (review). Asia Pac J Ophthalmol 2016;5(1):23-31.
23. Peng Y-F, Xie L-Q, Xiang Y, Xu G-D. Serum bilirubin and their association with c-reactive protein in patients with migraine. Journal of Clinical Laboratory Analysis 2016;30(6):982-5.

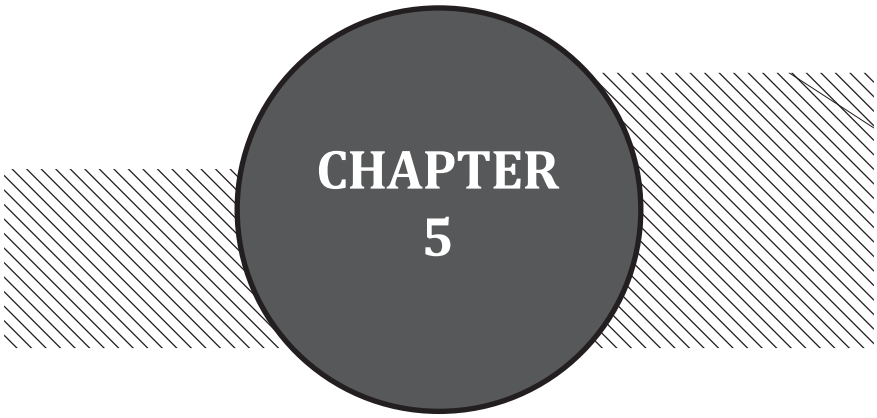
24. Akboga MK, Canpolat U, Sahinarslan A, Alsancak Y, Nurkoc S, Aras D, et al. Association of serum total bilirubin level with severity of coronary atherosclerosis is linked to systemic inflammation. Atherosclerosis 2015;240(1):110-14.
25. Canpolat U, Aytemir K, Yorgun H, Hazırolan T, Kaya EB, Şahiner L, et al. Association of serum total bilirubin levels with the severity, extent and subtypes of coronary atherosclerotic plaques detected by coronary CT angiography. Int J Cardiovasc Imaging 2013;29(6):1371-79.
26. Leibovitch I, Kurtz S, Kesler A, Feitliher N, Shaemesh G, Sela B-A. C-reactive protein levels in normal tension glaucoma. J Glaucoma 2005;14(5):384-6.
27. Lee NY, Park H-Y L, Park CK, Ahn MD. Analysis of systemic endothelin-1, matrix metalloproteinase-9, macrophage chemoattractant protein-1, and high-sensitivity c-reactive protein in normal-tension glaucoma. Current Eye Research 2012;37(12):1121-26.

	NTG group n=38	Control group n=38	p-value
Gender (male/female)	8/30	10/28	1.00
Age (years)	65.21±16.12	66.45±15.02	0.730
Direct bilirubin (mg/dl)	0.19	0.23	< 0.05
Indirect bilirubin (mg/dl)	0.36	0.48	< 0.05
Total bilirubin (mg/dl)	0.60	0.68	0.237

Table 1. Comparison of age, gender and bilirubin levels between patient and control groups.

Pancreas Transplantation, Types And The History

Hüseyin Kerem TOLAN[1]



[1] Dr., Sağlık Bilimleri Üniversitesi Ümraniye Eğitim ve Araştırma Hastanesi Genel Cerrahi AD.

BACKGROUND

Pancreas transplantation is currently the only known curative treatment method for restoring glycemic control thus treating the patients with type 1 diabetes mellitus. American Diabetes Association in 2004 defined clearly the indication of pancreas transplantation in diabetes patients such as; patients with end stage renal disease, the patients without substantial renal disease but having a history of frequent metabolic complications that are incapacitating clinical and emotional problems despite of having an exogenous insulin therapy, and/or consistent failure of insulin based management to prevent acute metabolic complications (1). The World Health Organization (WHO) estimates that 9% of the global population (> 600,000,000 people) is to be diabetic; requiring insulin and approximately 10% of this population having type 1 diabetes (2). Now a day also Type 2 diabetes patients are taken as candidates for pancreas transplantation if; they have longstanding insulin dependence with requirements less than 1 unit/kg/24 h and if their BMI is less than 32 kg/m2 and also without having any cardiovascular disease (3).

The pancreas transplantation is not a lifesaving procedure unlike other solid organ transplantations. But it prevents the life-threatening future complications of the diabetes such as; episodes of deep hypoglycemia and also significantly improves the quality of life of the diabetic patients (4) (5). It is known that the "insulin-induced hypoglycemic unawareness" has an estimated mortality of 3% to 6%, which can be prevented by the pancreas transplantation (6). Between 1991 and 2008, the incidence of type 1 diabetes increased from 7/100 000 to 10/100 000 person years (7). Thus in the close future it will be a more and more important global health problem.

TYPES OF PANCREAS TRANSPLANTATİON

1-*Pancreas transplant alone (PTA)*: Patients with type-I diabetes with normal kidney function glomerular filtration rate of 80-100 mL/min/1.73m2; but with diabetic complications that could be mortal like; hypoglycemic unawareness, ketoacidosis that requires multiple hospital admissions or with diabetic rapidly progressing and severe complications such as diabetic neuropathy, retinopathy, etc. are suitable for this type of transplantation (8) (9). With this technique patient can restore glucose homeostasis (10) but there is a higher risk of graft loss and acute rejection in this type of pancreas transplantation (11) (12).

2. *Simultaneous pancrease-kidney transplant (SPK)*: The most common performed type of pancreas transplant consisting around 90% of the pancreas transplant cases. The indications have been adapted by the UK Transplant Kidney and Pancreas Advisory Group and include type 1 diabetics with end-stage renal failure (13). The transplanted organs generally come from the same deceased donor, but sometimes combined organs could also be used (14).

3- *Pancreas-after-kidney transplant (PAK):* This approach is used for patients also with diabetes associated renal failure. The main aim is to decrease the morbidity and mortality rates associated with the dialysis therapy by first providing a healthy kidney (living or deceased) to the patient which is then followed by a second step of deceased donor pancreas transplantation. It is 8-15% of pancreas transplants. It has a reduced waiting time and a lower mortality rates compared with SPK (15).

4- *Simultaneous deceased donor pancreas and live donor kidney transplant*: It has the benefit of lower rate of delayed graft function, reduced waiting times thus resulting in improved outcomes compared to SPK (16).

5- ***Islet cell transplant:*** This is the transplantation of a purified preparation of isolated pancreatic islets. The candidates for the islet cell transplantation are the patients despite having the optimal insulin therapy that are unable to control their hypoglycemia. In most centers the islets of the endocrine component of the pancreas; which is about 2% of the gland, are infused via the portal vein into the liver. In some studies the islet cell transplantation has been shown to produce similar outcomes to PTA in selected patients (17). The multicenter trials using the Edmonton protocol showed a five year insulin independence rate of 11% in islet transplant cases, compared with 50% for PTA (18). So this promising era still needs additional technical and immunologic improvements to work on to increase the success rates (19).

HISTORY

The first pancreas transplantation attempt in history was an experimental xenotransplantation. Three pieces of pancreas taken from a sheep was transplanted subcutaneously to a Type 1 diabetic child. After the successful transplantation process the child passed away 3 days later due to the severe ketoacidosis (20).

The first clinical transplantation of the pancreas was performed only 3 years after the first kidney transplant performed at the University of Minnesota, USA; in 12/1/1966 by the team of doctors Kelly, Lillehei, Merkel, Idezuki and Goetz. This first case was a 28-year-old woman who had been diabetic since the age of 9. She also had a concomitant end-stage renal disease. In the transplantation operation; a pancreas, along with kidney and donor duodenum was transplanted to the patient. Here the transplanted pancreas was a segmental graft and the pancreatic duct was ligated in the operation. The operation went smooth and the patient was initially free of insulin after the operation. On the post-operative day 6 need for insulin to regulate the blood glucose emerged which was thought to be due to the steroids used in the immunosuppression protocol. Also due to the intra-operative ligation of the pancreatic duct the patient developed a pancreatitis and the graft had to be removed by the team on February 14, 1967. Short after this complication the kidney was also rejected. The patient passed away on post-operative day 13 due to a pulmonary embolism (21). After this failed attempt; in the laboratory canine models of segmental pancreas transplantation were developed in Minnesota group by Dr. Merkel working in the laboratory of Dr. Kelly (22). They tried to give radiation to the canine pancreas in order to decrease the pancreatic exocrine function in pancreatic allografts to increase the outcomes (23).

At the same time in the laboratory of Dr. Lillehei; Dr. Idezuki (24) from Japan and Dr. Largiader (25) from Zurich were working on a canine pancreatic-duodenal transplant model. In this technique the exocrine secretions of the pancreas was drained by a Roux-en-Y duodenojejunostomy. By using this promising new transplantation technique developed in the canine models; Lillehei performed the second pancreas transplantation in January 1967 with his team. The graft functioned for 4.5 months and the rejection was avoided by using the high dose prednisone. Getting good result with this new technique, until 1973 they performed 12 more pancreas transplantations in which they used the complete pancreas-duodenum graft from the donors and also performing a percutaneous duodenostomy to divert the exocrine secretions of the transplanted pancreas (26) (27). Until 1973 Lillehei kept on performing pancreas transplantations with the similar technique which in the future was also used by some teams (20) (21) In Lillehei series; the Pancreas Transplantation Alone (PTA) patients had fewer complications compared to the Simultaneous Pancreas- Kidney (SPK) patients; but the overall complication incidences were high which was later thought to be due to the use of the duodenal portion of the donor while transplanting the pancreas (28). After Najarian becoming the new director and also by the help of the addition of the new team members; Simmons, Kjellstrand and

Sutherland, who worked on Kidney transplantation and islet cell transplantation programs the researches and the efforts incressed (29) (30). In South America after the first pancreas transplantation in 1969; Teixeira (31) and Bortagaray (32) also reported their experiences in pancreas and islet cell transplantations. In 1979 from United States; the Colorado group Merkel and Starzl reported their first kidney and pancreas transplantation (33). After them in United States; Connolly from Irvine Medical Center also performed another case (34). In United Kingdom in London; the first pancreas transplantation was performed in 1972 by the team of Bewick (35). With the improvement of the surgical techniques and other improvements in the immunosuppression the number of cases gradually increased. In the techniques of implantation of the graft the pancreatic duct management was an important step of the transplant surgery. In 1971 Gliedman introduced the technique of performing an anastomosis of the Wirsung duct with the native ureter at the Montefiore Hospital in New York which was an opening of a new era (36). In 1976 Bewick (35) and in 1979 Sutherland (37) performed cases by leaving the duct open draining in the abdomen with having good results at that time. When this technique was used it was seen that in nearly half of the cases there were no problems estimating that the inactive exocrine excretions of the pancreas were absorbed by the peritoneal cavity. But in the other half of the cases the grafts had to be removed due to the chemical peritonitis caused by the pancreatic exocrine excretions (38).

Working on the pancreatic duct management at the same period in 1978 Dubernard from France described the new polymer injection technique in to the pancreatic duct; in order to induce fibrosis in the duct and thus abolishing the exocrine secretions of the pancreas (39). Also other materials were used in order to block the excretions by the other centers. While these pancreatic duct management experiments and trials were going on; Groth from Sweden described an enteric anastomosis technique that can be used in the segmental grafts (40). In the early 1980s; the technique that was first described by Gliedman (anastomosing the pancreatic duct to the recipient ureter) was modified by Sollinger; in which he directly anastomosed the whole pancreatic graft to the bladder of the recipient (41). This technique was again modified and improved by Corry and Ngheim at the University of Iowa, utilizing the bladder diversion of the entire pancreatic-duodenal graft with the bladder (42). Also by using this technique it was seen that the rejection of the implanted graft could be easily diagnosed by the decline in the urinary amylase levels. The decrease in urine amylase levels during the routine monitoring which always was preceded by the hyperglycemia as a manifestation of rejection; can be seen and the rejection can be manged immediately by the close monitoring of the urine amylase levels (43). This technique was being used nearly in all programs. If there were chronic urinary complications (urethritis, hematuria, metabolic acidosis, urinary fistula, etc.) due to the bladder drainage then the anastomosis were converted to the enteric drainage type (44). At the same period some centers started to use the enteric anastomosis technique described initially by Lillehei. For example; Starzl from the University of Pittsburgh used the original described technique (45) and Groth and Tyden from the Stockholm group used the segmental intestinal diversion technique for the pancreatic duct drainage procedure (46).

The improvement in techniques and also in the immunosuppression protocols the success rates of pancreatic transplantation were increasing. In order to prevent and treat rejection; Calne from Cambridge was the first one to use the cyclosporine (47), Sutherland introduced their quadruple immunosuppression with the induction therapy (48), Starzl was the first to use tacrolimus as an immunosuppressant (49), the mycophenolate mofetil was introduced by Sollinger (50) and in 1998 daclizumab was also used by the Sutherland group additional to their quadruple protocol (51).

The enteric anastomosis technique in SPK cadaveric graft cases is generally preferred if the patient is not a high risk patient (elderly, obese, chronic peritonitis due to peritoneal dialysis etc.). The preferred method of pancreatic drainage in solitary cadaveric transplants and the living donor pancreas transplants is the bladder drainage technique in most of the experienced centers with good outcomes (52).

REFERENCES

1. Robertson P, Davis C, Larsen J, et al. Pancreas transplantation in type 1 diabetes. Diabetes Care 2004;27(Suppl 1):S105doi:10.2337/ diacare.27.2007.S105.
2. World Health Organization. Diabetes. Available at: http://www.who.int/mediacentre/factsheets/fs312/en/. Accessed January 11, 2019.
3. Redfield RR, Scalea JR, Odorico JS. Simultaneous pancreas and kidney transplantation: current trends and future directions. Curr Opin Organ Transplant 2015; 20: 94-102.
4. Gruessner RWG. Recipient procedures. In: Gruessner RWG, Sutherland DER, eds. Transplantation of the Pancreas. New York: Springer-Verlag; 2004:150-178.
5. Kandaswamy R, Skeans MA, Gustafson SK, et al. OPTN/SRTR 2013 annual data report: pancreas. Am J Transplant 2015; 15 (Suppl 2):1–20.
6. Nathan DM, Fogel H, Norman D, et al. Long-term metabolic and quality of life results with pancreatic/renal transplantation in insulin dependent diabetes mellitus. Transplantation. 1991;52:85-91.
7. Imkampe AK, Gulliford MC. Trends in type 1 diabetes incidence in the UK in 0- to 14-year-olds and in 15- to 34-year-olds, 1991-2008. Diabet Med 2011;28:811-4.
8. Scalea JR, Butler CC, Munivenkatappa RB, et al. Pancreas transplant alone as an independent risk factor for the development of renal failure: a retrospective study. Transplantation. 2008;86:1789-1794.
9. Odorico JS, Voss B, Munoz DR. Kidney function after solitary pancreas transplantation. Transplant Proc. 2008;40:513-515.
10. Paty BW, Lanz K, Kendall DM, et al. Restored hypoglycemic counterregulation is stable in successful pancreas transplant recipients for up to 19 years after transplantation. Transplantation 2001;72:1103-7.
11. Finger EB, Radosevich DM, Dunn TB, et al. A composite risk model for predicting technical failure in pancreas transplantation. Am J Transplant 2013;13:1840-9.
12. Dong M, Parsaik AK, Kremers W, et al. Acute pancreas allograft rejection is associated with increased risk of graft failure in pancreas transplantation. Am J Transplant 2013;13:1019-25.
13. Rayhill SC, D'Alessandro AM, Odorico JS, et al. Simultaneous pancreas-kidney transplantation and living related donor renal transplantation in patients with diabetes: is there a difference in survival? Ann Surg. 2000;231:417-423.
14. Farney AC, Rogers J, Orlando G, et al. Simultaneous transplantation of the living donor kidney and deceased donor pancreas and other transplant options for diabetic and uremic patients. Curr Opin Organ Transplant 2015;20:103-7.
15. Gruessner AC, Sutherland DE, Dunn DL, et al. Pancreas after kidney transplants in posturemic patients with type I diabetes mellitus. J Am Soc Nephrol 2001;12:2490-9.
16. Farney AC, Cho E, Schweitzer EJ, et al. Simultaneous cadaver pancreas living-donor kidney transplantation: a new approach for the type 1 diabetic uremic patient. Ann Surg. 2000;232:696-703.

17. Moassesfar S, Masharani U, Frassetto LA, et al. A comparative analysis of the safety, efficacy, and cost of islet versus pancreas transplantation in nonuremic patients with type 1 diabetes. Am J Transplant 2016;16:518- 26.
18. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. Diabetes 2005;54:2060-9.
19. Farney AC, Sutherland DE, Opara EC. Evolution of islet transplantation for the last 30 years. Pancreas 2016;45:8-20.
20. Lillehei RC, Simmons RL, Najarian JS, et al. Pancreatico-duodenal allotransplantation: experimental and clinical experience. Ann Surg. 1970;172:405-436.
21. Kelly WD, Lillehei RC, Merkel FK, et al. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery 1967;61:827-37.
22. Merkel FK, Kelly WD, Goetz FC. Heterotopic dog pancreatic allografts. Surg Forum. 1967;18:381–3.
23. Merkel FK, Kelly WD, Goetz FC, Maney J. Irradiated heterotopic segmental canine pancreatic allografts. Surgery. 1968;63:291–7.
24. Idezuki Y, Feemster JA, Dietzman RH, Lillehei RC. Experimental pancreaticoduodenal preservation and transplantation. Surg Gynecol Obstet. 1968;126:1002–14.
25. Largiader F, Lyons GW, Hidalgo F, Lillehei RC. Orthotopic allotransplantation of the pancreas. Am J Surg. 1967;113:70– 6.
26. Lillehei RC, Idezuki Y, Feemster JA, Dietzman RH, Kelly WD, Merkel FK, et al. Transplantation of stomach, intestine, and pancreas: experimental and clinical observations. Surgery. 1967;62:721–41.
27. Lillehei RC, Idezuki Y, Kelly WD, Najarian JS, Merkel FK, Goetz FC. Transplantation of the intestine and pancreas. Transplant Proc. 1969;1: 230–8.
28. Lillehei RC, Ruiz JO, Aquino C, et al. Transplantation of the pancreas. Acta Endocrinol 1976; 83(suppl 205):303–320.
29. Sutherland DE, Matas AJ, Najarian JS. Pancreatic islet cell transplantation. Surg Clin North Am. 1978;58:365–82.
30. UCLA Tissue Typing Laboratory. History of transplantation: Thirty-five recollections. Los Angeles, California: The Regents of the University of California; 1991. ISBN: O-9604606-7-5.
31. Teixeira E, Monteiro G, de Cenzo M, Teixeira A, Bergan JJ. Transplantation of the isolated pancreas: report on the first human case. Bull Soc Int Chir. 1970;29:337–44.
32. Chapo Bortagaray M, Nusimovich B, Lori R, Viaggio J, Zelazco JF, Crouzel G, et al. Pancreas transplantation. Surgical technic and various clinical observations. Prensa Med Argent. 1969;56:767–8.
33. Sutherland DE. Pancreas and islet transplantation. II. Clinical trials. Diabetologia. 1981;20:435–50.
34. Connolly JE, Martin DC, Steinberg T, Gwinup G, Gazzaniga AB, Bartlett RH. Clinical experience with pancreaticoduodenal transplantation. Arch Surg. 1973;106:489–94.
35. Bewick M. Proceedings: clinical pancreatic allotransplantation: a review of world results. Ann R Coll Surg Engl. 1976;58:326.
36. Gliedman ML, Gold M, Whittaker J, Rifkin H, Soberman R, Freed S, et al. Clinical segmental pancreatic transplantation with ureter-pancreatic duct anastomosis for exocrine drainage. Surgery. 1973;74:171–80.

37. Sutherland DE, Goetz FC, Najarian JS. Intraperitoneal transplantation of immediately vascularized segmental pancreatic grafts without duct ligation. A clinical trial. Transplantation. 1979;28:485–91.

38. Sutherland DER, Goetz FC, Rynasiewicz JJ, et al. Segmental pancreas transplantation from living related and cadaver donors: a clinical experience. Surgery 1981; 90:159 –169.

39. Dubernard JM, Traeger J, Neyra P, et al. New method of preparation of segmental pancreatic grafts for transplantation: trials in dogs and in man. Surgery 1978; 84:633– 640.

40. Groth CG, Collste H, Lundgren G. Successful outcome of segmental human pancreatic transplantation with enteric exocrine diversion after modifications in technique. Lancet 1982; 2:522–524.

41. Sollinger HW, Cook K, Kamps D. Clinical and experimental experience with pancreaticocystostomy for exocrine pancreatic drainage in pancreas transplantation. Transplant Proc 1984; 16:749 –751.

42. Nghiem DD, Corry RJ. Technique of simultaneous pancreaticoduodenal transplantation with urinary drainage of pancreatic secretion. Am J Surg. 1987;153:405–6.

43. Prieto M, Sutherland DER, Fernandez-Cruz L, et al. Experimental and clinical experience with urine amylase monitoring for early diagnosis of rejection in pancreas transplantation. Transplantation 1987; 43:71–79.

44. Burke GW, Gruessner RWG, Dunn DL, et al. Conversion of whole pancreaticoduodenal transplants from bladder to enteric drainage for metabolic acidosis or dysuria. Transplant Proc 1990; 22:651– 652.

45. Starlz T, Iwatsuki S, Shaw BW, Greene DA, Van Thiel DH, Nalesnik MA, et al. Pancreaticoduodenal transplantation in humans. Surg Gynec Obstet. 1984;159:265–72.

46. Groth CG, Lundgren G, Ostman J, Gunnarson R. Experience with 9 segmental pancreatic transplant in preuremic diabetic patients at Stockholm. Transp Proc. 1980;12:688–771.

47. Calne RY, White DJ. The use of cyclosporin A in clinical organ grafting. Ann Surg. 1982;196:330–7.

48. Squifflet JP, Sutherland DE, Rynasiewick JJ, Field MJ, Heil JE, Najarian J. Combined inmunosuppressive therapy with ciclosporin A, and azathioprine. Transplantation. 1982;34:315–8.

49. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataranman R, Jain A. FK506 for liver, kidney, and pancreas transplantation. Lancet. 1989;2:1000–4.

50. Rayhll SC, Kirk AD, Odorico JS, Pirsh JD, D'Alesandro AM, Sollinger HW. Simultaneous pancreas-kidney transplantation at the University of Wisconsin. Clin Transpl. 1995;261–9.

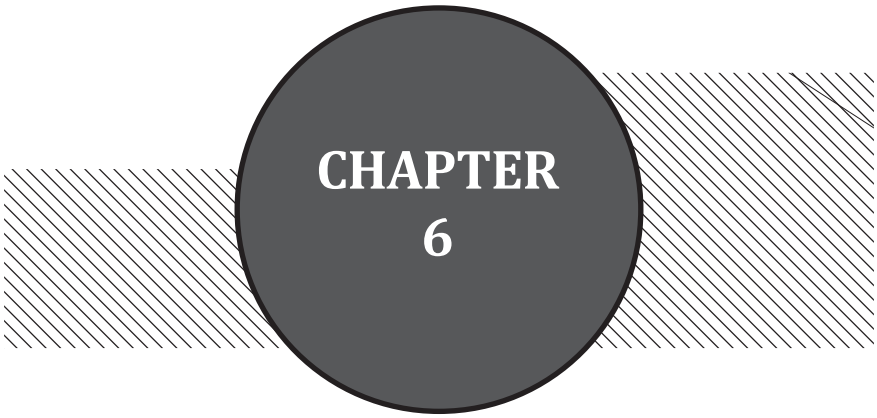
51. Schulz T, Martin D, Heimes M, et al. Tacrolimus/mycophenolate mofetil/steroid-based immunosuppression after pancreas-kidney transplantation with single-shot antithymocyte globulin. Transplant Proc 1998; 30:1533–1535.

52. Sutherland DE, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, Mauer SM, Kennedy WR, Goetz FC, Robertson RP, Gruessner AC, Najarian JS. Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Surg. 2001 Apr;233(4):463-501.

An Overview On Genetically Modified Organisms

Gamze YURTDAŞ[1]

Saniye BİLİCİ[2]



[1] MSc, Nutrition and Dietetic Department, Gazi University, Ankara, Turkey

[2] Phd, Nutrition and Dietetic Department, Gazi University, Ankara, Turkey

INTRODUCTION

As the population of the world is increasing rapidly the arable lands are becoming smaller and clean water resources are decreasing. Human being have been trying to search new solutions to increase the productivity in agriculture to meet the food and nutritional requirements against to fight with hunger (1, 2). Researchers have focused on the developments in biotechnology, making the gene transfer among organisms possible. The term genetically modified food refers to products developed through biotechnology (3). These are food items that have had their DNA changed through genetic engineering. The presence of genetically modified food products within our food system has been a topic of discussion around the world because of biotechnology offers a variety of potential benefits and risks. The controversy is whether or not the benefits of genetically modified foods have potential environmental and health impacts (1, 3). Thus, the aim of this chapter is to review the impacts of genetically modified organisms and their safety assessment in the view of current literature.

The Term of GMO: What is it meaning and what is GMO used for?

Genetically modified organisms (GMOs) is defined by World Health Organization (WHO) as "organisms (i.e. plants, animals or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination". The term genetically modified (GM) foods or GMOs is most commonly used to refer to crop plants created for human or animal consumption using the latest molecular biology techniques. This technology, commonly referred to as "modern biotechnology", "gene technology", or "recombinant DNA technology" or "genetic engineering", let not only the transfer of the selected individual genes to another but also the transfer among unrelated species (4).

The applications of modern biotechnology are used in such fields as agriculture, health, husbandry and industry. The main purposes of using GMOs in agriculture include: providing resistance in agricultural crops against viruses, fungus, bacteria, parasites, herbicides and insects, increasing the tolerance level against such negative factors as heat, drought, humidity and saltiness, chancing the taste-aroma-smell of agricultural crops, shortening the process of growing fruits, improving nutritional value (1, 5). In the health field, GMOs are used to diagnose and cure the illness, production of the blood coagulation factors, organ transplantation, gene therapy, production of vaccine and medication (3). GM microorganisms are used in food industry to produce bread, cheese, beer etc. and also as food additive (stabilizator, thickener, emulgator, sweetener, preservative, colouring agent and flavouring etc.) (6). In husbandry, GMOs are used to feed poultry and fish (3, 7).

A Short Brief Look at the Long History of GMOs

The DNA modification technology was first revealed in 1944 when it was showed that the genetic material could be transferred among different species (8, 9). The double helix structure of DNA was defined by Watson and Crick in 1954 and DNA recombinant technology was developed in 1973 (9, 10). The first genetically modified plants (antibiotic resistant tobacco and petunia) were grown by three independent groups in 1983 (9, 11). In the beginning of 1990s, China became the first country that commercialized a transgenetic crop by defining the virus resistant tobacco. Being the first genetically modified food grown for commercial purposes, the tomato named "FlavrSavr" was aimed to have a long shelf life by maturing without softening. In 1994, sale of the "FlavrSavr" was approved by Food and Drug Administration (FDA) in the United States of America (USA). Later on, transgenetic crops such as the modified oil "canola", herbicide resistant cotton and soybean were also approved by FDA (9). The glyphosate-resistant crops was a variety

of soybean, engineered by Monsanto in 1996. Now glyphosate-resistant technology has been applied to many other crops, including corn and sugar beets. Scientists have also genetically engineered crops to increase nutrition value. For instance, Golden Rice was developed in 2000 with the goal to combat vitamin A deficiency, which is estimated to kill over 500,000 people every year (12).

What GM crops are currently being grown and where?

The biotechnology crops planted worldwide in the late 20 years have increased substantially. According to International Service for the Acquisition of Agricultural Biotechnology Applications (ISAAA) latest report, the cultivation area of genetically modified crops was 189.8 million hectares in 2017 while it was 1.7 million hectares in 1996. Genetically modified crops are cultivated in a total of 24 countries (13). The planting areas and biotech crops of these countries are shown in Table 1.

Nowadays, modern biotechnology is mostly used in economically valuable agricultural crops such as soybean, cotton, maize and canola (1). According to ISAAA report, it was determined that 77% of the soybean, 80% of cotton, 32% of corn and 30% of canola cultivated in the world were GMO (13). Soybean which is the most important animal food of the European Union (EU) has been modified to increase the production. Some agricultural crops such as maize, rape seed (canola), cotton etc. have been genetically modified to add some agronomic features to them such as insect resistance and/or herbicide tolerance (14). Food made from GM plants (maize, canola, soybean and cotton) are generally used as additives in nutrition and as for these crops, they are consumed widely especially in America. While cornstarch containing GMO is used in baby biscuits, instant soup and dressings, wafers and mayonnaise; sweeteners such as glucose syrup and fructose syrup made from corn containing GMO are used in wafers, biscuits, coke, cakes and fruit juice; as for cotton seed oil and canola oil, they can be used also in mayonnaise, instant salad dressings, grain, bread and snacks (1, 14, 15).

Rank	Country	Area (million hectare)	Biotech crops
1	USA	75.0	Maize, soybean, cotton, canola, sugar beet, alfalfa, papaya, squash, apple, potato
2	Brazil	50.2	Maize, soybean, cotton
3	Argentina	23.6	Maize, soybean, cotton
4	Canada	13.1	Canola, maize, soybean, sugar beet, alfalfa, potato
5	India	11.4	Cotton
6	Paraguay	3.0	Soybean, maize, cotton
7	Pakistan	3.0	Cotton
8	China	2.8	Cotton, papaya, poplar, tomato, sweet pepper
9	South Africa	2.7	Maize, soybean, cotton
10	Bolivia	1.3	Soybean
11	Uruguay	1.1	Soybean, maize
12	Australia	0.9	Cotton, canola
13	Philippines	0.6	Maize
14	Myanmar	0.3	Cotton
15	Sudan	0.2	Cotton
16	Spain	0.1	Maize
17	Mexico	0.1	Cotton, soybean
18	Colombia	0.1	Cotton, maize
19	Vietnam	<0.1	Cotton
20	Honduras	<0.1	Maize
21	Chile	<0.1	Maize, soybean, canola
22	Portugal	<0.1	Maize
23	Bangladesh	<0.1	Maize
24	Costa Rica	<0.1	Cotton, soybean, pineapple
25	Slovakia	-	Maize
26	Czech Republic	-	Maize
	Total	189.8	

Table 1. Global Area of Biotech Crops in 2017 (13)

The İmplications of Using GMOs: Advantages and Disadvantages

Seeing such an increasing use of GMOs, it has been focused on what the advantages and disadvantages of using them and whether the benefits outweigh the risks. The implications of genetic engineering are both complex and contradictory, since genetic engineering uses new technologies and brings up very different opinions and conflicting viewpoints. It has been argued that public health could benefit from using GMOs and that there are some issues, which could be solved with current biotechnological instruments.

Enrichment of the content of food: Enriching food with vitamins A, C, E, unsaturated fatty acids and probiotics, nutritional value can be increased with some genetic modifications. For example, Golden Rice enriched with beta-caroten to prevent blindness due to vitamin A deficiency which is common problem in third world countries. In addition, some studies are carried out to increase the starch content of potatoes, quality of amino acid in grains and the quality of bread of wheat (7, 9).

In addition to enrichment of the nutritional value, GMOs are also produced to increase their benefits related to human health. It is known that antioxidants play an important role in preventing numerous illness. In accordance with this purpose, antioxidant (lycopene) in tomato and pepper and vitamin C in strawberry can be increased (16-18). To control cholesterol levels, it is possible to increase unsaturated fat rate in genetically modified soybean and rapeseed oil (6, 7, 16).

Production of edible vaccine: Modern biotechnology methods allow the virus or bacteria antigens to be synthesized in the edible part of plant cells. Therefore, transgenic food can assume the role of oral vaccine that can produce antibody through mucosal immunity that can stimulate the immune system. Edible vaccines against such microorganisms as *Escherichia coli, Helicobacter pylori* is trying to be produced by using some plants like rice, maize, soybeans and potato (9). These vaccines are stored and implemented more easily than traditional vaccines (19).

Agricultural benefits: Increasing of tolerance of agricultural crops against some negative environmental conditions (cold weather, drought and saltiness), the addition of desired taste, colour and structure to the fruit and vegetables and increase in shelf life can be the benefits of genetically modified crops (6). The increase in resistance of agricultural crops against insects and herbicides is one of the most important applications of biotechnology in agricultural area (20). Farmers use a high amount of pesticide to prevent the loss in the crops resulted in insects and this ends up with economical loss for farmers. Maize which is genetically modified using *Basillus thuringienses* can help decreasing the usage of chemical pesticides and the cost to get into the market (19). The resistance to insects is achieved by transferring the gene (Bt gene) produced by *Bacillus thuringiensis* bacteria to tomato, tobacco, cotton and corn plants. The herbicide resistance is also achieved by transferring a bacterial gene to plants to provide resistance to certain herbicides. Furthermore, plants are modified to increase resistance against diseases. For instance, when a viral disease had threatened the industry of papaya seriously, they were rendered resistant against diseases through genetic modification (20, 21). By transferring genes resistant to diseases and pests against agricultural crops, both productivity is increased and the use of pesticide is reduced. In a meta-analysis of 147 studies conducted between 1995 and 2014, it was stated that adoption of genetic modification technology reduced the use of chemical pesticides by an average of 37%, increased product efficiency by 22% and increased farmer profits by 68% (22). It is claimed that poverty and famine in third world countries like Africa can be prevented by increasing the crop yield (17, 18).

The usage in treatment of diseases and in organ transplantation: Transgenetic animals can be used to express recombinant proteins like fibrinogen. Cloned animals are suitable to be utilized for incurable human diseases thanks to their potential modelling. Moreover, these animals can be used to produce pharmacologically useful proteins such as the factor of coagulation in haemophilia patients and insulin in diabetics. Heart, liver,

kidney and fetal cells suitable to transplant into human beings can also be created by using cloned livestock (goat, pig and sheep) (23).

Bearing in mind all the possible benefits of GMO applications, the risks of application of biotechnology in agriculture and medicine should also be discussed. There are some potential well known impacts of GMO applications detailed as follows.

Environmental effects: The most important risk related to genetically modified plants is the genes escape due to ease of gene exchange among plants**.** Genetically modified plants by competing with natural species might either cause vanish them or the migration of new genetic features transferred to natural or wild species during outcrossing. It is reported that this might led to loss in genetic variability. Another possibility is the development of super wild species through the migration of genes, which had been transferred to plants to provide resistance against herbicides or pesticide. Besides, another environmental impact is also damaging the organisms that are not the targets. For example, in a result of the transfer of BT maize pollen through wind to silk vine consumed by monarch butterfly caterpillars, high mortality rate of them was detected (19, 24).

The effects on animal health: It was reported that genetically modified food caused some health problems such as gastrointestinal system diseases, infertility, immunity problems, aging, diseases related with insulin disorders in animal studies (19, 24). Some studies emphasizes the adverse effects of GM foods on animal health are shown in Table 2.

Author, year, referance	GM crop	Model	Duration	Results
Fares and El Sayed,1998 (25)	Potato	Mice	2 weeks	Hyperplastic cells were detected in the small intestine.
Ewen and Pusztai,1999 (26)	Potato	Mice	10 days	Gastric mucosa proliferation in different parts of the digestive tract were determined.
Malatesta et al., 2005 (27)	Soybean	Rat	3 months	Decrease in the form of liver cell nucleus, increase in metabolic rate of cells and increase in the number and size of the packets containing enzymes in the pancreatic cells were observed.
Ermakova., 2006 (28)	Soybean	Rat	2 weeks	Decreased sperm count, significant changes in embryo development and increased infant mortality.
Seralini et al., 2007 (29)	Maize	Rat	90 days	Rats had decreased phosphorus and sodium excretion, increased triglyceride levels and hepatorenal toxicity.
Cyran et al., 2008 (30)	Maize	Mice	3 weeks	After the 4^{th} generation, the immune system, reproductive genes and sperm counts of mice were found to be decreased.
Sissener et al., 2009 (31)	Soybean	Salmon	7 months	It was observed that triacylglycerol in which the middle intestine was shrunk in the group fed with genetically modified soybean.

Table 2. Effects of GM foods on animal health

It was found that after the consumption of MON863 maize, growth rate difference between male and female rats resulting in 3.3 % decrease in weight for male rats and 3.7 % increase in for female rats (29). The negative effects of GM food on gastrointestinal system were reported in some experiments carried out on animals (23, 24). Stomach erosion and necrosis were detected in rats those consumed the GM tomatoes so-called "FlavrSarv" (32). It was reported that Roundup Ready® R GM soya caused inflammation along the distal bowels of salmons (33).

It is also claimed that GM crops have impacts on the immune system and reproductive health (34). A particular increase of production related to Cry9C gene-specific IgG and IgG1, which has immunological characteristics was found in rats and mice consuming GM maize (35). An increase in the level of white blood cells was discovered in the male rats consuming GM maize (29). It was reported that the birth rate of the piglets consuming GM maize decreased by 80 % (34). In a study related to the effects of GM soya beans on testicles of mice, differences in mice sertoli cells were detected (36). DNA taken by the pregnant mice through foods was found in some organs of fetus and new-born animals. This shows us that there is a possible transfer from transplacental way and makes us think that the stranger DNA taken into the body is a potential mutagen for the fetus (37).

In addition, a decrease in blood glucose and increase in cholesterol, triglyceride and HDL concentration in the rats consuming GM rice sub-chronically were detected (38).

The Effects on Human Health: The effects of GMOs on human health can be classified under four subtitles: allergenic potential, toxicity, antibiotic resistance and cancer.

Allergenic potential: The possibility of creating new allergens has been identified as a risk that does not relate directly to the use of GM technology, but depends on the particular gene that has been added to a GM crop. It can reveal in these crops in various ways (39). The DNA and its metabolites transferred to GM food may cause allergic reaction since they are usually found in the protein structure. Furthermore, an allergenic substance can be transferred to another food through genetic modification and protect the allergenic feature (39, 40). Nordlle et al. (41) transferred genes from methionine-rich Brazil nuts to metionin-poor soybeans. They reported that the transgenic soybeans generated as a result of gene transfer caused allergy in individuals who are allergic to these kinds of nuts (41). In another study, it has been stated that the genetically modified maize to produce Bt endotoxin causes immune responses including allergic hypersensitivity (42). The use of GM soybeans has been reported to cause increasing by 50% in soybean-induced allergies in England (43). On the other hand it is mentioned that GM soybeans were uncommon in England and therefore there was a little amount of exposure. Thus, this increase of soya allergencity was explained through high acceptance and being very common of soybean products and processed food in English market (44). In a recent study which 83 studies reviewed, ıt has been stated that the allergens of GMOs were not higher than traditional foods (45). WHO doesn't recommend the transfer of genes from common allergic organisms to non-allergic ones unless the protein crop of the gene transferred is proved to be non-allergic. The food developed using traditional growing methods is usually tested for allergencity and the testing standards of GM foods are evaluated by Food and Agriculture Organization of United Nations (FAO) and WHO. WHO reported that there wasn't any allergic effect in GM foods found in the market (4).

Toxicity: It was reported that insect and weed killer genes and terminator genes in GM plants show their effects by producing toxins and gathering of these toxins in tissues might have been risky (1). It was indicated that a new disease called Eosinophilia Myalgia Syndrome (EMS) had revealed as a result of the consumption of GM L-tryptophan produced by a company (46). FDA reported that GM foods those they had evaluated up to now didn't cause allergic or toxic reaction (15).

Resistance against antibiotics: While producing GMOs, antibiotic resistant genes are used as markers to distinguish genetically modified cells from the original ones. It is expected that these genes will become resistant against antibiotics inserting them into bacteria inside the human and animal body (1). WHO recommends the usage of the gene transfer technology that does not contain any antibiotic resistant gene (4).

Cancer: It was claimed that GMOs might have had direct or indirect carcinogenic effect. It was particularly reported that chemical substances such as bromoxynil and glufosinate, resistant to herbicides used in cotton, maize, soya and colza may have carcinogenic effect (20). Hormones and hormone like substances may affect human health negatively. It is known that the bovine growth hormone (rBGH) injected to increase milk productivity of cows causes the increase of the insulin like growth factor (IGF-I) in milk. It is concerned that the increase of IGF-I level in blood may be risky in terms of lymphosarcomata, ovarian cancer, lung cancer, breast cancer, pancreatic cancer etc. since it causes tumour cell growth as well healthy cells (7, 47).

GMOs and Food Safety

Food safety is defined as following the necessary rules and taking precautions during the production, processing, preservation and distribution of foods in order to ensure healthy and ideal food production (48). In terms of food safety, the question of the possibility of recombinant DNA presence into human intestine flora or genome through horizontal gene transfer is serious. Considering that the source of foods are live organisms and one of the contents of these organisms is DNA, it is seen clearly that DNA is taken into the body together with food. DNA taken into the body can be both removed after digested via the enzymes in digestive system and in some cases it can remain undigested. In case DNA reaches the colon where the activity of enzyme is low but the bacteria concentration is high without degradation, there is a possibility that DNA can be taken into cell by micro flora. It is known that bacterial genes can be taken into the structure by the bacteria along the colon (49). In this sense, the bacterial antibiotic resistant genes used as reagent during GMO production may cause serious risks (6). On the other hand, Jonas et al. (50) reported that DNA derived from GMOs was equivalent to DNA in unmodified food. It is also stated that the potential risks associated with DNA consumption are independent from DNA. Therefore, DNA from GMOs is thought to be as safe as DNA in food (50). To better understand the effects of GMO-DNA on food safety, it is important to evaluate the gene transfer processes in nature and the mechanisms behind them (49).

National and International Regulations of GMOs

"Biosafety" concept and the need of legal regulation on the planting, importation, labelling and sale of these crops have emerged as a result of possible the negative effects of GMOs on health, environment, and biological diversity (51). Biosafety has been defined as realizing the activities related to GMOs and its products safe to protect human, animal and plant health together with the environment and to maintain the biological diversity (52). The legal regulations related to GMO and biosafety take form worldwide based on 2 different principles called "The Principle of Substantial Equivalence" and "The Precautionary Principle". "The Principle of Substantial Equivalence" states that the product acquired as a result of analysing a GM crop is substantially equivalent to the traditional one. The USA usually makes regulations based upon this principle. In the USA, the regulations of biotechnological crops and the studies evaluate the probable risks of GMOs on human health and ecosystem are made by United States Department of Agriculture (USDA), United States Food and Drug Administration (FDA) and United States Environmental Protection Agency (EPA). Legal Regulations in EU are made based on "The Precautionary Principle". According to this principle, GM crops are evaluated diversely from the traditional ones and different arrangements are made to them (51).

There is no institution in the world that works directly in the field of biosafety at an international or regional level. The EU makes investigations relative to this matter together with Food and Agricultural Organization (FAO), Organization for Economic, Cooperation and Development (OECD) and World Trade Organization (WTO) and European Union (EU) work groups and committees. These institutions have published some binding regulations as well nonbinding ones that have the characteristics of a guide. Convention on Biological Diversity (1992) and Cartagena Protocol on Biosafety (2003) are biosafety regulations that have a binding characteristic. Cartagena Protocol on Biosafety is important due to being the first legal binding document at an international level about cross-border movements of GMOs (53, 54). This protocol contains protecting of biodiversity by taking into consideration the risks related to human health and the transfer process, cross-border commercial activities of all GMOs that may affect sustainable usage (55).

Similar to EU, Turkey makes legal regulations based upon "The Precautionary Principle". The first biological arrangement in Turkey made by The Ministry of Agriculture and Rural Areas in 1998 is "The Direction of the Testing Area of Transgenetic Cultivated Plants" (20, 51). The first international initiative is "The Cartagena Protocol on Biosafety" accepted by Grand National Assembly of Turkey in 2004 (55). The importation, processing, exporting, controlling and supervising of GM foods were legalized through "The Biosafety Law number 5977" on 18 March 2010. In accordance with this law, launching of GMO and its crops on the market without approval, the usage of GMO and its crops in defiance of the decision of the committee, growing GM plants and breeding GM animals, the usage of GMOs and its crops out of purpose determined by the committee and the addition of GMO and its crops in infant foods and formulae, follow on formulae and in nutritional supplements of infants and toddlers were banned (52). In Turkey, with the decision taken by Biosafety Committee, only 10 types of soya beans and 26 types of maize can be imported to use as bait (56).

Labelling Regulations for GMOs

Many countries apply certain rules for labelling of GMOs. In each country these rules differ in many factors such as contents, limits, exceptions, implementations and obligations. In the countries which apply labelling rules, obligation to the labelling of the products, obtained from a GM plant, which are not equivalent to the traditional products (i.e. rice with increased nutritional values and canola with higher oleic acid) is a common rule. Nevertheless, there are differences in the labelling of the products which are equivalent to the traditional products and these differences rise between the countries with obligatory labelling rules and the ones with volunteer labelling rules. While there aren't any rules to differentiate the GM products from the similar ones in the USA markets, in other countries such as the EU countries, Japan, China, Australia and New Zealand it is obligatory to label the GMO products. The countries with voluntary labelling rules, leave the preference of labelling the product by its GMO coverage and printing the relevant statement on the product, to the producer. The countries that apply obligatory labelling rules, require the statement of the GMO coverage of the product on the label (51).

In the countries applying labelling rules, limit values change from 0,9 % to 5%. In Turkey, it is obligatory to label the approved GMO products with a 0,9 % or higher threshold value, while lesser values are accepted as infected (51). The labelling rules and limit values of some countries are shown in Table 3.

Countries	Food safety approval regulations	Labeling regulations	Specificity
European Union	Process-based mandatory	Stringent mandatory, includes derived products	Traceability requirements, 0.9% threshold
Brazil, China, Russia	Process-based mandatory	Stringent mandatory, includes derived products	No traceability, low threshold
Australia, Japan, Korea, Saudi Arabia, Thailand, Taiwan	Process-based mandatory	Mandatory labeling based on product content	With labeling exemptions, 1 to 5% threshold levels
United States, Canada, Argentina, Hong Kong, Philippines, South Africa	Substantial equivalence, mandatory (U.S.: voluntary consultation)	Voluntary for substantial equivalence	5% threshold level for labeling
Chile, Ecuador, Indonesia, Vietnam	Mandatory (in place or pending)	Mandatory, introduced but not implemented	Product-based labeling
İndia, Kenya	Mandatory (in place or pending)	Intention to require labeling	Slow regulatory process
Bangladesh, most African countries	Considering mandatory	No clear position	Wait-and-see approach
A few African countries	No	No	GM free

Table 3. Characteristics of Trade-Related Regulations in Some Countries (57)

Safety Evaluation of GMOs

The purpose of the safety evaluation of the genetically modified food is to evaluate its safety in comparison with the traditional food, in regards to the modifications done to their nutritional values. This evaluation is a multi-step, comprehensive process and it is based on the equivalency to the traditional food (19). In this case, the GM food should not be any different from the traditional one in nutritional elements, allergens and toxin levels or should not contain new allergens or toxins. In general, the safety evaluation contains the genetically modified food effects on health (toxicity), it's potential of causing allergic reactions (allergenicity); specific components those thought to have nutritional or toxic characteristics; the stability of the added gene; the nutritional effects related to the genetic modification and the unwanted effects those can be the result of the implementation of a gene (4).

In case of detection of a new or a different hazard during the safety evaluation, the risk that corresponds to the hazard should be defined and its effect to human health should be specified. The main components of the risk assessment for food safety are shown in Table 4. WHO suggests the usage of the Codex Alimentarius for the safety evaluations of GMOs (4). According to the Codex Alimentarius guide, the safety evaluation of food produced

from a DNA recombinant plant is realized with the steps below (58).

A. description of the recombinant-DNA plant;
B. description of the host plant and its use as food;
C. description of the donor organism(s);
D. description of the genetic modification(s);
E. characterization of the genetic modification(s);
F. safety assessment:
   - expressed substances (non-nucleic acid substances),
   - compositional analyses of key components,
   - evaluation of metabolites,
   - food processing,
   - nutritional modification; and
G. other considerations.

Assessment of possible toxicity	✓ Potential toxicity of introduced novel proteins ✓ Amino acid sequence homology ✓ Acute toxicity tests
Assessment of possible allergenicity	✓ Source of the protein ✓ Amino acid sequence homology ✓ Pepsin resistance ✓ Specific serum screening
Potential changes in nutritional value	✓ Composition analysis of macro and micro nutrients, toxins and allergens

Table 4. The main components of the risk assessment for food safety (19, 59)

World Health Organization indicates that the safety of the GM aliments should be evaluated on a case basis and that it is not possible to make general statements on the safety of all genetically modified foods. Remarking also that in the international market there are a lot of GM foods those have passed the safety evaluations and these aliments do not pose a risk to the human health, WHO states the opinion that in the countries in which these foods are approved, the consumption of such food by general population does not risk the human health. WHO reports that the continuous implementation of safety assessments based on Codex Alimentarius principles and the relevant and adequate follow-ups after being put in the market is a key principle in providing the safety of the GM foods (4).

Food and Drug Administration emphasizes that foods derived from GM plants must meet the same requirements as of the food derived from traditionally grown plants including safety requirements. FDA offers a consulting process which prompts the developers of GM food to hold consultation with FDA before they commercialize their goods. This process helps the developers of the GM food in determining the necessary steps for ensuring the legality and safety of the food that they produce from GM food. The purpose of the consulting process is to provide the abolishment of all the question marks about safety and other regulating factors before the commercial allocation. The food, coming from the genetically engineered plants which are intended for cultivation in the USA and evaluated by FDA with the help of consulting process, hasn't been launched on the market till FDA resolves the safety issues of these kinds of products (15).

Organization for Economic Co-Operation and Development has mentioned that further studies are needed to evaluate the long term effects of GM food on human health, food safety and upon environmental impacts. The standards of safety evaluation of GM food must be coherent (58).

American Medical Association has stated GM foods have been consumed more than 20 years and any clear result on human health hasn't been reported. However, because of horizontal gene transfer, these foods have possible negative effects in terms of toxicity and allergenity potential. Safety evaluations before marketing are made for identifying and obviating the health risks of human beings. While consumers support that the GM foods to be labelled, FDA compels that they must be labelled only when they are far beyond their traditional equivalents or only when the production process changes the profile of the food (e.g. if it includes a prevalent allergen) (60).

US Department of Agriculture supports the safe and secure implementation of science and technology to cover up the agricultural difficulties and to meet the consumer needs including biotechnology. USDA has an important role for the evaluation of safe production and usage of biotechnology plants and crops in the USA (60, 61).

European Commission has precuationary approach about the GM food to provide protection of environment and health of human beings at the highest level. GMOs are allowed depending on company descriptions based upon occasions and positive health and environment safety. Safety of all GMOs approved by EU were proved before launching to the market. EFSA is responsible for risk evaluation and the EU Commission is responsible of risk management. In the annual environmental monitoring reports for all permissible GMOs, any negative effect of GMOs to the environment wasn't reported (62).

CONCLUSION AND RECOMMENDATIONS

The production of genetically modified products and usage of them in many fields increase worldwide day by day. Although GMOs have lots of advantages in respect of economical, agricultural and medical issues, the possibility of creating some negative effects over human health, animal health and environment caused suspicion about them. The safety of GM food has still being discussed globally and has caused discord among countries. More studies are required to ensure the advantages and disadvantages of GM foods more clearly and to be sure that these foods do not cause health problems.

It's important to eliminate the possible drawbacks before they are presented to be used. In this context, the related institutions and organizations should do the safety evaluations and should act in accordance with the law and regulations of biosafety.

REFERENCES

1. Şen S, Altınkaynak S. Genetiği değiştirilmiş gıdalar ve potansiyel sağlık riskleri. Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 2014;18(1):31-8.
2. Meseri R. Beslenme ve genetiği değiştirilmiş organizmalar. TAF Preventive Medicine Bulletin. 2008;7(5):455-60.
3. Denli, M. "Genetiği Değiştirilmiş Organizmalar (GDO)", İstanbul Ticaret Odası Yayınları, 2012, Yayın no:2010 – 90, ISBN 978-9944-60-8ı5-2, İstanbul.
4. World Health Organization. Frequently asked questions on genetically modified foods. http://www.who.int/foodsafety/areas_work/food-technology/faq-genetically-modifiedfood/en/ 2014
5. Demir A, Seyis F, Orhan K. Genetik Yapisi Değiştirilmiş Organizmalar: I. Bitkiler. Anadolu Tarım Bilimleri Dergisi.21(2):249-60.

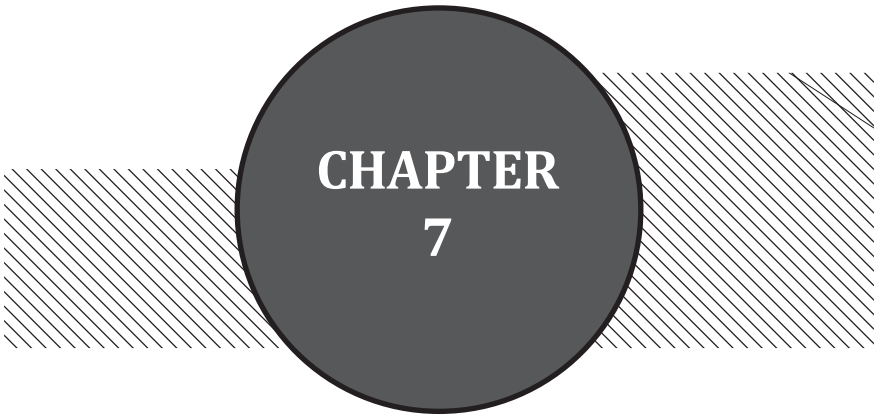
6. Çelik V, Balik DT. Genetiği değiştirilmiş organizmalar (GDO). Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi. 2007;23(1):13-23.
7. Ergin SÖ, Yaman H. Genetiği değiştirilmiş gıdalar ve insan sağlığı üzerine etkileri. Gümüşhane Üniversitesi Sağlık Bilimleri Dergisi. 2013;2(2).
8. Avery OT, MacLeod CM, McCarty M. Studies on the chemical nature of the substance inducing transformation of pneumococcal types: induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. Journal of experimental medicine. 1944;79(2):137-58.
9. Zhang C, Wohlhueter R, Zhang H. Genetically modified foods: A critical review of their promise and problems. Food Science and Human Wellness. 2016;5(3):116-23.
10. Cohen SN, Chang AC, Boyer HW, Helling RB. Construction of biologically functional bacterial plasmids in vitro. Proceedings of the National Academy of Sciences. 1973;70(11):3240-4.
11. Fraley RT. Liposome-mediated delivery of tobacco mosaic virus RNA into petunia protoplast. Plant molecular biology. 1983;2(1):5-14.
12. FDA. (Genetically Engineered Animals: Consumer Q&A." U.S. Food and Drug Administration, June 2015. http://www.fda.gov/animalveterinary/developmentapprovalprocess/geneticengineering/geneticallyengineeredanimals/ucm113672.htm).
13. The International Service for the Acquisition of Agri-biotech Applications (ISAAA). Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Year. 2017.
14. De Santis B, Stockhofe N, Wal J-M, Weesendorp E, Lallès J-P, van Dijk J, et al. Case studies on genetically modified organisms (GMOs): Potential risk scenarios and associated health indicators. Food and Chemical Toxicology. 2017.
15. FDA.https://www.fda.gov/Food/IngredientsPackagingLabeling/GEPlants/ucm346030.htm.
16. Kramkowska M, Grzelak T, Czyzewska K. Benefits and risks associated with genetically modified food products. Annals of Agricultural and Environmental Medicine. 2013;20(3).
17. DellaPenna D. Nutritional genomics: manipulating plant micronutrients to improve human health. Science. 1999;285(5426):375-9.
18. Falk MC, Chassy BM, Harlander SK, Hoban IV TJ, McGloughlin MN, Akhlaghi AR. Food biotechnology: benefits and concerns. The Journal of nutrition. 2002;132(6):1384-90.
19. Eroğlu E, Ayaz A. Genetiği Değiştirilmiş Organizmalar: Beslenme ve Sağlık Yönü, Güncel Konular, Hatipoğlu yayınları,175-197 2015.
20. Haspolat I. Genetiği değiştirilmiş organizmalar ve biyogüvenlik. Ankara Üniv Vet Fak Derg. 2012;59:75-80.
21. Arya D. Genetically modified foods: benefits and risks. Massachusetts Medical Society Committee on nutrition and physical activity; 2015.
22. Klümper W, Qaim M. A meta-analysis of the impacts of genetically modified crops. PLoS One. 2014;9(11):e111629.
23. Uzogara SG. The impact of genetic modification of human foods in the 21st century: A review. Biotechnology advances. 2000;18(3):179-206.
24. Verma C, Nanda S, K Singh R, B Singh R, Mishra S. A review on impacts of genetically modified food on human health. The Open Nutraceuticals Journal. 2011;4(1).

25. Fares NH, El-Sayed AK. Fine Structural Changes in the Ileum of Mice Fed on -Endotoxin-Treated Potatoes and Transgenic Potatoes. Natural toxins. 1998;6(6):219-34.
26. Ewen SW, Pusztai A. Effect of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. The Lancet. 1999;354(9187):1353-4.
27. Malatesta M, Baldelli B, Battistelli S, Tiberi C, Manuali E, Biggiogera M. Reversibility of hepatocyte nuclear modifications in mice fed on genetically modified soybean. European journal of histochemistry: EJH. 2005;49(3):237.
28. Ermakova I. Genetically modified soy leads to the decrease of weight and high mortality of rat pups of the first generation. Preliminary studies. Ecosinform. 2006;1(2996):4-9.
29. Séralini G-E, Cellier D, de Vendomois JS. New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal toxicity. Archives of environmental contamination and toxicology. 2007;52(4):596-602.
30. Cyran N, Gülly C, Handl S, Hofstätter G, Meyer F, Skalicky M, et al. Biological effects of transgenic maize NK603xMON810 fed in long term reproduction studies in mice. Unpublished report: Institute fur Ernahrung, Austria. 2008.
31. Sissener N, Sanden M, Bakke AM, Krogdahl Å, Hemre G-I. A long term trial with Atlantic salmon (Salmo salar L.) fed genetically modified soy; focusing general health and performance before, during and after the parr–smolt transformation. Aquaculture. 2009;294(1-2):108-17.
32. Pusztai A, Bardocz S, Ewen S. 16 Genetically Modified Foods: Potential Human Health Effects. Food Safety. 2003;347.
33. Bakke-McKellep A, Koppang E, Gunnes G, Sanden M, Hemre GI, Landsverk T, et al. Histological, digestive, metabolic, hormonal and some immune factor responses in Atlantic salmon, Salmo salar L., fed genetically modified soybeans. Journal of Fish Diseases. 2007;30(2):65-79.
34. Dona A, Arvanitoyannis IS. Health risks of genetically modified foods. Critical reviews in food science and nutrition. 2009;49(2):164-75.
35. 手島玲子, 渡邉敬浩, 奥貫晴代, 五十鈴川和人, 穐山浩, 小野寺博志, et al. Effect of subchronic feeding of genetically modified corn (CBH351) on immune system in BN rats and B10A mice. 食品衛生学雑誌. 2002;43(5):273-9.
36. Vecchio L, Cisterna B, Malatesta M, Martin T, Biggiogera M. Ultrastructural analysis of testes from mice fed on genetically modified soybean. European Journal of Histochemistry. 2009;48(4):449-54.
37. Doerfler W, Schubbert R. Uptake of foreign DNA from the environment: the gastrointestinal tract and the placenta as portals of entry. Wiener Klinische Wochenschrift. 1998;110(2):40-4.
38. Poulsen M, Kroghsbo S, Schrøder M, Wilcks A, Jacobsen H, Miller A, et al. A 90-day safety study in Wistar rats fed genetically modified rice expressing snowdrop lectin Galanthus nivalis (GNA). Food and Chemical Toxicology. 2007;45(3):350-63.
39. Lee T, Ho H, Leung T. Genetically modified foods and allergy. Hong Kong Med J. 2017;23(3):291-5.
40. Çetinkaya PG, Soyer ÖU, Şahiner ÜM. Genetiği değiştirilmiş organizmalar ve alerji arasındaki ilişki. 2015.
41. Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK. Identification of a Brazil-nut allergen in transgenic soybeans. New England Journal of Medicine. 1996;334(11):688-92.

42. Conner AJ, Glare TR, Nap JP. The release of genetically modified crops into the environment. The Plant Journal. 2003;33(1):19-46.
43. Smith JM. Point of view: genetically modified foods unsafe? Evidence that links GM Foods to allergic responses mounts. Genetic Engineering News. 2007;27(19).
44. Herman EM. Genetically modified soybeans and food allergies. Journal of Experimental Botany. 2003;54(386):1317-9.
45. Dunn SE, Vicini JL, Glenn KC, Fleischer DM, Greenhawt MJ. The allergenicity of genetically modified foods from genetically engineered crops: a narrative and systematic review. Annals of Allergy, Asthma & Immunology. 2017;119(3):214-22. e3.
46. Mayeno AN, Gleich GJ. Eosinophilia-myalgia syndrome and tryptophan production: a cautionary tale. Trends in Biotechnology. 1994;12(9):346-52.
47. Ozkok GA. Genetically Modified Foods and the Probable Risks on Human Health. Int J Nutr Food Sci. 2015;4(3):356-63.
48. Gıda T, Federasyonu İSD. Çiftlikten Çatala Gıda Güvenliği. TGDF Yayınları, Ankara. 2011:70.
49. Van den Eede G, Aarts H, Buhk H-J, Corthier G, Flint HJ, Hammes W, et al. The relevance of gene transfer to the safety of food and feed derived from genetically modified (GM) plants. Food and Chemical Toxicology. 2004;42(7):1127-56.
50. Jonas D, Elmadfa I, Engel K-H, Heller K, Kozianowski G, König A, et al. Safety considerations of DNA in food. Annals of Nutrition and Metabolism. 2001;45(6):235-54.
51. Erdoğan SM. Dünya'da GDO Mevzuatı, Ticareti ve Uygulamalarının Karşılaştırılması ve Türkiye. AB Uzmanlık Tezi; 2015.
52. Anonim. Biyogüvenlik Kanunu. 26 Mart 2010 tarihli Resmi Gazete, Sayı: 27533. .
53. AFAD. 2014-2023 Genetik Yapıları Değiştirilmiş Organizmaların Biyogüvenliği Yol Haritası Belgesi. Ankara: AFAD.
54. Kıvılcım Z. Cartagena protokolü ve Türkiye biyogüvenlik mevzuatı. Marmara Avrupa Araştirmalari Dergisi. 2012: 20(1):99-121.
55. Korkut D, Soysal A. Genetiği Değiştirilmiş Organizmalar. Ankara: Halk Sağlığı Uzmanları Derneği (HASUDER) Lönnerdal, B(2003) Genetically Modified Plants for Improved Trace Element Nutrition(133), 1430. 2013;1433.
56. Türkiye Biyogüvenlik Bilgi Değişim Mekanizması http://www.tbbdm.gov.tr/Home/RegulationsHome/NationalRegulation s.aspx Erişim Tarihi: 14.07.2013
57. Gruère GP. International trade-related regulations of GM food: What policies for developing countries?: International Food Policy Research Institute, Program for Biosafety Systems; 2007.
58. Codex Alimentarius. Foods derived from modern biotechnology. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Rome. 2009.
59. FAO. The comparative approach for safety assessment of foods derived from recombinant-DNA plants. GM Food Safety Assessment: Tools for Trainers. 2009:19-20.
60. American Medical Association, "Report 2 of The Council on Science and Public Health: Labeling of Bioengineered Foods" (2012), http:// factsaboutgmos.org/sites/default/files/AMA%20Report.pdf, accessed on Dec 1, 2018
61. USDA. "Biotechnology Frequently Asked Questions,"http://www.usda. gov/wps/portal/usda/usdahome?navid=AGRICULTURE&contentid= BiotechnologyFAQs.xml, accessed December 10, 2018.
62. European Commission, "Questions and Answers on the EU's New Approach to the Cultivation of GMOs," MEMO/10/325 13/07/2010, http:// europa.eu/rapid/press-release_MEMO-10-325_en.htm?locale=en, accessed Dec. 1, 2018.

Using Genetic and Epigenetic Information in Response to Occupational Toxicants

Esra EMERCE[1]



[1] Gazi University, Pharmacy Faculty, Department of Toxicology, Ankara, Turkey

Workers might be confronted with health problems due to various risk factors that may be found in the work environment. These risk factors are typically physical, chemical, biological agents as well as psychosocial and ergonomic factors. However, with a multidisciplinary approach, workers can be protected from workplace hazards by identifying occupational hazards and by providing effective control and prevention methods. Considering recent data, the burden of registered occupational diseases is very high. According to the International Labour Organization (ILO) estimations for 2017, 2.78 million workers all over the world die every year from work-related diseases (86.3 %; 2.4 million workers) and occupational accidents (13.7 %; 380000 workers). The first three places in work-related diseases are circulatory diseases (31%), malignant neoplasms (26%) and respiratory diseases (17%). Considering work-related mortality rates all over the world, Asia's death rates (65.0%) are almost six times higher in Africa (11.8%), Europe (11.7%) and America (10.9%) (Hämäläinen, Takala & Kiat, 2017).

Diseases caused by exposure to risk factors arising from working life are defined as occupational diseases. Occupational diseases are generally associated with only one causal agent with a specific or strong relationship to the occupational activity (i.e. noise-induced hear loss, asbestosis). Another health problem that is more common than occupational diseases is work-related diseases. These diseases have a complex etiology and occupational factors together with other risk factors contribute to disease formation (e.g. cardiovascular diseases, various cancers). There are two main elements in the definition of occupational diseases:

a) Requires the establishment of a causal relationship between a specific working environment and/or work activity and a specific disease (exposure-effect relationship);

b) These diseases occur in exposed people with a frequency rate above the mean morbidity of the rest of the population, or in other worker populations (Lesage, 2011).

The list of occupational diseases prepared by the ILO has four main groups: occupational diseases caused by exposure to substances resulting from work activities (such as chemical, physical and biological agents); occupational diseases by target organ systems; occupational cancers; and other diseases. Given these listed diseases and potential work-related diseases, chemicals are one of the most common hazards for workers. In this context, this review is limited to chemical substances.

There are several factors that affect the toxicity of a toxic substance. These are physicochemical property, dose, toxicokinetic properties, duration of exposure to the agent, exposure route, and interaction with other chemicals. In addition, individual factors such as age, gender, health status, genetic factors, personal habits and hygiene might affect the toxicity of the agent. As a result of exposure to occupational toxicants, effects can occur from asymptomatic physiological and biochemical changes to the disease and ultimately to death. Monitoring by biomarkers that can indicate biological changes and susceptibilities in the processes ranging from exposure to a toxic agent in the workplace to the occurrence of disease in individuals provides important information about the causality relationship in occupational diseases (Fig 1). Both biological and environmental monitoring can help assess exposure to chemicals, characterization of exposure pathways and potential risks, and reduction of these risks.

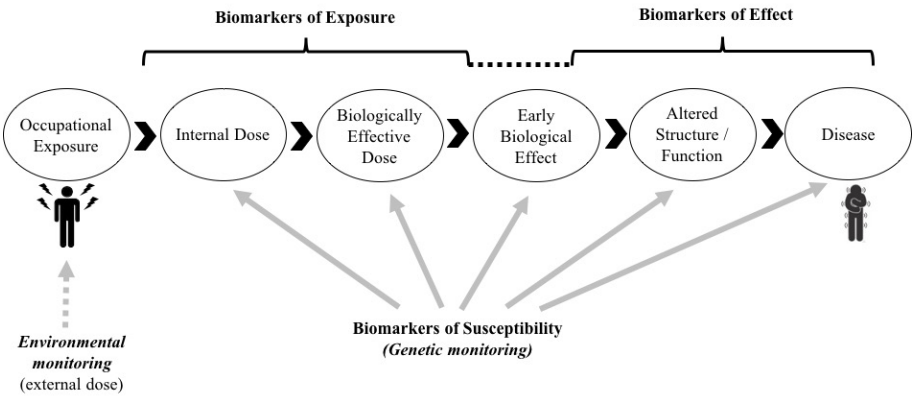


Figure 1. Continuum from exposure to disease (Adapted from: NRC, 1987)

Biomarkers are basically divided into three categories as biomarkers of exposure, effect, and susceptibility (Duffus, Nordberg, & Templeton, 2007; WHO, 2001).

- Biomarker of exposure: Toxicant or its metabolite(s) or the product of an interaction between a toxicant and some target molecule or cell that is measured in a compartment within an organism.
- Biomarker of effect: A measurable biochemical, physiological, behavioral or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease.
- Biomarker of susceptibility: An indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific toxicant.

It is necessary to know the amount of exposure to the relevant chemicals in determining the health risks of workers. This can be achieved by environmental measurements in the workplace and biomarkers of exposure. In this context, environmental measurements, which are generally easier to apply, are used in practice. These measurements are an indirect way to determine the exposure level. The external dose obtained from the environmental measurements is the dose calculated from the concentration of the substance obtained from the immediate environment of the worker and from the daily intake of the individual. Although measurement of the external dose provides a basis for understanding the relationship of the chemical to the disease process, it may be misleading by giving values above or below the actual internal dose even if the personal monitoring is present. Therefore, more accurate and informative results can be achieved with biological monitoring. Biological monitoring provides additional information that can improve occupational risk assessment at the individual and/or group level. These include evaluation of the total uptake of a chemical absorbed by all exposure routes, estimation of current, recent or long-term exposure, a degree of metabolism, early biological effects, and the chemical tolerance of the organism and toxic response (ICOH, 2010). The disadvantage of biological monitoring is the inability to distinguish only occupational originated exposure. Therefore, in the occupational risk assessment, biological monitoring and environmental monitoring are not alternatives to each other but complementary.

The biomarkers of exposure include the internal dose, the biologically effective dose and the determination of early biological effects. The internal dose is the amount of the chemical or metabolite present in a biological specimen. A biologically effective dose is the amount of a chemical or metabolite that interacts with important subcellular, cellular, and target tissues. The early biological effects represent an event which is related to the health

effect of toxic agent and predicts the disease. Because biomarkers are in continuity, it may be difficult to distinguish between exposure and effect biomarkers. Early biological effects can be used as a biomarker for both exposure and effect and may be the bridge between the two processes. The biomarkers of the effect show biochemical or functional changes with a wide range of biological responses, from physiological adaptation to disease. It is used as an early determinant of clinical disease. The biomarkers of susceptibility determine the genetic susceptibility that can cause differences in the response of workers to an agent at every stage from the exposure to the formation of the disease.

Both genetic and epigenetic information is now being used increasingly in these three types of biomarkers, which help to identify the toxicity of occupational toxicants and can be summarized above. Genetic information can reveal whether there is a change in the genetic material of a worker as a result of exposure to a toxic agent, or it may also show hereditary properties at the gene level that increase or decrease the risk of disease. As an area of application, changes in the epigenetic processes induced by occupational exposure have recently gained popularity. The increase in genetic/epigenetic research in the field of occupational health, the scientific developments caused by the human genome sequence, and the exponential increase in the advances in molecular biology techniques are an important factor. Developments in genetic technologies have been useful in understanding occupational diseases and chemical exposure studies, in particular, their mechanisms of effect and their mode of action. Occupational health and safety professionals are increasingly interested in understanding gene-environment interactions that can contribute to preventive and control strategies at both mechanistic and population levels.

Fundamental considerations of genetic and epigenetic factors in chemical risk assessment processes (Schulte, Whittaker & Curran, 2015);

- Does the toxicant change DNA or gene regulation?
- Does response alter when genotype changes?
- Does internal dose change when genotype changes?
- Can a genetic change display early disease?

Monitoring the effects of occupational exposures on genetic material

Genotoxicity refers to the ability of a chemical to damage the genetic material (structure, sequence, and/or the number of genes) by causing cell damage. It can occur mainly by direct or indirect mechanisms. Direct genotoxicity occurs when the genotoxic agent or its metabolite interacts with DNA (such as DNA adducts, DNA strand breaks, chromosomal aberrations) while the indirect mechanism of action interacts with non-DNA targets to increase genetic damage (such as DNA repair enzymes, cell cycle, spindles) (Kirsch-Volders, Vanhauwaert, Eichenlaub-Ritter & Decordier, 2003). In addition, changes in gene regulation may be observed due to various changes in the genetic material when exposed to a genotoxic agent. Occupational toxic agents can have genotoxic effects in both somatic cells and germ cells. Damage to germ cells is important due to the ability to pass to the next generation in addition to being associated with spontaneous abortions and infertility (Sakkas & Alvarez, 2010). Genotoxic effects in somatic cells are associated with many health problems, mainly cancer, cardiovascular diseases, neurodegenerative diseases, and accelerated aging (Coppedè & Migliore, 2015; Hoeijmakers, 2001; ICPEMC, 1990). The fact that mutagenic/genotoxic chemicals are effective even at low doses is important in terms of occupational risk.

A large number of occupationally exposed chemicals can cause various diseases and health problems by affecting the genetic material of workers. To date, many chemicals have been evaluated by the International Agency for Research on Cancer (IARC) and carcinogenicity classifications have been made considering the evidence from human and

experimental animals. IARC classified chemicals were first evaluated by Siemiatycki et al. (2004) and then by Loomis, Guha, Hall & Straif (2018) in terms of occupational exposures and some of the chemicals were listed as occupational carcinogens. Although significant advances have been made in the identification of occupational carcinogens through the studies, a need for research on the etiology of work-related cancer is continuing. Many of the risk factors currently present in the workplace have not been evaluated in terms of carcinogenic potentials due to inadequate epidemiological evidence on toxic effects and lack of exposure data.

In the context of occupational toxicology, the determination of the mutagenic and genotoxic effects of the chemicals in workplace by studying *in vitro* and *in vivo* designs is contributed to the step of hazard identification of the risk assessment. Short-term genotoxicity tests have been used for many years in bacteria, yeast, various cell cultures and biological materials of experimental animals (Bajpayee, Pandey, Parmar & Dhawan, 2005). Studies have shown that genotoxic compounds cannot be determined by a single assay. For this reason, a tiered test approach is used in order to evaluate the genotoxicity of the chemicals (Madle, Kasper, Pabel & Speit, 2008). Chemicals that are mutagenic and / or carcinogenic can be identified with clinical observations, epidemiological studies, and experimental tests. Genotoxicity biomarkers applied in *in vitro* and *in vivo* are now being used at increasing rates in workers exposed to chemicals. In occupational health field, short-term genotoxicity assays offer a promising possibility for monitoring exposure to genotoxic chemicals and their health effects in workers. When biological monitoring for exposure is concentrated on genetic material in biologic specimens, it is known as genetic monitoring. Somatic mutations, DNA and protein adducts and cytogenetic changes are frequently used as exposure biomarkers and in some cases as biomarkers of effect.

Protein and DNA adducts are often used as a biomarker of exposure. Since these biomarkers show interaction with the target molecule, they determine the biologically effective dose in addition to being biomarker of effect. For genetic biomarkers used for marker of effect, chromosomal aberrations, sister chromatid exchanges, micronucleus, DNA strand breaks, oncogen mutations, and gene expression can be given as examples. As with all biomarkers, genotoxic biomarkers are also considered as significant data in risk assessments in the case of validation of the link between biomarker and disease (IPCS, 2001). Among the genotoxicity tests, chromosomal aberration test and cytokinesis-block micronucleus assay were validated in predicting cancer risk with international cooperation (Fenech, 2002; Hagmar et al., 1998). In the last decade, international efforts have continued to validate the use of comet assay which determines DNA damage and the buccal micronucleus assay, which are commonly used in biomonitoring for chemicals with genotoxic potential (Bolognesi et al., 2017; Bonassi et al., 2009; Collins et al., 2014). With improving these techniques, these methods are used as highly effective biomarkers in determining both exposed and unexposed groups and in monitoring the effect.

Thanks to technological advances in molecular biology and genetics, omics techniques such as genomics, transcriptomics, and adductomics have now revealed methods to assist in the evaluation of various biological responses that may arise as a result of exposure to toxic agents. These methods can be promising tools for the development of biomarkers of exposure and early effect biomarkers by focusing on interaction with occupational factors. Toxicogenomics allows the determination of differences in expression levels of gene or gene products in a variety of genes due to exposure to a toxic agent. High-throughput technologies such as microarrays, which can show the expression level of thousands of genes after exposure to occupational toxicants, provide important information in determining the etiology of occupational diseases (Judson et al., 2011).

There are many studies evaluating changes in genetic material in workers exposed

to occupational toxicants. In these studies where the genotoxic effect of the chemical is determined the quantitative grouping by using exposure biomarkers instead of grouping exposed and unexposed individuals based on the person's declaration ensures more reliable results (Anlar et al., 2018; Bonassi, Milić & Neri, 2016; Duydu et al., 2012; Karahalil, Burgaz, Fişek & Karakaya, 1998; Li et al., 2014; Liu et al., 2017; Sardas, Omurtag, Tozan, Gül & Beyoglu, 2010).

In occupational epidemiological studies, cytogenetic changes that are generally associated with exposure to genotoxic chemicals are determined using cross-sectional designs. However, although there are many challenges in its application, longitudinal epidemiological study designs in which specific individuals can be monitored for a long time with continuous or repeated measures should be considered as the most appropriate study method in determining which genetic biomarkers are significant in the formation of toxicant-induced disease.

Effects of genetic susceptibility on occupational exposure

The important information about genetics had begun to be obtained in the early 1950s with the discovery of DNA and after the Human Genome Project was completed in 2003, it has gained the momentum. In recent years, there has been an increase in the knowledge that genetic characteristics of individuals which play a role in the response of workers exposed to occupational risk factors, mainly chemicals. In a workplace, some individuals may exhibit adverse health effects, even at significantly lower levels or shorter exposure durations to a chemical than other workers. These individuals constitute risky groups defined as hyper-susceptible. The role of hereditary genetic factors on individual differences in toxicity to occupational hazards should be understood.

The genes which have polymorphisms can be used as biomarkers of susceptibility in an exposure-disease relationship. The genes which cause individual susceptibility can be divided into three groups. In the first group, there are genes acting in mechanistic cellular pathways that are not affected by exposure to any environmental factor (i.e. tumor suppressor genes such as p53, BRCA1, oncogenes such as myc). Polymorphisms in these genes increase susceptibility to disease in individuals. The second group are polymorphisms in genes affecting the toxicokinetic behavior of a toxic agent. Polymorphisms in genes encoding phase I and phase II enzymes that play a role in the metabolism of a xenobiotic can lead to changes in the detoxification and bioactivation processes of the toxic agent by affecting the activity or inducibility of their enzymes which are expressed by the genes. Another category is the genes encoding DNA repair enzymes. Changes in enzyme activity due to polymorphisms in genes which are capable of repairing genotoxic chemical-induced DNA damage can make individuals more susceptible to normal populations (Garte, 2008).

The study which investigated the effects of genetic polymorphisms in biotransformation and DNA repair genes on hemoglobin adducts and genotoxicity biomarkers in workers exposed to styrene is a good example related to the subject (Godderis et al. 2004). Among the genetic association studies, glutathione S-transferases (GSTs) take an important place. It is known that the genes encoding phase II metabolic enzymes, GSTM1 and GSTT1 do not show enzymatic functional activity if they are null genotype (Geisler and Olshan, 2001). It was showed that GSTM1 and GSTT1 genotypes have been related to an increase number of cancer, likely due to an increased susceptibility to occupational toxic agents (Stojanovic, Milovanovic, Pastorino, Iavicoli & Boccia, 2018). Decreased DNA repair is associated with many diseases, especially cancer (Torgovnick & Schumacher, 2015). For this reason, studies demonstrating that polymorphisms in DNA repair genes change the risk of cancer in workers exposed to chemicals in the work environment is enlightening to identify risky subgroups (Wang et al., 2010).

Most diseases include a complex gene pathway system that cannot be represented by a single gene polymorphism. Therefore, it is problematic to find the relationship between single gene polymorphism and disease and to determine susceptible subgroups in the exposed group based on the polymorphism of this single gene. In this context, genome-wide association studies (GWAS) which is used to identify SNPs and CNVs in human DNA, are a powerful tool for identifying potential genetic polymorphisms associated with susceptibility to disease (Chu et al., 2014).

Epigenetic changes in occupational exposure

Epigenetic changes can switch genes on or off without changes in DNA base sequences and determine which proteins are transcribed. Epigenetic changes may also pass on to next generations. There are different mechanisms that control epigenetic changes, but they act in association with each other in epigenetic processes. Many types of epigenetic mechanisms have been identified including DNA methylation, RNA methylation, and histone modifications. Abnormalities in gene expression when a deviation from normal epigenetic patterns can lead to various clinical outcomes. These changes can occur both spontaneously and as a result of exposure to environmental and occupational factors or from certain mutations.

Environmental and occupational chemicals can alter many biological processes that affect epigenetic mechanisms. As a result of these changes, chromatin organization and gene expressions change and the risk of disease increases. Epigenetic modifications are intended to be used as a biomarker for exposure to occupational toxicants and carcinogens. Epigenomic profiling is considered to be a promising biomarker for providing information on the mechanisms of effect in the toxicological risk assessment of, particularly potentially carcinogenic and teratogenic chemicals (Miousse et al. 2015).

Many studies have been conducted on the subject, considering that many chemicals in the workplace could cause epigenetic changes in workers. Examples of epidemiological studies in various occupational exposures rather than *in vitro* and *in vivo* studies are summarized below. In a study conducting in workers exposed to solvents, DNA hypermethylation induced by solvent exposure was found to be associated with the development of chronic toxic encephalopathy (Godderis et al., 2012). Chrome exposure in chrome plating workers has been observed to cause hypomethylation in mitochondrial DNA (Yang et al., 2015). It was found that exposure to welding fumes causes DNA hypomethylation in F2RL3 (thrombin receptor-like 3) gene, which is a marker of cardiovascular disease prognosis and mortality (Hossain, Li, Hedmer, Tinnerberg, Albin & Broberg, 2015). Some other examples leading to modifications in epigenetic processes include benzene (Seow et al. 2012), vinyl chloride monomers (Wu et al., 2013), pesticide (Rusiecki et al., 2017), and polyaromatic hydrocarbons (Duan et al., 2013).

Defining epigenetic changes that may develop due to occupational toxicants will provide a significant contribution to risk assessment in occupational health and safety. However, a few important points should be considered during the use of epigenetic endpoints in risk assessment. Since some epigenetic effects are hereditary and others can be acquired in a person's life, it is necessary to distinguish between the contribution of these resources and whether they are independent or interactive risk factors. In addition, it is important to determine whether an epigenetic effect is associated with the causal pathway of the disease concerned. As studies on the relationship between epigenetic and occupational toxicants increase, the need to establish appropriate paradigms, approaches and methods for the use of these data in risk assessment will also gain importance (Schulte et al., 2015).

CONCLUSION

Understanding the role of genetic and epigenetic knowledge in work-related health problems can improve occupational risk assessments. Moreover, although occupational exposure standards are basically developed based on the assumption that all workers are at a similar risk (Schulte & Howard, 2011), identifying genetically susceptible workers and developing standards in view of the individual variability in responding to hazardous substances should be considered in the near future. Considering that the frequencies of polymorphisms vary between ethnic groups and populations in different regions of the World, widespread population-specific genetic susceptibilities may be also addressed by local organizations.

Although there are ethical, legal, social and political considerations which are not mentioned in this chapter in obtaining and using genetic/epigenetic information (MacDonald & Williams-Jones, 2002), with the development of high-throughput techniques over time, this information and their applications are expected to be widespread in practical life for protecting the health of workers.

REFERENCES

1. Anlar, H.G., Taner, G., Bacanli M, Iritas, S., Kurt, T., Tutkun, E. … Basaran, N. (2018). Assessment of DNA damage in ceramic workers. *Mutagenesis*, 33(1), 97-104.
2. Bajpayee, M., Pandey, A.K., Parmar, D., & Dhawan, A. (2005). Current Status of Short-Term Tests for Evaluation of Genotoxicity, Mutagenicity, and Carcinogenicity of Environmental Chemicals and NCEs. *Toxicology Mechanisms and Methods*, 15(3), 155-180.
3. Bolognesi, C., Knasmueller, S., Nersesyan, A., Roggieri, P., Ceppi, M., Bruzzone, M. … Fenech, M. (2017). Inter-laboratory consistency and variability in the buccal micronucleus cytome assay depends on biomarker scored and laboratory experience: results from the HUMNxl international inter-laboratory scoring exercise. *Mutagenesis*, 32(2), 257-266.
4. Bonassi, S., Milić, M., & Neri, M. (2016). Frequency of micronuclei and other biomarkers of DNA damage in populations exposed to dusts, asbestos and other fibers. A systematic review. *Mutation Research*, 770(Pt A), 106-118.
5. Bonassi, S., Biasotti, B., Kirsch-Volders, M., Knasmueller, S., Zeiger, E., Burgaz, S. … Fenech, M. (2009). State of the art survey of the buccal micronucleus assay--a first stage in the HUMN(XL) project initiative. *Mutagenesis*, 24(4), 295-302.
6. Chu, M., Ji, X., Chen, W., Zhang, R., Sun, C., Wang, T. … Shen, H. (2014). A genome-wide association study identifies susceptibility loci of silica-related pneumoconiosis in Han Chinese. *Hum Mol Genet.,* 23(23), 6385-6394.
7. Collins, A., Koppen, G., Valdiglesias, V., Dusinska, M., Kruszewski, M., Møller, P. … ComNet project. (2014). The comet assay as a tool for human biomonitoring studies: the ComNet project. *Mutat Res Rev Mutat Res.*, 759, 27-39.
8. Coppedè, F., & Migliore, L. (2015). DNA damage in neurodegenerative diseases. *Mutation Research,* 776, 84-97.
9. Duan, H., He, Z., Ma, J., Zhang, B., Sheng, Z., Bin, P. … Zheng, Y. (2013). Global and MGMT promoter hypomethylation independently associated with genomic instability of lymphocytes in subjects exposed to high-dose polycyclic aromatic hydrocarbon. *Arch Toxicol.,* 87(11), 2013-2022.

10. Duydu, Y., Başaran, N., Ustündağ, A., Aydin, S., Undeğer, U., Ataman, O.Y. … Bolt, H.M. (2012). Assessment of DNA integrity (COMET assay) in sperm cells of boron-exposed workers. *Arch Toxicol*., 86(1), 27-35.

11. Fenech, M. (2002). Chromosomal Biomarkers of Genomic Instability Relevant to Cancer. *Drug Discovery Today*, 7, 1128-1137.

12. Garte, S. (2008). Individual Susceptibility and Gene-Environment Interaction. Wild, C.P., Vineis, P., & Garte, S. (Eds.), in *Molecular Epidemiology of Chronic Diseases*. West Sussex, England: Jobn Wiley & Sons.

13. Geisler, S.A., & Olshan, A.F. (2001). GSTM1, GSTT1, and the risk of squamous cell carcinoma of the head and neck: a mini-HuGE review. *Am J Epidemiol.,* 154(2), 95–105.

14. Godderis, L., De Boeck, M., Haufroid, V., Emmery, M., Mateuca, R., Gardinal, S., … Lison, D. (2004). Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. *Environ Molec Mut,* 44, 293–303.

15. Godderis, L., De Raedt, K., Tabish, A.M., Poels, K., Maertens, N., De Ruyck, K. … Viaene, M.K. (2012). Epigenetic changes in lymphocytes of solvent-exposed individuals. *Epigenomics*, 4(3), 269-277.

16. Hagmar, L., Bonassi, S., Strömberg, U., Brøgger, A., Knudsen, L.E., Norppa, H., & Reuterwall, C. (1998). Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). *Cancer Res,* 58, 4117-4121.

17. Hämäläinen, P., Takala, J., & Kiat, T.B. (2017). *Global Estimates of Occupational Accidents and Work-related Illnesses 2017.* Singapore: Workplace Safety and Health Institute. Retrieved from http://www.icohweb.org/site/images/news/pdf/Report%20Global%20Estimates%20of%20Occupational%20Accidents%20and%20Work-related%20Illnesses%202017%20rev1.pdf

18. Hoeijmakers, J.H. (2001). Genome maintenance mechanisms for preventing cancer. *Nature*, 411, 366–374.

19. Hossain, M.B., Li, H., Hedmer, M., Tinnerberg, H., Albin, M., & Broberg, K. (2015). Exposure to welding fumes is associated with hypomethylation of the F2RL3 gene: a cardiovascular disease marker. *Occup Environ Med*, 72(12), 845-51.

20. ICOH. (2010). Biomonitoring for occupational health risk assessment (BOHRA). *Toxicology Letters*, 192, 3–16.

21. ICPEMC. (1990). The possible involvement of somatic mutations in the development of atherosclerotic plaques. Report of Subcommittee 7/1. Conclusions and recommendations. *Mutat Res*., 239(3),143-148.

22. IPCS (International Programme on Chemical Safety). (2001). *Biomarkers in Risk Assessment: Validity and Validation.* Geneva: World Health Organization.

23. Duffus, J.H., Nordberg, M., & Templeton, D.M. (2007). Glossary of Terms used in Toxicology, 2nd edition (IUPAC Recommendations 2007). *Pure Appl. Chem.*, 79(7), 1153–1341.

24. Judson, R.S., Kavlock, R.J., Setzer, R.W., Cohen-Hubal, E.A., Martin, M.T., Knudsen, T.B. … Dix, D.J. (2011). Estimating toxicityrelated biological pathway altering doses for high-throughput chemical risk assessment. *Chem. Res. Toxicol.* 24(4), 451–462.

25. Karahalil, B., Burgaz, S., Fişek, G., & Karakaya, A.E. (1998). Biological monitoring of young workers exposed to polycyclic aromatic hydrocarbons in engine repair workshops. *Mutat Res,* 412(3), 261-269.

26. Kirsch-Volders, M., Vanhauwaert, A., Eichenlaub-Ritter, U., & Decordier, I. (2003). Indirect mechanisms of genotoxicity. *Toxicology letters*, 140, 63-74.
27. Lesage, M. (2011). Work-related Diseases and Occupational Diseases: The ILO International List. In: Topics in Workers' Compensation Systems. (P. Rey, & M. Lesage, (Chapter ed.s), *Encyclopedia of Occupational Health & Safety. ILO; Part III.* Retrieved from http://www.iloencyclopaedia.org/part-iii-48230/topics-in-workers-compensation-systems/36-26-workers-compensation-systems-topics-in/work-related-diseases-and-occupational-diseases-the-ilo-international-list
28. Li, K., Jing, Y., Yang, C., Liu, S., Zhao, Y., He, X. … Li, G. (2014). Increased leukemia-associated gene expression in benzene-exposed workers. *Sci Rep.,*4, 53-69.
29. Liu, N, Guan, Y., Xue, L., Yu, Y., Xiao, J., Chang, Z. … Guan, W. (2017). Assessment of DNA/Chromosome Damage in the Peripheral Blood Lymphocytes of Workers Exposed to Indium Compounds. *Toxicol Sci*, 157(1), 41-49.
30. Loomis, D., Guha, N., Hall, A.L., & Straif, K. (2018). Identifying occupational carcinogens: an update from the IARC Monographs. *Occup Environ Med*, 75(8), 593-603.
31. MacDonald, C., & Williams-Jones, B. (2002). Ethics and genetics: susceptibility testing in the workplace. *J Bus Ethics* 35, 235–241.
32. Madle, S., Kasper, P., Pabel, U., & Speit, G. (2008). Methods in Toxicology. In H. Greim, & R. Snyder (Eds.), *Toxicology and Risk Assessment: A Comprehensive Introduction.* (pp. 406-417). West Sussex: John Wiley & Sons
33. Miousse, I.R., Currie, R., Datta, K., Ellinger-Ziegelbauer, H., French, J.E., Harrill, A.H. … Thompson, K. (2015). Importance of investigating epigenetic alterations for industry and regulators: An appraisal of current efforts by the Health and Environmental Sciences Institute, *Toxicology*, 335, 11-19.
34. NRC (National Research Council) (1987). Biological markers in environmental health research. *Env Health Perspect* 74, 3–9.
35. Rusiecki, J.A., Beane Freeman, L.E., Bonner, M.R., Alexander, M., Chen, L., Andreotti, G. … Baccarelli, A. (2017) High pesticide exposure events and DNA methylation among pesticide applicators in the agricultural health study. *Environ Mol Mutagen*, 58(1), 19-29.
36. Sakkas, D., & Alvarez, J.G. (2010). Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. *Fertil Steril*, 93(4), 1027-1036.
37. Sardas, S., Omurtag, G.Z., Tozan, A., Gül, H., & Beyoglu, D. (2010). Evaluation of DNA damage in construction-site workers occupationally exposed to welding fumes and solvent-based paints in Turkey. *Toxicol Ind Health*, 26(9), 601-608.
38. Schulte, P., & Howard, J. (2011). Genetic Susceptibility and the Setting of Occupational Health Standards. *Annu. Rev. Public Health*, 32, 149–159.
39. Schulte, P.A., Whittaker, C., & Curran C.P. (2015). Considerations for Using Genetic and Epigenetic Information in Occupational Health Risk Assessment and Standard Setting, *Journal of Occupational and Environmental Hygiene*, 12(1), 69-81.
40. Seow, W.J., Pesatori, A.C., Dimont, E., Farmer, P.B., Albetti, B., Ettinger, A.S. … Baccarelli, A.A. (2012). Urinary benzene biomarkers and DNA methylation in Bulgarian petrochemical workers: study findings and comparison of linear and beta regression models. *PLoS One*, 7(12), e50471.
41. Siemiatycki, J., Richardson, L., Straif, K., Latreille, B., Lakhani, R., Campbell, S. … Boffetta, P. (2004). Listing occupational carcinogens. *Environ Health Perspect.* 112(15), 1447-1459.

42. Stojanovic, J., Milovanovic, S., Pastorino, R., Iavicoli, I., & Boccia, S. (2018). Occupational exposures and genetic susceptibility to urinary tract cancers: a systematic review and meta-analysis. *Eur J Cancer Prev,* 27(5), 468-476.

43. Torgovnick, A., & Schumacher, B. (2015). DNA repair mechanisms in cancer development and therapy. *Front Genet,* 6, 157.

44. Wang, Q., Wang, A.H., Tan, H.S., Feng, N.N., Ye, Y.J., Feng, X.Q. … Xia, Z.L. (2010). Genetic polymorphisms of DNA repair genes and chromosomal damage in workers exposed to 1,3-butadiene. *Carcinogenesis.* 31(5), 858-863.

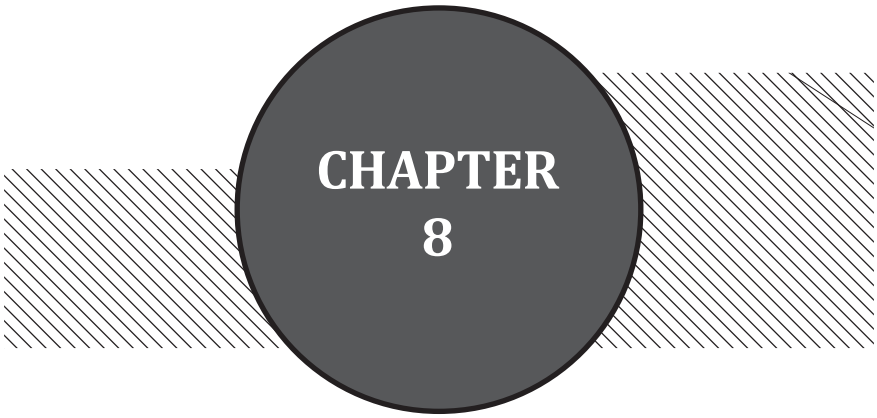
45. WHO (World Health Organization). (2001). *Biomarkers in Risk Assessment: Validity and Validation. Environmental Health Criteria 222.* Geneva: World Health Organization. Retrieved from http://www.inchem.org/documents/ehc/ehc/ehc222.htm

46. Wu, F., Liu, J., Qiu, Y.L., Wang, W., Zhu, S.M., Sun, P.…. Xia, Z.L. (2013). Correlation of chromosome damage and promoter methylation status of the DNA repair genes MGMT and hMLH1 in Chinese vinyl chloride monomer (VCM)-exposed workers. *Int J Occup Med Environ Health.* 26(1), 173-182.

47. Yang, L., Xia, B., Yang, X., Ding, H., Wu, D., Zhang, H. … Zhuang, Z. (2015). Mitochondrial DNA hypomethylation in chrome plating workers. *Toxicol Lett*, 243, 1-6.

Approach To Peripheral Vertigo

Mehmet Erkan KAPLAMA[1]



[1] ????

LOGIN

Vertigo is an illusion of movement in which the vestibular system is caused by sudden neural activity imbalance. Sometimes the patient can describe it like a sensation of tilt. Instead, the word dizziness indicates a sensation of disturbed relation to surrounding objects in space with feelings of rotation or whirling characteristic of vertigo as well as non-rotatory swaying, weakness, faintness and unsteadiness characteristic of giddiness. The incidence of vertigo in the general population is around 20 to 30%. Generally, symptoms and symptoms are unclear, non-specific and difficult to identify. However, it can usually be diagnosed with a systematic approach. Vertigo is not a specific diagnosis. Central and peripheral causes should be differentiated in patients with vertigo. The majority of the vessels are caused by the peripheral. Peripheral vertigo is typically sudden onset. Patients usually indicate that the floor slides under the feet or the walls move. Changes in head position and rapid movements often increase symptoms. It is often accompanied by autonomic findings such as nausea, vomiting, sweating and pallor. The peripheral vestibular system forms the vestibular labyrinth and vestibular nerve. Pathologies that disrupt the functioning of peripheral systems cause peripheral vertigo

PATHOPHYSIOLOGY

Ensuring balance and adapting the body to the environment depend on the integrity and coordination between the vestibular system, the visual system and the somatosensory system. These systems work in mutual communication and provide orientation and balance continuity under the guidance of immediate afferent warnings. The impulses from the eyes, muscles, joints and otic labyrinths are constantly sent about the position of the body. The sensory organs of these three systems are connected to the cerebellum via the vestibular nucleus. A problem in any of the centers involved in providing balance leads to symptoms such as vertigo, imbalance and vertigo. The vestibular system determines the body's orientation according to gravity through the otoliths. The vestibular apparatus is located in the labyrinth of the petrous part of the temporal bone and consists of three parts. They form semicircular canals, utricul and sacculus (otoliths). It can also be classified as peripheral and central vestibular system. Semicircular canals, otoliths and vestibulocohlear nerve include peripheral vestibular system. The central vestibular system includes the cerebellar parts and connections related to the nuclei and the vestibular system. While semicircular canals provide information about movement and momentum, otoliths provide information about body's gravitational fit. Semicircular canals are double circle canals that respond symmetrically to the angular movements of the head. The semicircular canals are filled with a fluid called endolymph. The movement of the fluid in the semicircular canals causes the movement of specialized hairy cells in the canal and the activation of the afferent vestibular stimuli. The membranous labyrinth with head movements moves towards the cupola. Includes the following buyers. The nerve cells in the canals have resting and action potentials. With the movement of the head, the hairy cells in the cupola are activated while the ones on the opposite side are in the desires. Utricus and saccharine are sensitive to the linear movements of the head. The movement of the autoconces with the movement of the head moves the cells attached to it. The urethra is activated by the linear movements of the head, while the saccular is more involved in maintaining the balance against gravity. In this balanced system, any asymmetric activity will result in vertigo.

STORY

A careful history from the patient with vertigo is a priority and important for physical examination and special vestibular tests. In the history taken from the patient, it can be distinguished whether he described a true vertigo or other types of balance disorders. Recent illnesses such as upper respiratory tract infections, traumas, surgical interventions, ototoxic drugs, bad habits, long-term travel history, long-term bed rest, stress and fatigue, and other symptoms accompanying vertigo should be evaluated. The duration of the vertigo provides important information. In seconds, BPPV, vascular causes lasting for several minutes, Meniere disease lasting for several hours, vestibular neurinitis lasting days, lasting for weeks, months suggest central and psychological reasons. Autologous or neurological symptoms should be questioned in the anamnesis. The provoking situations should be investigated. Sudden changes in head position, abrupt dislocation, presence of upper respiratory tract infections, stress, trauma, changes in ear pressure should be questioned provoking provictive symptoms.

	Peripheral vertigo	Central vertigo
Onset	Acute	Subacute or slow
Nausea, vomiting	May be severe	Varies
Otological symptoms	Common	Rare
Neurological symptoms	Rare	Common
Duration	Short duration, may decrease after a few days	Persists
Nystagmus	Horizontal-rotatory	Vertical
Vertigo severity	severe	less

Table 1. Central and Peripheral Vertigo distinctive Features

PHYSICAL EXAMINATION

Otological Examination: Although usually otoscopic examinations are normal, the examination may also reveal signs of acute or chronic otitis media, cholesteatoma and previous operations. An audiogram should be routinely performed in cases with hearing loss. Meniere's disease and acoustic neuroma may be associated with fluctuating hearing loss and unilateral hearing loss may help to determine localization.

Cranial nerve examination: External auditory pathway hypoesthesia (Hitselberger's sign) and loss of corneal reflex may be a sign of acoustic neuroma.

Neuro-otological examination: Neuro-otologic examination of both sides of the body is performed, and nystagmus, cerebellar functions, muscle strength and deep tendon reflexes should be examined.

Nystagmus is the name given to the movement of the eyes to the other side with and without recurrence. It has two phases, slow and fast phase. The slow phase is generated by the vestibulo-ocular reflex, while the fast phase is the correction movement generated by the parapontin reticular formation. Physiological, peripheral and central nystagmus are divided into three. Vestibular nystagmus are horizontal-rotatory type and typically have two components. Typically, the slow phase of horizontal nystagmus strikes the peripheral pathology side in the acute stage (irritative stage) and the fast phase toward the solid side.Vertebral nystagmus is never seen in vestibular diseases. Sometimes congenital disease may present with vertical nystagmus.The separation distance of the eyeballs from the middle line determines the amplitude of the nystagmus. In peripheral vestibular

nystagmus, amplitude is large and small in power plants. Spontaneous vertical nystagmus or diagonal nystagmus has a high diagnostic value in central nervous system diseases. Nystagmus occurring in different directions in two eyes is always indicative of central pathologies. DixHallpike maneuver used for the diagnosis of benign positional vertigo is a test that can be easily applied. In the diagnosis of BPPV, 83% were found to be positive and 52% were negative.

Cerebellar System Examination: Cerebellar system examination should not be neglected in balance disorders, and the patient's gait should be monitored during the examination. The ataxic walk in the cerebellar disease is characterized by broad-based irregular steps, swinging, wobbling, tremor in the trunk, and side-to-side wobbling. Findings of incoherence such as dysmetry and disdiadocokinesis are determined. Hypotonia, intense tremor and explosive speech are available. Vertebral nystagmus towards the lesion side is observed.

Romberg Sign: Used in the examination of proprioceptive functions and is an indicator of loss of postural control in darkness. Body stability against gravity provides a qualitative measurement of information reaching the vestibulospinal system with reflexes involved in adjusting muscle tone and joint movements. In this test, while the patient is standing, the feet and arms are symmetrically joined together and the eyes are closed. Normal individuals can remain in this position for 30 seconds without shaking. In vestibular system disorders, when the patient closes his / her eyes, the lesion falls to the side. Lack of posture is an indicator of abnormal proprioception. In the sharpened Romberg test, the patient keeps his feet in a line with the foot of one foot and the other foot of the foot at the tip. In this position, the sharpened Romberg test (+) is found when the loss of balance, which does not appear when the eyes are open, occurs when the eyes are closed. This is considered to be an indication of unilateral and uncompensated mild peripheral vestibular pathologies. While the open or closed eyes in the cerebellar attack during the Romberg test has no effect on the balance, somatosensory pathologies that hold the posterior column in the eye open when the eye is open, the eye closes. In somatosensory pathologies, the patient may fall not only to one side but also to both sides. In central pathologies, when the eyes are open, the patient falls on the pathology side and closing the eyes does not increase the imbalance. In acute vestibular diseases, the patient falls to the side of the pathology and her imbalance increases. When the eyes are open, there is imbalance, the patient falls on the pathology side. Stance is stable when eyes are open in bilateral vestibular function loss or unilateral chronic compli- cation. However, when the eyes are closed, the patient does not fall all the time, there may be fall due to shaking and unbalance. In psychogenic vertigo, while the eyes are closed during the Romberg test, severe imbalance occurs. However, if the patient falls, almost never falls.

Whole Body Examination: A complete cardiovascular and neurological system examination should be performed and diagnostic tests such as cardiac monitoring, echocardiography, vascular examination, imaging of the central nervous system and electroencephalography should be performed. Cardiovascular system disorders, especially in elderly patients, may increase the risk of cerebrovascular disease. A complete cranial nerve and cerebellar system examination can help with the localization of the lesion.

Common Peripheral Vertigosis

Benign paroxysmal positional vertigo (BPPV)

It is the most common cause of recurrent vertigo in adults , in order for the BPPV to occur, autoconin should be disrupted from the utricular macula and fall into one of the semicircular canals. The lifetime prevalence of BPPV is 2.4% . Since BPPV is the most common cause of vertigo in neurotology clinics, it should be well recognized and treated.

In BPPV, the posterior canal is the most frequently affected channel and the right posterior canal is most commonly affected by the right side.

The BPPV clinical causes calcium carbonate crystals to fall off the utricular reasonable and fall into one of the semicircular canals. The posterior duct is affected by the majority of cases due to its location. In canalolithiasis, the calcium carbonate crystals circulate freely in the canal and cause a bulblapedal or ampullafugal deflection of the cup with certain head movements. In cupulolithiasis, calcium carbonate crystals adhere to the canal and cause similar clinical picture. In both cases it causes vertigo and nystagmus specific to the affected canal. Patients describe vertigo in the form of illusion of movement of the environment when they lie on the bed, get out of bed, return to bed, or reach for something in the shelf. Rotation attacks last less than 30 seconds, but patients may perceive these attacks as longer. Especially when getting out of bed in the morning, vertigo attacks are very severe. This is due to the fact that calcium carbonate crystals are concentrated in a part of the canal during sleep, and the severity of getting out of bed causes vertigo.Patients may complain of the feeling of drowsiness throughout the day. BPPV can be seen after head trauma, viral labyrinthitis, Meniere's disease, migraine, inner ear operations.

One of the most important examinations to be performed in the patient presenting with vertigo is positional test. First, the Dix-Hallpike test should be performed (Figure 1). If the test is performed with Frenzel goggles which suppress the visual fixation, it is better because it allows to see low and short-lived nystagmus. It is often appropriate to start the positional test from the left because the right side is affected.

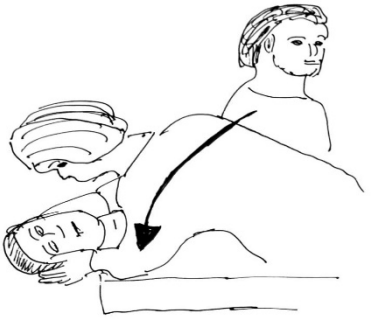


Figure 1. Dix-Hallpike test should be performed for right.

As a result of positional test, calcium carbonate crystals in the posterior canal cause the cup to move away from the utricle (ampullofugal). This excitatory stimulus is caused by nystagmus, which has a torsional component towards the lower ear with a mixed up stroke. In BPPV, nystagmus occurs 1-15 seconds after positional testing; The duration of the attack is between 5 and 40 seconds. When the patient is seated, the direction of the nystagmus is reversed when the patient is seated. Because the cup is stimulated in the opposite direction (ampullopedal) direction. After the diagnosis is made, corrective maneuver the particles must be removed from the posterior duct. Epley maneuver is one of the most effective treatments in the medical sciences. (Figure 2). The benefits of maneuvering to the complaining side in patients with only vertigo or nausea sensation without positional nystagmus in the positional test have been shown in studies. After the

maneuver; for example, if the patient is maneuvered to the right side, it is recommended that the patient sleep with two pads on the left side for a week. Approximately one minute after maneuver

One week may be the complaint of ongoing imbalance. If the position-triggered vertigo persists, the maneuver is repeated a second time.

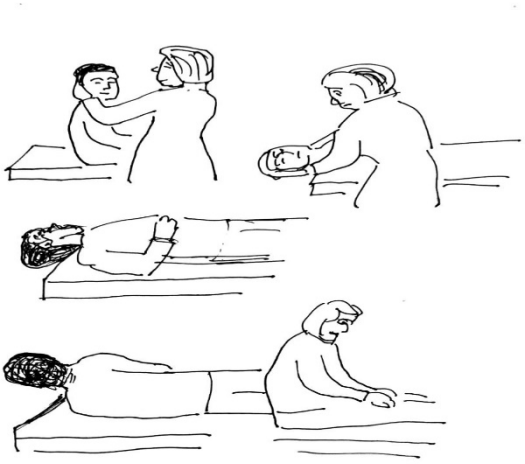


Figure 2. Epley maneuver for right.

MENIERE'S DISEASE

Meniere's disease is characterized by vertigo, fluctuating sensorineural hearing loss and tinnitus triad. There is a feeling of pressure and fullness in the affected ear. Vertigo attacks last from a few minutes to several hours. In early stage, hearing loss is reversible but becomes permanent in the late period. Tinnitus is strong and is defined by the patient as the sound of the ocean. Episodes are repeated at irregular intervals and the remission period is long. Bilateral Meniere's disease may develop in 1 \ 3 patients. Drop attack may be seen without loss of consciousness or neurological symptoms. When hearing loss is deep, episodic vertigo fluktuan hearing levels and tinnitus may not accompany. In Meniere's disease, there is a sensorineural hearing loss in the early stage holding low frequencies and the caloric examination may be normal. Treatment: Diuretics and salt restriction are the basis of medical treatment. 90% of patients respond to medical treatment. Endolymphatic sac decompression or shunt applications, vestibular nerve resection or labirintectomy may be performed in cases resistant to medical treatment. Chemical ablation of the vestibular structure is a treatment that is considered to be a great benefit. For this purpose, intratympanic Gentamycin injection is applied. Although it is less aggressive than surgery, there is an increased risk of hearing loss in chemical ablation.

VESTIBULAR NEURINITIS

Vestibular neurinitis is the third most common cause of vertigo after BPPV and Menire's disease. Vestibular neurinitis as a result of acute inflammation of the vestibular nerve is characterized by sudden onset episodic episodes of vertigo and vertigo is severe and usually lasts longer than 24 hours.48

There are no accompanying symptoms such as tinnitus and hearing loss. Patients are usually young to middle age adults. Spontaneous nystagmus is present and is usually horizonto-rotate. Reduction of the vestibular signal in the affected ear causes vestibular excitation in the intact ear and consequently the rapid phase of the nystagmus is in the intact ear, the slow phase in the affected ear. Almost all patients have a history of an upper respiratory tract infection in a few weeks.

The basis of treatment in the acute phase is symptomatic and supportive treatment. For this purpose vestibular suppressants and antiemetics can be used. Vertigo attack and vomiting are reduced during the day, reducing their severity and generally recovering without any sequela. There are also ongoing discussions on the use of steroids and antiviral drugs in the treatment of vestibular neurinitis. Supports that corticosteroid treatment improves in a shorter time and affects long-term peripheral vestibular function positively in most studies. results have been shown to be better. However, more extensive studies are needed to evaluate the efficacy of antiviral therapy.

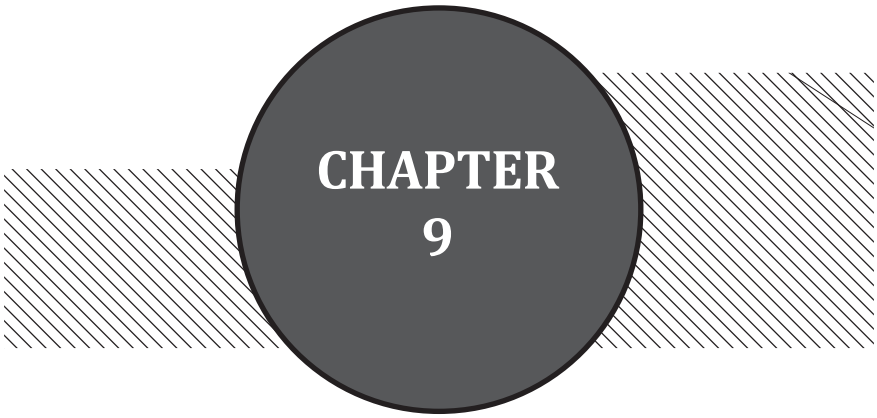
RESOURCES

1. Akyıldız N. Kulak Hastalıkları ve Mikrocerrahisi. 2002;2:84-140.
2. Bahadır C, Dıraçoğlu D, Kurtuluş D, Garipoğlu İ. Efficacy of canalith repositioning manevuers for benign paroxysmal positional vertigo. Clinical Chiropractic 2009; 12(3):95–100.
3. Baloh RW, Honrubia V. Clinical Neurophysiology of the Vestibular System, 2nd ed. Philadelphia, F.A. Davis 1990.
4. Baloh RW. Clinical practice. Vestibular neuritis. N Engl J Med 2003;348:1027-32.
5. Brandt T. Benign paroxysmal positioning vertigo. In: Brandt T eds. Vertigo: ıt‟s multisensory syndromes. London: Springer-Verlag 2003:242–55.
6. Buttner U, Helmchen C, Brandt T. Diagnostic criteria for central versus peripheral positioning nystagmus and vertigo: a review. Acta Otolaryngol 1999;119:1-5.
7. Ceryan K, Şerbetçioğlu MB. Kulak Burun Boğaz Hastalıkları ve Baş Boyun Cerrahisi. Editör Çelik O. 2. Baskı. İzmir. Asya Tıp Kitabevi. 2007; 1:36–63.
8. Diamond C, O'Connell DA, Hornig JD, Liu R. Systematic review of intratympanic gentamicin in Meniere's disease. J Otolaryngol 2003;32:351-61.
9. Furman, J.M. and S.P. Cass, Benign paroxysmal positional vertigo. New England Journal of Medicine, 1999. 341(21): p. 1590-1596.
10. Ganança CF, Caovilla HH, Gazzola JM, Ganança MM, Ganança FF. Epley's maneuver in benign paroxysmal positional vertigo associated with Meniere's disease. Brazz J Otorhinolaryngol. 2007; 73:506–12.
11. Hanley K, O'Dowd T, Considine N. A systematic review of vertigo in primary care. Br J Gen Pract 2001;51:666-71.
12. James A, Burton MJ. Betahistine for Ménière's disease or syndrome. Cochrane Database Syst Rev 2011;(3):CD001873.
13. Kentala E, Rauch SD. A practical assessment algorithm for diagnosis of dizziness. Otolaryngol Head Neck Surg 2003;128:54-9.
14. Kerber, K.A., et al., Use of BPPV processes in emergency department dizziness presentations: a population-based study. Otolaryngol Head Neck Surg, 2013. 148(3): p. 425-30.

15. Korkut N Ozturan O, Cokkeser Y, Saydam L, et al. Benign Paroksismal Pozisyonel Vertigo ve Kanalit Repozisyon Prosedürü. Kulak Burun Boğaz İhtis Derg 1998;5:16-21.. Vertigoya genel bakış. Klinik Gelişim 2005;18(1):6572.
16. Korkut N. Vertigoya genel bakış. Klinik Gelişim 2005;18(1):6572.
17. Labuguen RH, Initial Evaluation of Vertigo. Am Fam Physician 2006;73:244-51.
18. Lempert T, Neuhauser H. Vertigo and dizziness rekated to migraine: a diagnostic challange. Cephalalgia 2004; 24: 83-91.
19. Neuhauser HK, Radtke A, von Brevern M, Lezius F, Feldmann M, Lempert T. Burden of dizziness and vertigo in the community. Arch Intern Med. 2008; 168(19):2118-24.
20. Seemungal, B.M. and A.M. Bronstein, A practical approach to acute vertigo. Practical neurology, 2008. 8(4): p. 211-221.
21. Viirre E., Purcell I, Baloh R. The Dix-Hallpike Test and The Canalith Repositioning Maneuver. The Laryngoscope. January 2005;115:184-7.

Hyperbaric Oxygen Therapy

Ali Erdal GÜNEŞ[1]



[1] Assistant Professor. Harran University, School of Medicine, Department of Underwater and Hyperbaric Clinical Medicine, Sanliurfa, Turkey

1. INTRODUCTION

Hyperbaric oxygen therapy (HBOT) is an inhaler therapy that is administered to the patient in a completely closed pressure chamber, independent of the external environment, with 100% oxygen inhalation at a certain pressure, at a pressure higher than 1 atmosphere.[1] The main purpose of treatment is to increase the partial oxygen pressure by increasing the amount of dissolved oxygen in the blood. During treatment, the arterial oxygen pressure rises to 2000 mm HG and this increase is about 200-400 mm HG in tissue level.[2] High dissolved oxygen reaches more distances in the tissue, in other words, increasing of the oxygen diffusion distance in the tissue. In addition, this treatment contributes to tissue healing by increasing neo-angiogenesis and collagen production.[3] Other benefits are improving oxygen-dependent phagocytosis mechanisms, controlling edema and restoring circulation in affected tissues.[4]

2. HISTORY

HBOT dates back to the 1600s. The first known pressure chamber in the records is called 'Domicilium' which was made in 1662 by the researcher and religious man Henshaw (Figure 1).[5] In 1879, the French surgeon Fontaine put forward to the idea of operating patients in a pressurized environment.[6] Anesthesiologist Dr Cunningham founded the Hyperbaric Hospital in 1928 in Lawrence, Kansas. According to the Central Records, known as the *Steel Ball Hospital*, it weighed 900 tons and consisted of six floors and was 64 feet wide. It was pressurized up to 3 times the normal atmospheric pressure.

Figure 1. In Domicilium is targeted treatment with compressed air. Mostly used for the treatment of asthma and chronic bronchitis.[5]

It was scientifically thought that Steel Ball Hospital was not good for some diseases and was closed two years later. In the Second World War, the hospital was broken down and used in the war.[6]

In the following years, researches on HBOT continued with the military studies of Paul Bert. Paul Bert carried out studies on the effects of HBOT on the central nervous system and lung, which have two toxic effects. Measured and tested seizures in different dive depths and different bottom time.[6]

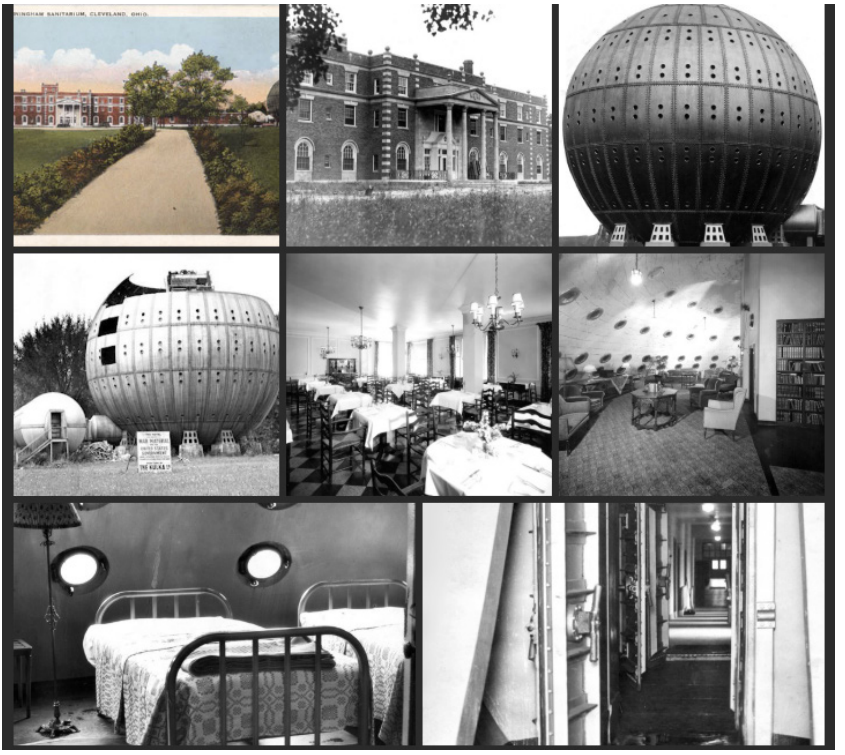


Figure 2. Photos of Steel Ball Hospital, The Cunningham Sanitarium.[7]

In 1935, Behnke showed that the cause of decompression sickness nitrogen gas. Behnke and Shaw successfully treated decompression sickness with HBOT.[8]

3. APPLICATION METHODS

HBOT can be applied in different ways depending on the device to be treated. However, the sessions are consecutive and last about 2 hours in all treatments. In the same way, the preparation of the treatment may vary depending on the device. The devices are divided into mono-place and multi-place. Each treatment type has its advantages and disadvantages.

3.1. Multi-Place Pressure Chamber

Multi-place pressure chamber is the treatment device used by 3rd stage health centers.[9] In particular; treating multiple patients at the same time and taking intubated intensive care patients are the most important advantages. Basic intensive care instruments such as intravenous treatments, ventilator and monitarization can be used in chamber. Ambient pressure is generated by well-filtered air, in the multi-person pressure chamber. Patients are treated with a hood, mask or endotracheal tube. (Figure 1a).[10] With the help of the internal staff, the patient can be urgently intervened when the first and emergency help is needed.[11] The disadvantage of multi-place chamber is the cost. It is very expensive for purchase.

3.2. Mono Place Pressure Chamber

Ambient pressure is provided in mono-palce chamber via oxygen gas. Patients no need to use mask or hood for treatment (Figure 1b).[10] One of the most important advantages of this type of pressure chamber is that the device is mobile. If necessary, the patient can be transferred by ambulance or airplane in a pressurized chamber.

Another important advantage is that patients with a history of facial burn or laryngectomy may be treated without mask by direct breathing through the environment. In recent years, mono-place devices have been developed with air pressurized systems. The disadvantage of this type of device is the formation of claustrophobia in patients. It is also the height of the risk of fire as it is pressurized by oxygen.

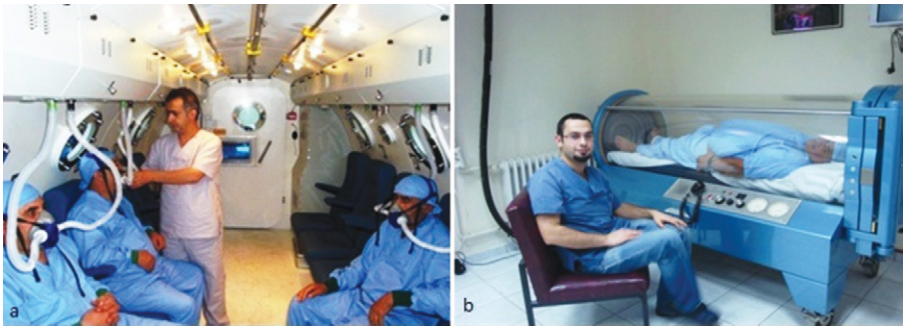


Figure 3. a: Multi-Place Pressure Chamber; b: Mono Place Pressure Chamber.[10]

4. MECHANISM OF HBOT

According to the Underwater and Hyperbaric Medicine Society, the environmental pressure during HBOT application must be at least 1.4 atmosphere absolut (ATA) and above.[1] Amount of dissolved oxygen in the plasma increases as pressure increases.[12]

4.1. Physics of HBOT

The mechanism of action of HBOT based from gas laws. These gas laws constitute the content of the ideal gas law.

The Boyle Gas Law ($P_1xV_1=P_2xV_2$) is used to explain the physical effect of HBOT. This effect of HBOT is used in the treatment of new gas formations in the body which cause embolism in particular. The only indication of this effect is the use of HBOT in decompression sickness and arterial gas embolism.

The Charles Gas Law ($P_1/T_1=P_2/T_2$) explains that the temperature will increase when a closed pressure chamber is pressurized. In the same way, the pressure will decrease than chamber cool down. This situation is especially important in elderly, children and intubated patients. Multi-place pressure chambers have air conditioning system. In this way, patients are given a comfortable treatment. There is no such facility in mono-place pressure chambers.

According to Henry gas law, the solubility of the gas depends on the ambient pressure and the number of dissolution layers in the liquid in which the gas is contacted. In particular, we use this law when calculating the amount of oxygen dissolved in the plasma. Using this effect of HBOT, we treat diseases such as wound healing, burns, peripheral arterial disease and crush injuries.

4.2. Physiologic Effect of HBOT

The physiological effect of HBOT is divided into two as direct and indirect. The direct effect is the continuation of tissue viability by the high amount of dissolved oxygen reaching the hypoxic area. Indirect effects include vasoconstriction, decreased inflammation and angiogenesis.[13] HBOT enhances the reduced immune response in the hypoxic area. HBOT increases bactericidal defense by regenerating neutrophils.

Oxygen decreases the edema and ischemia at the points reached by HBOT, and increases the diffusion distance of oxygen by 4 times in the tissue and positively affects the treatment results. HBOT increases the oxygen pressure in the hemoglobin and removes carbon monoxide molecules from the body. It shows bactericidal and bacteriostatic effects in necrotizing infections and intracranial abscesses. It provides control of the infection and reduces the release of toxins. HBOT also increases the effect of antibiotics such as gentamicin and quinolone.

Retina and cochlea are very sensitive to oxygen deficiency. HBOT provides oxygen supply to the remaining ischemic areas, provides neovascularization and affects the recovery of hearing-vision.[14]

5. ADVERSE EFFECT

Side effects are more common in older people. Many of the side effects are reversible myopia and cataract.[15] Brain and lung are two organs in which HBOT shows toxic effects. After lung toxication respiratory system symptoms can be listed as a; chest pain, cough, decreased vital capacity, pulmonary edema and atelectasis.[16] HBOT affects the central nervous system causing vertigo, tonic-clonic convulsion, tunnel vision, tremor and loss of consciousness.[16]

6. CONTRAINDICATIONS

Single and definite contraindications to HBOT are untreated pneumothorax. Relative contraindications can be listed as; chemotherapeutic agents (cisplatin, bleomycin, etc.), pulmonary emphysema, epilepsy, high fever, optic neuritis and congenital spherocytosis.[16]

7. COMPLICATIONS

The most common complication is barotrauma in the middle ear and paranasal sinuses. However, complications include reversible myopia and cataract can also be listed.[1] Despite the aforementioned complications and contraindications, HBOT is a safe treatment method. Especially in multi-place chambers, the complication rate decreases with the help of internal staff.

8. CONCLUSION

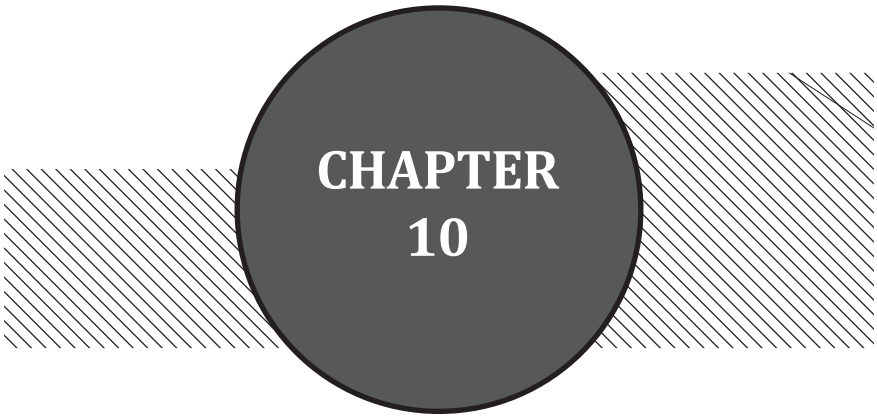
HBOT enhances angiogenesis and neutrophil mediated immune response, and reducing tissue ischemia. Studies have shown the cellular and physiological benefits of HBOT. It has been shown that HBOT can be given as main or additional treatment in the treatment of cases such as postradiotherapy tissue damage, wound healing, avascular necrosis, compromised graft-flap, osteomyelitis, thermal burns, crush, compartment syndrome, sudden vision and sudden hearing loss. However, controlled double-blind randomized trials are needed to demonstrate the efficacy of HBOT in many diseases or in ongoing new indications.

REFERENCES

1. Tibbles PM, Edelsberg JS. Hyperbaric-oxygen therapy. N Engl J Med 1996;334:1642Y1648.
2. Thom SR. Hyperbaric oxygen therapy. J. Intensive Care Med. 1989; 4:58–74.
3. Cianci P. Advances in the treatment of the diabetic foot: Is there a role for adjunctive hyperbaric oxygen therapy? Wound Repair Regen 2004;12(1)-10.
4. Bakker D. Selected aerobic and anaerobic soft tissue infections. Hyperbaric Medicine Practice. Kindwall EP and Whelan HT, eds. Best Publishing Company. 2002.p.576-597
5. Henshaw IN, Simpson A.Compressed Air as a Therapeutic Agent in the Treatment of Consumption, Asthma, Chronic Bronchitis and Other Diseases. Edinburgh: Sutherland and Knox; 1857.
6. Kindwall E, Whelan H.Hyperbaric Medicine Practice. 2Nd ed. Flagstaff, AZ: Best Publishing Company; 2004:chap 1, 18, 19, 20, 25, 29, 30.
7. Morgan Choffin, "The Cunningham Sanitarium," Cleveland Historical, accessed January 31, 2019, https://clevelandhistorical.org/items/show/378.
8. Thiagarajan B, Arjunan K. Hyperbaric Oxygen therapy Concepts and Myths. WebmedCentral:ENT Scholar 2012;3(4):WMC003309.
9. Sualtı Hekimleri Derneği, Basınç Odası Merkezleri Ve Sualtı Hekimliği Ve Hiperbarik Tıp Uzmanı Doktorlarımız, accessed February 1, 2019, http://sualti.org/?page_id=11
10. Kati B, Pelit ES, Gunes AE. Hyperbarıc Oxygen Therapy and Applications in Urology. J Reconstr Urol 2017;7(1):8-18.
11. Ozdemir Y, Uzun G, Mutluoglu M, Gulec B. Hyperbaric oxygen therapy for the management of postsurgical wounds in hidradenitis suppurativa. Am Surg. 2010;76(12):E237-8.
12. Grim PS, Gottlieb LJ, Boddie A, et al. Hyperbaric oxygen therapy. JAMA 1990;263:2216Y2220
13. Devaraj D, Srisakthi D. Hyperbaric oxygen therapy – can it be the new era in dentistry? J Clin Diagn Res. 2014;8(2):263–265.
14. Jones MW, Wyatt HA. Hyperbaric, Physics. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018-.2018 Oct 27.
15. Palmquist BM, Philipson B, Barr PO. Nuclear cataract and myopia during hyperbaric oxygen therapy. Br J Ophthalmol 1984;68:113Y117.
16. Undersea and Hyperbaric Medical Society. Indications for hyperbaric oxygen therapy: approved indications. Available at: http://www.uhms.org/indications/indications.htm. Accessed May 10, 2018.

Allergen Immunotherapy

Ceyda TUNAKAN DALGIÇ[1]



[1] Cumhuriyet Üniversitesi Tıp Fakültesi, Alerji ve Klinik İmmünoloji Bilim Dalı

1. ALLERGEN IMMUNOTHERAPY FOR ALLERGIC DISEASE: THERAPEUTIC MECHANISMS

Introduction

Allergen immunotherapy (AIT) is the only disease-modifying treatment available for several common allergic diseases. Subcutaneous immunotherapy (SCIT) is the best studied form of AIT and is effective for allergic rhinitis and rhinoconjunctivitis, allergic asthma, and Hymenoptera venom allergy. SCIT involves the repeated subcutaneous injection of increasing amounts of allergen beginning with very small doses of allergen and gradually increasing to higher doses. Another popular method involves sublingual administration (SCIT) in the form of dissolvable tablets or extracts. AIT alters the immune system's reaction to causative allergens and induces long-lasting tolerance to these allergens. Multiple cell types in the blood and affected organs show changes and contribute to the development of allergen-specific immune tolerance. The following changes are consistently observed: Decreases in mast cell and basophil activity and degranulation leading to fewer allergic symptoms upon allergen exposure; changes in allergen-specific antibody isotypes. There is an early increase in allergen-specific immunoglobulin (Ig) E levels, which later decreases. There is an early and continuous increase in allergen-specific IgG4 levels; the generation of allergen-specific regulatory T and B cells (Tregs and Bregs) and suppression of allergen-specific effector T cell subsets and innate lymphoid cells; decreases in tissue mast cells and eosinophils, which is accompanied by a decrease in type I skin test reactivity [1,2].

Subcutaneous versus sublingual

SLIT and SCIT induce immune tolerance to allergens through similar mechanisms. Oral mucosa and tonsil immunity are important during SLIT because allergens are mostly captured by tolerogenic dendritic cells before reaching mast cells [1,2].

Clinically evident changes

Altered responses to allergen challenge

Allergic patients who are challenged with an aeroallergen to which they are sensitive often display a biphasic inflammatory response. The biphasic response consists of an early phase of symptoms that develops within minutes of challenge and, a later phase that begins several hours later [2].

Changes in skin test reactivity

Repeat skin testing is not recommended for monitoring the effectiveness of SCIT, because although immunotherapy can inhibit both the immediate and late responses to intradermal allergen skin testing, changes in skin reactivity as assessed with office-based skin testing do not always correlate with improvement in symptoms of allergic rhinitis or asthma [2,3].

Immunologic changes with SCIT

Changes in allergen-specific antibodies

Subcutaneous immunotherapy (SCIT) characteristically results in changes in several types of allergen-specific antibodies; including allergen-specific IgE levels in serum initially increase, then decrease slowly; allergen-specific IgG levels in serum increase and remain elevated, beginning several weeks to months after the changes in IgE; allergen-specific IgG4 levels continuously increase as long as SCIT continues; allergen-specific IgA levels in the serum and secretions increase [3].

IgE

The efficacy of SCIT is not dependent upon simple reductions in levels of allergen-specific IgE, and clinical improvement develops before specific IgE antibody levels decrease. Allergen-specific IgE antibody levels initially increase in most patients and may continue to rise, even after the maximum prescribed dose has been reached. This trend reverses over time in some patients, with levels gradually decreasing within several months after reaching maintenance. Levels may even drop to below pretreatment levels. Allergen-specific IgE levels typically increase after seasonal allergen exposure in individuals not receiving SCIT. This postseasonal spike is reduced or eliminated by SCIT. Although regulatory mechanisms start within days, a significant decrease in IgE occurs over years [3-5].

IgG

Allergen-specific serum IgG antibodies are often present at low levels in allergic patients. Immunotherapy generally results in an increase in allergen-specific IgG antibodies, which lags behind the rise in IgE antibodies by weeks to a few months. Levels may continue to rise over many months of maintenance immunotherapy, and elevated levels may persist for many years after immunotherapy is discontinued [4].

"Blocking" IgG4

Before starting SCIT, allergic patients demonstrate allergen-specific IgG in serum that consists mostly of the isotypes IgG1 or IgG2. With immunotherapy, IgG4 becomes more prominent, a pattern which is called the "modified T helper type 2 (Th2) response." Specific IgG antibodies (both IgG4 and other isotypes) are capable of blocking in vitro mediator releases from allergen-stimulated mast cells and basophils (the early-phase response) and are sometimes referred to as "blocking" antibodies for this reason. IgG4 may be functionally monovalent and therefore more effective as a blocking antibody than other IgG isotypes. A protective role for allergen-specific IgG4 antibodies is also suggested by the finding that beekeepers who develop immunity after multiple stings have high levels of venom-specific IgG4. In contrast, individuals who have been stung only a few times typically have mostly IgG1 antibodies to the venom. Thus, it may be that chronic stimulation with allergen induces a predominantly IgG4 response, while limited allergen exposure results in an IgG1-dominated response. Precisely how IgG4 modifies the allergic response is an area of ongoing research. The two leading theories are: Allergen-specific IgG4 molecules may compete for allergen with IgE bound to mast cells (ie, the blocking antibody theory). IgG4 displays unique structural features of its hinge region that results in a lower affinity for certain Fc receptors, and allergen-specific IgG4 may reduce the sensitivity of antigen-presenting B cells and therefore T cells to an allergen by competing with IgE in a mechanism called "IgE-facilitated antigen (or allergen) presentation". IgE-facilitated antigen presentation refers to the observation that antigen-presenting B cells with the low-affinity IgE receptor CD23 can be activated by low levels of allergen in the presence of allergen-specific IgE [4,5].

B cell changes

There is growing evidence that SCIT induces IgG4-positive regulatory B cells (Bregs) that produce high levels of interleukin-10 (IL-10) and suppress antigen-specific T cell proliferation.

Role of IL-10

Interleukin-10 (IL-10) is the leading cytokine produced by regulatory T cells (Tregs) during interactions with B cells that suppress specific IgE production, and IL-10 also

induces specific IgG4 production. In addition, B cells overexpressing IL-10 potently suppressed production of proinflammatory cytokines by peripheral blood mononuclear cells and suppressed antigen-specific proliferation in vitro [5,6].

T cell changes

During AIT, allergen-specific Treg cells are generated, which produce IL-10 and transforming growth factor-beta (TGF-beta), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), and program cell death protein 1 (PD1). These cytokines suppress proliferative and cytokine responses against major allergens. During AIT, CD4+ T helper (Th) cells shift from producing Th2 (especially IL-4) cytokines following stimulation with the allergen to producing Th1 and Treg cytokines (eg, IFN-gamma, IL-10). Increased production of IL-12, a strong inducer of Th1 responses, contributes to this shift [5-7].

Regulation of innate lymphoid cells

Innate lymphoid cells (ILCs) are lymphocytes that unlike adaptive T and B cells, do not express rearranged antigen-specific receptors. Type 2 ILCs in the lung play a critical role in priming the adaptive type 2 immune response to inhaled allergens, including recruitment of eosinophils and Th2 cytokine production [6,7].

Changes in tissue cellularity

AIT decreases the recruitment of mast cells, basophils, and eosinophils in the skin, nose, eye, and bronchial mucosa after provocation or natural exposure to allergens. It has been proposed that histamine and leukotrienes are released at low subthreshold levels from mast cells and basophils during AIT [7,8].

2. SUBCUTANEOUS IMMUNOTHERAPY FOR ALLERGIC DISEASE: INDICATIONS AND EFFICACY

Indications

SCIT using preparations of aeroallergens may be indicated in the management of the following disorders: Allergic rhinitis, with or without allergic conjunctivitis, including seasonal allergic rhinitis/conjunctivitis, perennial allergic rhinitis/conjunctivitis, both seasonal and perennial allergic rhinitis/conjunctivitis; allergic asthma, seasonal allergic asthma, perennial allergic asthma, both allergic rhinitis and allergic asthma. A patient is a candidate for allergen immunotherapy only if it has been established that there is a clinically important allergic component to his/her disease. The clinician should ensure that the patient has maximized environmental control measures and is on an optimal medication regimen. SCIT is usually recommended for the treatment of allergic respiratory disease only after a period of pharmacologic management and observation [7-9].

It is appropriate to consider a trial of allergen immunotherapy in patients with significant allergic disease, for whom management is suboptimal for any of the following reasons: Persistent symptoms on a seasonal and/or perennial basis, an inadequate or partial response to environmental control and pharmacotherapy, noncompliance with maintenance medication regimen or suboptimal use of medication devices, side effects related to medication use, cost burden associated with chronic medication use [9,10].

The composition of allergen extracts

Variation in the composition of extracts used for immunotherapy has also been a problem historically. Efforts are underway to “standardize” important aeroallergens, which is a process that ensures the composition and potency of specific extracts. The use of standardized allergens should further improve the consistency and quality of allergen immunotherapy [8-10].

Dosing

The dose of allergen administered in immunotherapy is another important variable. It has been established that there is an effective dose range for most allergens, and a coordinated international effort is ongoing to define that range for each of the important aeroallergens, although the process is far from complete [9].

Efficacy

Efficacy in allergic rhinitis

A number of randomized trials have demonstrated that allergen immunotherapy with the following specified is efficacious in allergic rhinitis: birch, mountain cedar, grass, ragweed, and *Parietaria* pollens; cat, dog, *Alternaria* and *Cladosporium* molds; cockroach; and dust mites [10].

Allergic asthma

Studies of specific allergens (short ragweed, mixed grass, *D. pteronyssinus*, *Cladosporium*, cat, or dog) have shown significant benefit in carefully selected patients with allergic asthma. Part of the difficulty in assessing the benefit of immunotherapy in allergic respiratory disease has been the reliance on subjective measures of effect [7-10].

Careful selection of patients

Proper selection of patients is crucial for clinical success. Asthma triggers vary significantly among individuals, and allergen exposure may be just one of several triggers that are important for a given patient. Thus, patients for whom allergen exposure is clearly an important trigger are more likely to experience meaningful benefit. Another important factor may be the duration of allergic disease. New-onset allergic asthma may be more responsive to SCIT than the longstanding disease [9].

Persistence of benefit after discontinuation

We counsel patients that several more years of continued relief is typical after SCIT is discontinued. In the case of relapse, it is possible to start immunotherapy again. Reliable biomarkers for determining which patients will experience lasting benefit from SCIT have not been identified.

Specıal patient groups

Severe or unstable asthma

The administration of SCIT may impart unacceptable risk for patients with severe or unstable asthma because these individuals are at greater risk for systemic allergic reactions involving severe bronchospasm. This includes patients who require chronic oral glucocorticoid therapy or have had severe exacerbations requiring hospitalization or intubation in the previous six months. These high-risk patients can sometimes be managed with anti-IgE therapy first, and once stabilized, they may then be able to undergo SCIT while continuing to receive anti-IgE [5,7].

Pregnancy

SCIT is not usually initiated during pregnancy, although it may be continued in women who were receiving the therapy prior to becoming pregnant.

Beta-blocker and ACE-inhibitor therapy

Patients on chronic beta-blocker treatment for cardiovascular disease are at some risk for a poor outcome if they have an anaphylactic reaction to an immunotherapy injection, since they may not respond properly to epinephrine or other vasopressor agents used to treat the reaction. Patients receiving angiotensin-converting enzyme (ACE) inhibitors are theoretically at increased risk during anaphylaxis, possibly due to the inhibition of conversion of angiotensin I to angiotensin II, resulting in poorer response to hemodynamic stress during hypotension or to accumulated bradykinin, a potent vasodilator [8].

Autoimmune disease

Questions about the safety of administering SCIT to patients with autoimmune disease have been raised, based upon theoretical concern about modulating the immune response in patients who may have aberrant immune regulation. Immunotherapy does not activate complement or anaphylatoxins (C3a and C5a). We suggest the decision to administer SCIT to a patient with concomitant allergic and autoimmune disease be made cooperatively with the patient after careful consideration of the potential risks and benefits for that individual [3-7].

Age of patient

SCIT is appropriate for both adults and children, and there are no defined age limits for its administration.

Adherence

Traditional SCIT requires multiple, regularly scheduled visits to a health care provider. In the United States, it is common practice to administer one or two sets of injections each week, beginning with low doses and gradually increasing to the effective therapeutic range for each allergen. Effective doses are typically reached within three to six months, after which visits are needed every two to four weeks for the remainder of the treatment period. Clinicians or staff should discuss the time commitment required with patients/caregivers in detail when planning to begin SCIT since adherence to the treatment schedule is necessary to obtain maximal benefit. Accelerated schedules of SCIT have been developed, which allow patients to reach effective therapeutic doses within a few weeks or days. Rates of adverse allergic reactions are generally higher with the most accelerated protocols, although many patients tolerate cluster schedules well and prefer the greater convenience [6].

Duration of therapy

There is a consensus that an initial course of immunotherapy should consist of three to five years of maintenance treatment. A minimum of three years has been identified in several studies as an effective initial treatment period and one which provides some lasting benefit after the injections are discontinued [5].

Monitoring

Practice parameters suggest that patients receiving SCIT should be evaluated clinically each year to determine if any adjustments in dose or allergen mix are indicated. Neither repeat skin testing nor laboratory tests provide useful information about how well SCIT is working for a specific patient.

Disorders that are not treated with immunotherapy

Clinical studies do not support the routine use of allergen immunotherapy for food hypersensitivity, chronic urticaria and/or angioedema, latex allergy, or drug allergies.

Clinical indications for allergen immunotherapy

Symptoms of allergic rhinitis, allergic conjunctivitis, allergic asthma, or any combination of these disorders after natural exposure to aeroallergens, demonstrable evidence of clinically relevant specific IgE, and at least one of the following: Poor response to pharmacotherapy, allergen avoidance, or both unacceptable adverse effects of medications, wish to reduce or avoid long-term pharmacotherapy and the cost of medication, possible prevention of asthma in patients with allergic rhinitis [8-10].

Sublingual immunotherapy for allergic rhinoconjunctivitis and asthma

The alternate approach of administering allergens orally, and more specifically with a sublingual methodology in which the allergen is given as either a dissolvable tablet (under the tongue) or as an aqueous or liquid extract.

- **Sublingual immunotherapy tablets (SLIT-tablets)**

Allergen is formulated into a rapidly dissolving tablet that is held under the tongue until completely dissolved. The tablets are self-administered, once daily.

- **Sublingual aqueous or glycerinated liquid allergen extracts (SLIT-drops)**

An aqueous or liquid (eg, glycerinated) extract of allergen, generally administered as drops, is held under the tongue for a specified period of time, and then the residual is swallowed.

Types of allergens

The majority of studies of SLIT have been performed with pollen allergens in patients with allergic rhinitis.

Mechanisms of action

The normal response of the gut immune system to nonpathogenic proteins is tolerance, a fact which forms the basis for the concept of oral immunization. Allergen extracts given sublingually are primarily taken up by dendritic cells in the mucosa and presented to T cells in the draining lymph nodes. Likely mechanisms of action include activation of T regulatory cells and downregulation of mucosal mast cells. Allergenic proteins that reach the small intestine are processed through columnar mucosal cells and presented to T lymphocytes within Peyer's patches. Local tolerance is believed to arise through stimulation of antigen-specific T helper cells to increase IgA production with concomitant suppression of immunoglobulin G (IgG) and immunoglobulin M (IgM) production [9,10].

Immunologic changes following SLIT

The following changes in the humoral responses to allergens are seen with SLIT : Increases in allergen-specific immunoglobulin G4 (IgG4), several studies suggest that IgG4 production is under the control of interleukin-10 (IL-10), blunting of seasonal increases in allergen-specific IgE.SLIT also results in changes in the cellular response to allergens, including : Increases in CD8+ T cells and decreases in the CD4: CD8 T cell ratio, increases in IL-10 production and interleukin-12 (IL-12)/interferon-gamma by peripheral blood monocytes [4-6].

Patient selection

Indications and patient preparation

Each of the SLIT-tablet products is indicated for the treatment of allergic rhinitis (with or without conjunctivitis) induced by the allergen contained in the product.

Contraindications

SLIT-tablets are labeled as contraindicated in patients with severe, unstable, or uncontrolled asthma. Other contraindications include a history of eosinophilic esophagitis, a history of any severe systemic reaction or severe local reaction after taking SLIT, and hypersensitivity to any of the inactive ingredients.

Administration

Labeling of specific products

SLIT-tablet therapy is initiated with a full dose or a short escalation in dose, with the first dose given under medical supervision, and then administration continues once daily and is self-administered by the patient or caregiver at home [4-7].

Duration of therapy

The optimal duration of a course of SLIT has not been defined.

Safety

The major safety benefit with SLIT-tablets is the markedly reduced incidence of anaphylaxis compared with subcutaneous immunotherapy (SCIT). The most common adverse effects of SLIT are oral mucosal itching and swelling.

Oral and pharyngeal adverse effects

The most prominent adverse effects observed with SLIT-tablets are local oral mucosal side effects including oral pruritus (lips, inside of mouth, throat) and ear pruritus, occurring in up to one-quarter of patients.

Eosinophilic esophagitis

A possible association has been reported between eosinophilic esophagitis and aeroallergen SLIT, as well as with oral immunotherapy with food allergens.

Use in pregnancy

Published data addressing the safety of SLIT in pregnancy are lacking.

Comparison of SLIT and SCIT

Advantages and disadvantages of SLIT

There are several potential advantages of SLIT compared with subcutaneous immunotherapy (SCIT): SLIT is safer, with fewer allergic reactions than SCIT; SLIT is more comfortable for patients; SLIT is more convenient for patients and clinicians because therapy is self-administered by the patient (or caregiver) at home. The disadvantages of SLIT include: Benefit is reliant upon consistent patient self-administration [8-10].

3. SCIT ADMINISTRATION, ADVERSE REACTIONS, AND MONITORING

Conventional Schedules

Conventional immunotherapy schedules involve one to three injections per week during a build-up phase that lasts a number of weeks, followed by a maintenance phase, during which injections are given every two to four weeks over a period of years.

Choosing a build-up Schedule

There are faster and slower versions of conventional schedules. The clinician may choose a schedule with a slower (more gradual) build-up for the patient at increased risk for a systemic allergic reaction to the immunotherapy itself. The primary advantage of slower schedules is the reduced risk of systemic allergic reactions. More conservative schedules are appropriate for patients with any of the following characteristics: Multiple large reactions during allergen skin testing, systemic symptoms during skin testing, persistent asthma, a history of systemic allergic reactions to previous immunotherapy. Advantages of faster schedules include patient and clinician convenience and a shorter time to maintenance and clinical improvement [1-3].

Maintenance phase

During the maintenance phase, the interval between injections is usually increased from once weekly up to a maximum of once monthly for inhalant allergens [4,5].

The total duration of therapy

The recommended duration of SCIT is three to five years of maintenance therapy.

Administration of injections

Issues surrounding the administration of SCIT injections include ensuring that the patient is given the correct injection, screening the patient for changes in his/her health that might alter the safety of immunotherapy, and optimal injection techniques [4-6].

Safety precautions at every visit

Personnel responsible for administering immunotherapy injections should be trained to perform the following measures methodically at the beginning of each patient visit to increase safety.

Injection technique

Immunotherapy injections should be given using a calibrated small volume syringe (usually a 1 mL syringe) with a 26- to 27-gauge nonremovable needle (usually three-eighths to one-half inch [0.9 to 1.3 cm] in length). The site should be wiped with an alcohol swab before giving the injection. Injections should be given subcutaneously in the lateral or posterior middle portion of the arm [7-10].

Training and preparation for managing systemic reactions

Personnel and site

Allergen immunotherapy should be administered in a setting where prompt recognition and treatment of a systemic reaction are optimized. The preferred location of administration of allergen immunotherapy is in the office of the allergy expert who prepared the patient's immunotherapy extract. There should be a clinician or clinician extender appropriately trained in emergency treatment immediately available in the medical facility [10].

Adverse reactions to immunotherapy

Local reactions

Injection site reactions consist of redness, pruritus, and swelling. A local reaction can range from a few millimeters in diameter to swelling and erythema that encompasses most of the patient's upper arm. Local reactions can be reduced by premedication with an antihistamine [1-3].

The relationship between local and systemic reactions

Local reactions are not immediate harbingers of systemic reactions and that adjusting immunotherapy doses based on local reactions did not impact the incidence of systemic reactions. There was no increase in systemic reactions [4,5].

Systemic reactions

Systemic allergic reactions to SCIT involve organ systems distant from the injection site. Most serious systemic reactions occur within 30 minutes after the injection. Therefore, a 30-minute waiting period following injection is recommended. These reactions may range in severity from mild rhinitis to fatal cardiopulmonary arrest. Delayed and biphasic systemic reactions are also reported following SCIT injections [6-10].

Following systemic allergic reactions

It is common practice following a systemic reaction to reduce the next injection to the previously tolerated dose and in cases of very severe reactions, to an even lower dose. In the case of very severe reactions, the prescribing clinician should review the course of immunotherapy to determine whether the risk/benefit assessment justifies the continuation of immunotherapy [9,10].

New vials

When new vials of allergen immunotherapy extract are provided, it is customary to reduce the dose by one-third to one-half, even though the same extracts from the same provider are used to compound the new extract and build back up to the dose the patient was previously receiving.

Monitoring

Patients should be re-evaluated at least every 6 to 12 months while they are receiving immunotherapy. Neither laboratory tests nor repeat skin testing is routinely used to monitor a patient's progress during aeroallergen SCIT.

4. ALLERGEN EXTRACTS

An allergen immunotherapy extract is a solution of one or more allergens that are used for immunotherapy.

Types of allergen extracts

Several types of allergen stock solutions are available from commercial sources. The allergen may be in an aqueous solution. A specific allergen may only be available in a limited number of forms. Most pollens are available in either aqueous solutions or 50 percent glycerin solutions. Glycerin is a stabilizing and bacteriostatic agent, but it can cause pain at the injection site in a dose-dependent manner. The standardized extracts of house dust mites, cat dander, and grasses are only available in 50 percent glycerin. Some pollens are also available in an alum-precipitated formulation. Fungal (mold), fire ant, and cockroach extracts are often available in aqueous or 50 percent glycerin solutions. Dog

extracts exist in aqueous and glycerinated forms, although only the acetone-precipitated (AP dog) product has been shown to be sufficiently potent. The only lyophilized products are the Hymenoptera venoms [8-10].

Standardized

Standardized extracts are preferred over nonstandardized extracts, although standardized extracts are not available for all allergens. Standardization refers to the way in which an allergen extract is produced and takes into account multiple factors, such as selection and collection of raw materials, extract preparation, storage, validation of assays, and reagents for batch-to-batch control. Standardized extracts offer the advantage of consistent potency, although a certain degree of variability is allowed. Despite these limitations, standardized extracts are preferred over nonstandardized extracts [6-8].

Storage

Once an allergen immunotherapy extract has been formulated, it should be stored at all times when not in use at 4° C. Storage should be in a dedicated refrigerator and not the one used for food or specimens.

Transport of extracts

Allergen extracts that have been made for a specific patient can be transported by the patient with relative ease. Extracts can be kept at ambient temperature for brief periods of one to two days, and vials can be mailed without refrigeration as long as they are not exposed to extreme temperatures or conditions.

5. HYMENOPTERA VENOM IMMUNOTHERAPY: EFFICACY, INDICATIONS, AND MECHANISM OF ACTION

Types of reactions to Hymenoptera stings

There are three common types of allergic reactions to Hymenoptera sting: anaphylactic reactions, cutaneous systemic reactions, and large local reactions. An anaphylactic reaction involves signs and symptoms of immunoglobulin E (IgE)-mediated allergy, typically affecting more than one organ system. A cutaneous systemic reaction (or a generalized cutaneous reaction) consists of signs and symptoms limited to the skin (ie, pruritus, erythema, urticaria, and/or angioedema), which is usually widespread and involves skin that is not contiguous with the sting site. A large local reaction consists of painful swelling and erythema limited to skin and subcutaneous tissues contiguous with the sting site. The affected area is typical>10 cm and may be much larger [6-8].

Effectiveness of VIT

VIT is the most effective form of immunotherapy in use. Protection from recurrent anaphylactic reactions appears to be established in most patients within one week of reaching maintenance doses. In addition to reducing the risk of recurrent systemic reactions, VIT improves the quality of life by reducing anxiety and allowing patients to participate in outdoor activities as they desire [9,10].

Reduced risk of recurrent reactions

An adult's risk of a recurrent systemic reaction is approximately 30 to 60 percent in the absence of VIT. After completing a course of VIT, the risk is reduced to 5 percent or lower. The few patients who do have recurrent anaphylactic reactions tend to have much milder symptoms than their pretreatment sting reaction.

Honey bee versus vespid VIT

Honey bee stings cause higher rates of recurrent anaphylactic reactions compared with strings of vespids (ie, yellow jackets, hornets, and wasps). Unfortunately, honey bee VIT is less effective in preventing future anaphylactic reactions than vespid VIT and the protection provided by honey bee VIT does not last as long as that from vespid VIT.

Indications and patient selection

Patients with past anaphylactic reactions

An individual is a candidate for VIT if **both** of the following are true: There is a reliable history of an anaphylactic reaction to an insect sting and the patient has either a positive venom skin test **or** elevated serum levels of venom-specific immunoglobulin E (IgE).

Note that a positive venom skin test or the presence of venom-specific IgE in the **absence** of a history of a sting-induced anaphylactic reaction is **not** an indication for therapy since approximately one-quarter of the general population has demonstrable venom-specific IgE [7-9].

Children

Children with moderate or severe anaphylactic reactions to a Hymenoptera sting have an increased risk of anaphylactic reactions during adulthood if not treated with VIT. Therefore, these children should be treated with VIT [5,7].

Adults

Adults with past anaphylactic reactions to stings and positive skin tests have a 30 to 60 percent chance of a similar reaction to a future sting. The decision to pursue VIT should be a shared decision between the clinician and the patient. The risk is relatively greater for those with the following characteristics: Severe past reactions, allergy to honey bee venom rather than vespid (ie, yellow jacket, hornet, and wasp) venom, elevated baseline tryptase. VIT should be firmly advocated for individuals who had life-threatening anaphylaxis, and these patients should be advised that insect avoidance/access to epinephrine is not considered an adequate alternative to VIT [4,8].

Special populations

Pregnant women

During pregnancy, VIT is not initiated nor are doses increased, which is the practice with other forms of injection immunotherapy. However, women who are already receiving VIT when they become pregnant may continue treatment.

Patients with known or possible mast cell disorders

In patients with mastocytosis, sting-induced anaphylaxis is more common and more likely to be life-threatening compared with patients without mast cell disorders. VIT is effective in this group and is felt to be sufficiently safe in that the benefits outweigh the risks for most patients. Serum tryptase is an easily obtained screening test for mast cell disorders and the evaluation of patients with sting-induced anaphylaxis [3,5].

Children

Children with past reactions limited to the skin are at low risk of developing an anaphylactic reaction to subsequent stings, even if VIT is not administered. Thus, although VIT is effective in children with cutaneous systemic reactions, it is not necessary.

Adults

There may be situations in which VIT is still appropriate for adults with cutaneous

systemic reactions. Specifically, if the individual has underlying medical conditions or medications that could affect the outcome of an anaphylactic reaction, frequent unavoidable exposure to Hymenoptera, or impaired quality of life, then it is appropriate to proceed with VIT.

Patients with large local reactions

Individuals with large local reactions are not usually candidates for VIT, because the risk of anaphylaxis to future stings is low [1].

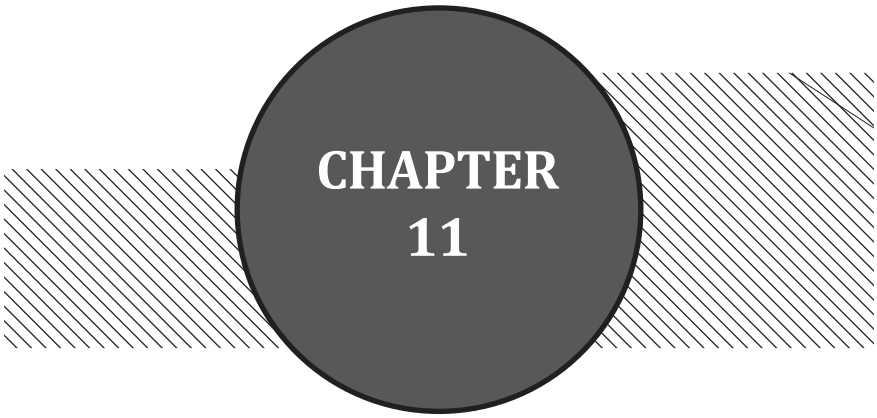
REFERENCES

1. International Consensus on Allergen Immunotherapy II: Mechanisms, standardization, and pharmacoeconomics. Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, Cox L, Demoly P, Frew AJ, O'Hehir R, Kleine-Tebbe J, Muraro A, Lack G, Larenas D, Levin M, Martin BL, Nelson H, Pawankar R, Pfaar O, van Ree R, Sampson H, Sublett JL, Sugita K, Du Toit G, Werfel T, Gerth van Wijk R, Zhang L, Akdis M, Akdis CA. J Allergy Clin Immunol. 2016 Feb;137(2):358-68. doi: 10.1016/j.jaci.2015.12.1300. Review.
2. International consensus on allergy immunotherapy.
3. Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, Cox L, Demoly P, Frew AJ, O'Hehir R, Kleine-Tebbe J, Muraro A, Lack G, Larenas D, Levin M, Nelson H, Pawankar R, Pfaar O, van Ree R, Sampson H, Santos AF, Du Toit G, Werfel T, Gerth van Wijk R, Zhang L, Akdis CA.
4. J Allergy Clin Immunol. 2015 Sep;136(3):556-68. doi: 10.1016/j.jaci.2015.04.047. Epub 2015 Jul 7. Review.
5. Allergen immunotherapy for allergic asthma: A systematic review and meta-analysis.
6. Dhami S, Kakourou A, Asamoah F, Agache I, Lau S, Jutel M, Muraro A, Roberts G, Akdis CA, Bonini M, Cavkaytar O, Flood B, Gajdanowicz P, Izuhara K, Kalayci Ö, Mosges R, Palomares O, Pfaar O, Smolinska S, Sokolowska M, Asaria M, Netuveli G, Zaman H, Akhlaq A, Sheikh A.
7. Allergy. 2017 Dec;72(12):1825-1848. doi: 10.1111/all.13208. Epub 2017 Jul 6. Review.
8. Allergen immunotherapy. Mannan S. Immunotherapy. 2017 Nov;9(15):1199-1200. doi: 10.2217/imt-2017-0157.
9. Advances and highlights in allergen immunotherapy: On the way to sustained clinical and immunologic tolerance. Berings M, Karaaslan C, Altunbulakli C, Gevaert P, Akdis M, Bachert C, Akdis CA. J Allergy Clin Immunol. 2017 Nov;140(5):1250-1267. doi: 10.1016/j.jaci.2017.08.025. Epub 2017 Sep 20. Review.
10. Novel approaches and perspectives in allergen immunotherapy. Hoffmann HJ, Valovirta E, Pfaar O, Moingeon P, Schmid JM, Skaarup SH, Cardell LO, Simonsen K, Larché M, Durham SR, Sørensen P. Allergy. 2017 Jul;72(7):1022-1034. doi: 10.1111/all.13135. Epub 2017 Mar 20. Review.
11. Allergen Immunotherapy: History and Future Developments. Passalacqua G, Canonica GW. Immunol Allergy Clin North Am. 2016 Feb;36(1):1-12. doi:10.1016/j.iac.2015.08.001. Epub 2015 Oct 21. Review.
12. Allergen immunotherapy now and in the future. Nelson HS. Allergy Asthma Proc. 2016 Jul;37(4):268-72. doi: 10.2500/aap.2016.37.3966. Review.
13. An update on allergen immunotherapy. Arshad SH. Clin Med (Lond). 2016 Dec;16(6):584-587.
14. Applications and mechanisms of immunotherapy in allergic rhinitis and asthma. Kappen JH, Durham SR, Veen HI, Shamji MH. Ther Adv Respir Dis. 2017 Jan;11(1):73-86. doi: 10.1177/1753465816669662. Epub 2016 Sep 27. Review.

The Dark Side Of Drug Addiction In Women

Hilal ÖZBEK[1]

Şükran ERTEKİN PINAR[2]



[1] M.Sc. Midewife, Sivas Numune Hospital, Sivas

[2] Asst. Prof. Dr. Cumhuriyet University Faculty of Health Sciences, Midwifery Department, Sivas

INTRODUCTION

Substance abuse is defined as the unauthorized use of a psychoactive substance outside the scope of their medical indication (Kaya & Şahin, 2013; Koçak, Hotun Şahin, & Büyükkayacı Duman, 2015; Reid, 2016). Although addiction cause harm to psychological, physical health and social life of the individual, it is a disease that is defined as "an indispensable desire to repeat a certain behavior" that causes physical and mental symptoms when cannot be reached as a result of the tolerance to the substance by its use outside the scope (Asi Karakaş & Ersöğütçü, 2016; Koçak et al., 2015; Reid, 2016; Toker Uğurlu, Ceyhan Balcı, & Şengül, 2012). The self-control of the individual is affected by substance use and thus individual's original behaviors change and this leads to the creation of new attitudes and behaviors in the individual. In the long-term use of the substance, drug addiction problem arises (Özen Bekar, 2014). The purpose of this section is to emphasize the drug addiction problem in women.

History of Drug Addiction

The use of these psychoactive substances in almost all societies and cultures is as old as human history (Toker Uğurlu et al., 2012; Koçak et al., 2015). While eating the plants in their environment, the first people discovered that some of them had medical effects, and some of them made them feel different (Kaya & Şahin, 2013). Cuneiform scripts were found showing that Sumerians living in Mesopotamia in 4000 B.C had grown poppy and hemp and used these plants as syrup and powder. There is detailed information about poppy cultivation, opium production and medicines made in Egypt in 2000 BC. (Ögel, 1997; Devlet Denetleme Kurulu, 2014; Latuskie et al., 2018). Indians named the hashish as "vişema" which means success and happiness. In the sacred book of Persians called Zoroaster, it is stated that the cannabis plant gives happiness and joy by removing human grief and sorrow. Famous historian Herodotus wrote about the Assyrians and Sumerians living in Mesopotamia and the Scythians living in the Aral region that they got drunk and cheered up by breathing smoke from a cannabis-like plant (Uzbay, 2009; Kennedy, 2014). As seen in many civilization, many plants were grown for the purposes of treatment and entertainment (Kennedy, 2014).

When scientists began to study addictive behavior in the 1930s, they thought drug addicts were morally flawed and lacking the willpower. As a result of these views, it was considered as a moral failure rather than a health problem while shaping the society's point of view towards drug use and punishment was emphasized instead of prevention and treatment (Volkow, 2018). Illicit drug use has emerged widely among Turkish and European youth in the 1960s and there have been dramatic changes in the use of almost all drugs since then (Güvendeğer Doksat et al., 2016). It is now accepted that drug addiction is a medical disease that affects the brain and changes its structure. As a result of these studies, many biological and environmental risk factors have been determined to contribute to the development and progression of addiction, and effective prevention and treatment approaches have been started to be developed to reduce substance use among individuals, families and communities (Volkow, 2018). In addition, substance use represents family structure, socioeconomic level, gender, age, education level and the difficulties in emotional, social and academic development of the individual (Elmanna, Albaggar, & Taha, 2016; İzci & Bilici, 2015).

Drug Addiction in Turkey and in the World By Statistics

Drug addiction is an important public health problem in the world as well as in Turkey (Kaya & Şahin, 2013; Genç & Mihmanlı, 2014; Avşar, Koç, & Aslan, 2016). Drug addiction does not only affect the individual, but also affects every part of the society. It is also a

multidimensional biopsychosocial problem that causes too much damage to the individual and to the society, such as traffic accidents, suicide, crime, family fragmentation, business life deterioration, occupational losses and other economic problems (Özen Bekar, 2014; Avşar et al., 2016). Therefore, it is necessary to focus on social protection rather than individual treatment (Asi Karakaş & Ersöğütçü, 2016).

In 2016, the number of people who used drugs at least once was approximately 275 million and accounted for about 5.6% of the global population between the ages of 15-64. Addiction of about 31 million people who use drugs is at a point where they may need treatment (UNODC, 2018a, 2018b). The highest substance use rate is in Eastern Europe and the United States with 5-6% of the population. This shows that one out of every 20 people in these countries is addicted to drugs (Ritchie & Roser, 2018).

It is estimated that more than 92 million adults or more than a quarter of those aged 15-64 in the European Union have tried illegal substances at some point in their lives. Substance use is reported to be more common among men (56.0 million) than women (36.3 million) (EMCDDA, 2018).

According to the World Health Organization, approximately 450.000 people died in 2015 as a result of substance use. 167.750 of these deaths were directly related to substance use disorders (mostly overdoses) (UNODC, 2018a, 2018b). While 129.978 million people lost their lives in 2010, this rate increased to 143.775 million in 2016 (Ritchie & Roser, 2018). In Turkey, 326 people lost their lives due to drug addiction in 2010. This rate increased to 449 in 2016 (Ritchie & Roser, 2018).

When we look at gender differences in substance use disorders, it is seen that the prevalence in men is higher than women. According to the world data in 2010, while 48.8 million of 153.69 million alcohol and substance users were women, this number was 104.89 million for men. In 2016, this rate increased to 162.8 million, to 52.05 million for women and 110.75 million for men (Ritchie & Roser, 2018). The experience of substance use in the European Union in women (36.3 million) is less than men (56.0 million) (EMCDDA, 2018).

When we look at the drug use rate in Turkey, we see that while 604.643.69 people were addicted to drugs in 2010, this rate was 664.906.55 in 2016 (Ritchie & Roser, 2018). According to 2018 Turkey National Drug Report (TUBİM), the number of applications made only to outpatient treatment centers was 211.126 in 2017. The number of inpatient treatment for treatment centers in 2017 was 12.501. 48.55% of those receiving treatment in 2017 stated that they were receiving treatment for the first time, while 51.45% had been previously treated (TUBİM, 2018).

Drug addiction prevalence in Turkey is higher in men than women as it is in the worldwide prevalence. Again, when we look at the TUBİM 2018 report, 95.67% of the inpatients were male and 4.33% were female (TUBİM, 2018).

Drug Addiction and Women

The statistics on substance use show us the dark side of the addiction that does not appear very clearly. Substance use of women and its results are often ignored because of the false belief that supports less women become addicted than men. However, one of the important factors to remember is that the dark side of drug addiction in women can affect society more. In recent years, that the prevalence of substance use rates of women and men have become very close in the world is noteworthy in terms of women's health (Kutlu, 2011; Koçak et al., 2015).

In the literature, one of the reasons for the differences in the prevalence of drug use among men and women is the lack of equal access to drugs (social and cultural norms). It is stated that there would be no difference between men and women in terms of substance

use if they had equal Access to drugs (Lal, Deb, & Kedia, 2015; McHugh, Votaw, Sugarman, & Greenfield, 2018; UNODC, 2018c).

Scientists examining substance use have stated that women who have drug addiction may experience problems related to hormones, menstrual cycle, amenorrhea, fertility, pregnancy, breastfeeding and menopause (Lal et al., 2015; NIDA, 2018a). It is reported that women often use drugs because of weight control, combating fatigue, coping with pain, and self-treatment of mental health problems (NIDA, 2018a).

Adolescent women and substance use; The substance, which is seen as a method of coping in adolescence and young adulthood periods, affects the decision to use substance and the development of addiction (Yüncü et al., 2014). Most studies show that early (12-14 years) and late (15-17 years) adolescence are a critical risk period for the onset of substance use. However, it is reported that substance use may peak in young people between the ages of 18-25 (UNODC, 2018a). Adolescent girls and boys have different developmental and social problems (NIDA, 2014). In adolescence, girls are vulnerable to depression and anxiety, which may be a risk factor for substance use (Schwinn, Schinke, Hopkins, & Thom, 2016). The reasons for starting substance use among adolescent girls are usually adolescent depression, adjustment problems, mood disorders, having an older male partner, peer pressure, feelings of magic and power, exposure to physical or sexual abuse (NIDA, 2014; Lal et al., 2015). Substance use also causes other risky behaviors such as traffic accidents, suicides, violence, running away from the house, unwanted pregnancies at an early age, unprotected sexual intercourse and insecure sexual intercourse, especially among adolescent girls (Akkuş, 2010; Arabacı, Taş, & Dikeç, 2017; Kuhn, 2015). Young people using drugs can fail at school, be absent from school or leave school (Volkow, 2018).

In the literature, although the number of adolescent boys using drugs is higher than the girls, it is stated that this difference has begun to decrease (Mason et al., 2015). In the late adolescent period, substance use is higher in boys than in girls (Johnston et al., 2018). However, the difference in prevalence related to the socio-demographic characteristics decrease in the 12th grade, the use of synthetic cannabinoids was 10.3% among boys and 6.4% among girls, and in the 8th grade, there were minimal differences between boys and girls in the prevalence of cannabis (Patrick et al., 2016). When it is considered that the adolescents will be the parents of the future, it is an important issue to prevent the increasing rate and to get rid of the substance use.

Pregnant women and substance use; In the long term, substance use during pregnancy may pose a risky situation for both the health of the woman and the newborn (Latuskie et al., 2018; NIDA, 2018b). Infants who are exposed to drugs while they are still in the mother's womb are born below preterm or normal birth weight. These problems may disrupt behaviors, the ability to learn and perception of newborns in their later lives (NIDA, 2018a). These substances are found at high levels in fetus blood after the maternal substances reach the fetus through the placenta during pregnancy. These substances may make the infant dependent on opioids or other drugs with neonatal deprivation syndrome in the postnatal period (NIDA, 2018a; Volkow, 2018). The fact that the woman is both an adolescent and a drug addict can cause many complications (Oğuz et al., 2016). Studies have shown that the risk of perinatal mortality, preterm labor and low birth weight infant increases in heroin-dependent mothers. It is also reported that the use of prescription painkillers with tobacco or cannabis, or the use of drugs during pregnancy increases the risk of stillbirth by two or three times (Can, Bülbül, Uslu, Güran, & Nuhoğlu, 2010; NIDA, 2018a).

Fear, guilt and embarrassment prevent many women from disclosing drug use during pregnancy. The use of substance during pregnancy is known to have negative effects

on both mother and fetus, and may often be subject to stigmatization due to the use of pregnant women (Wendell, 2013). There are studies in the literature showing that many health professionals have stereotypical views and negative attitudes towards women using drugs (Adams, 2008; Miles, Francis, & Chapman, 2010). The antenatal care of pregnant women who use addictive substances is also insufficient compared to other pregnants (Genç & Mihmanlı, 2014). For this reason, pregnant women with drug addiction should be treated more sensitively and the effects on mother and fetus should be minimized.

Women exposed to violence and substance use; Violence among women who use drugs is higher and there is a strong correlation between the incidence of post-traumatic stress disorder, depression and suicidal behavior among women with experience of violence (Trevillion, Feder, & Howard, 2011; Bailey, Trevillion, & Gilchrist, 2018; UNODC, 2018c).

Studies show that women using drugs are two to five times more likely to have sex-based violence than those who do not (NatCen, 2013; UNODC, 2018c). Women can use drugs to self-treat after abuse and to cope with the emotional and physical pain they experience for violence (Simonelli, Pasquali, & De Palo, 2014; UNODC, 2018c). Therefore, it is necessary to be more careful about drug addiction in women who are exposed to violence.

Midwifery and Substance Use

The health, social and support services that drug addict women, and especially new mothers need when they use illegal drugs are composed of many components. It covers a wide range of services, including rehabilitation, which often go beyond the limits of medical treatment (Economidoy, Klimi, & Vivilaki, 2012; Polat, 2014). In this context, multidisciplinary cooperation and coordination are important to include women in the treatment and provide preventive services. Health care institutions and health professionals are critical in providing the necessary care for both mother and baby, and in helping women (Economidoy et al., 2012). Especially women in the adolescent period should be considered primarily because they are in the risk group (Kaya & Şahin, 2013).

In drug addiction, woman's stop using the substance completely should be the main target of the treatment. Two main objectives in this process should be (i) the avoidance of the addicted individual from the substance, (ii) ensuring physical, psychiatric and psychosocial well-being (Ögel, 1997; Kaya & Şahin, 2013; Koçak et al., 2015).

In England, where the quality of midwifery is strong, there are special midwives for women who are addicted to substances such as cigarettes, alcohol and heroin, and women and pregnant women who use these substances constitute the primary target group of midwives in education (Toker & Aktaş, 2010). The application is developed within professional, ethical and legal frameworks. Midwives are responsible for providing women-centered care in midwifery practices and for developing appropriate life skills (Miles et al., 2010). The midwife, specialized in drug addiction, works with the mental health team and provides support to pregnant women who use these substances before pregnancy, during the entire pregnancy and during the 6 months after the baby is born (Toker & Aktaş, 2010). There are not specialized team of midwives about drug addiction in Turkey yet.

CONCLUSION

Drug addiction problem has gradually increased among women and has taken on a worse dimension. For this reason, strong policies should be developed especially for women related to drug addiction and midwives should be included in this system. Midwives should raise awareness of women in their fight against drug addiction in

particular and raise awareness of society about drug addiction in general. In this context, midwives, who have key roles in women's health, should use their prophylactic, health promoting and curative roles more effectively in terms of drug addiction.

REFERENCES

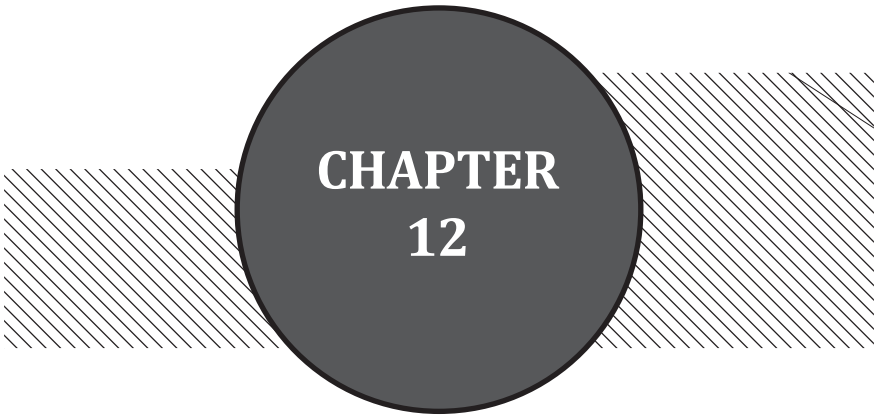
1. Adams, M. W. (2008). Comorbidity of mental health and substance misuse problems: a review of workers' reported attitudes and perceptions. *Journal of Psychiatric and Mental Health Nursing*, *15*(2), 101–108.
2. Akkuş, D. (2010). Ergende Esrar Kullanımı: Toplum Ruh Sağlığı Yaklaşımı (Olgu Sunumu). *Psikiyatri Hemşireliği Dergisi*, *1*(1), 43–46.
3. Arabacı, L. B., Taş, G., & Dikeç, G. (2017). Çocuk ve Ergenlerde Madde Kullanımı, Suça Yönelme, Ruhsal Bozukluklar ve Hemşirelik Bakımı. *Bağımlılık Dergisi*, *18*(4), 135–144.
4. Asi Karakaş, S., & Ersöğütçü, F. (2016). Madde Bağımlılığı ve Hemşirelik. *HSP* , *3*(2), 133–139.
5. Avşar, G., Koç, F., & Aslan, G. (2016). Sosyal Destek ve Benlik Saygısı. *ACU Sağlık Bil Dergisi*, *2016*(1), 44–49.
6. Bailey, K., Trevillion, K., & Gilchrist, G. (2018). What works for whom and why: A narrative systematic review of interventions for reducing post-traumatic stress disorder and problematic substance use among women with experiences of interpersonal violence. *Journal of Substance Abuse Treatment*.
7. Can, E., Bülbül, A., Uslu, S., Güran, Ö., & Nuhoğlu, A. (2010). Neonatal yoksunluk sendromu. *Şişli Etfal Hastanesi Tıp Bülteni, 44*(3), 124–127.
8. Devlet Denetleme Kurulu. (2014). *Madde ve Diğer Bağımlılıklar ile Mücadele Kapasitesinin ve Bu Bağlamda Türkiye Yeşilay Cemiyetinin Değerlendirilmesi*. *Hizmete Özel Araştırma ve İnceleme Raporu*.
9. Economidoy, E., Klimi, A., & Vivilaki, V. G. (2012). Caring for substance abuse pregnant women: The role of the midwife. *Health Science Journal*, *6*(1), 161–169.
10. Elmanna, A. H., Albaggar, A. K. A., & Taha, I. M. T. (2016). Knowledge , Attitudes and Practices of Rakshes Drivers Towards Drugs Abuse Knowledge , Attitudes and Practices of Rakshes Drivers Towards Drugs Abuse in Kosti-Sudan-2015. *American Journal of Environmental and Occupational Health*, *1*(1), 15–19.
11. EMCDDA. (2018). *Avrupa Uyuşturucu Raporu Eğilimler ve Gelişmeler 2018*. Lüksemburg.
12. Genç, S., & Mihmanlı, V. (2014). Madde Bağımlılığı ve Gebelik. *Okmeydanı Tıp Dergisi*, *30*(Ek Sayı 2), 120–123.
13. Güvendeğer Doksat, N., Demirci Çiftci, A., Zahmacioğlu, O., Tekden, M., Özbek, F., Günay, G., … Erdoğan, A. (2016). Trends and gender differences in alcohol and substance use among children and adolescents admitted to an addiction treatment center in Turkey: comparison of 2014 with 2011. *Anadolu Psikiyatri Dergisi*, *17*(4), 325–331.
14. İzci, F., & Bilici, R. (2015). Gebelerde Madde Kullanımı: Görülme Sıklığı ve Etkileri. *Bağımlılık Dergisi*, *16*(1), 26–34.
15. Johnston, L. D., Miech, R. A., O'malley, P. M., Bachman, J. G., Schulenberg, J. E., & Patrick, M. E. (2018). *2017 Overview Key Findings on Adolescent Drug Use*. *Monıtorıng Future Natıonal Survey Results On Drug Use 1975–2017*. Michigan. Erişim Tarihi: 15.01.2019 https://deepblue.lib.umich.edu/bitstream/handle/2027.42/142406/Overview 2017 FINAL.pdf?sequence=1&isAllowed=y1

16. Kaya, Y., & Şahin, N. (2013). Kadınlarda Madde Kullanımı ve Hemşirenin Rolü Substance. *Hemşirelikte Eğitim ve Araştırma Dergisi*, *10*(1), 3–7.
17. Kennedy, D. O. (2014). *Plants and the Human Brain*. Newyork: Oxford University Press. Erişim Tarihi: 16.01.2019 https://books.google.com.tr/books?id=YUNDAgAAQBAJ&lpg=PP1&hl=tr&pg=PR4&redir_esc=y#v=onepage&q&f=false
18. Koçak, D. Y., Hotun Şahin, N., & Büyükkayacı Duman, N. (2015). Alkol ve Sigara Bağımlılığı, Kadın Sağlığına Etkileri ve Hemşirelik Girişimleri. *Sempozum*, *1*(5), 43–47.
19. Kuhn, C. (2015). Emergence of sex differences in the development of substance use and abuse during adolescence. *Pharmacology & Therapeutics*, *153*, 55–78.
20. Kutlu, Y. (2011). Kadının Madde Kullanımı ve Bağımlılığı. *Psikiyatri Hemşireliği Dergisi*, *2*(2), 90–93.
21. Lal, R., Deb, K., & Kedia, S. (2015). Substance use in women: Current status and future directions. *Indian Journal of Psychiatry*, *57*(6), 275.
22. Latuskie, K. A., Leibson, T., Andrews, N. C. Z., Motz, M., Pepler, D. J., & Ito, S. (2018). Substance Use in Pregnancy Among Vulnerable Women Seeking Addiction and Parenting Support. *International Journal of Mental Health and Addiction*, 1–14.
23. Mason, M., Mennis, J., Way, T., Light, J., Rusby, J., Westling, E., … McHenry, C. (2015). Young adolescents' perceived activity space risk, peer networks, and substance use. *Health & Place*, *34*, 143–149.
24. McHugh, R. K., Votaw, V. R., Sugarman, D. E., & Greenfield, S. F. (2018). Sex and gender differences in substance use disorders. *Clinical Psychology Review*, *66*, 12–23.
25. Miles, M., Francis, K., & Chapman, Y. (2010). Challenges for midwives: pregnant women and illicit drug use. *Australian journal of advanced nursing*, *28*(1), 83–90.
26. NatCen. (2013). *Violence, abuse and mental health in England*. *Technical report*. England. Erişim Tarihi: 15.01.2019 http://www.natcen.ac.uk/media/1058443/REVA-Strand-1-technical-report.pdf
27. NIDA. (2014). Principles of adolescent substance use disorder treatment: A research-based guide. *National Institutes of Health*, 1–42.
28. NIDA. (2018a). *Substance Use in Women*. Erişim Tarihi:13.09.2019, https://www.drugabuse.gov/publications/research-reports/substance-use-in-women
29. NIDA. (2018b). *Substance Use in Women Sex and Gender Differences in Substance Use*. Erişim Tarihi:13.09.2019, https://www.drugabuse.gov/publications/drugfacts/substance-use-in-women
30. Ögel, K. (1997). *Uyuşturucu Maddeler ve Bağımlılık* (1. Baskı). İstanbul: Sena Ofset. Erişim Tarihi:13.09.2019, http://www.ogelk.net/Dosyadepo/tarihce_kogel.pdf
31. Oğuz, M. M., Acar, M., Polat, E., Akçaboy, M., Tuygun, N., Açoğlu, E. A., … Şahin Dağlı, F. (2016). Madde bağımlısı adolesan anne ve bebeği, (59), 68–71.
32. Özen Bekar, E. (2014). Hemşirelerde Madde Kullanımı ve Hemşirelik Hizmetleri. *Sağlık ve Hemşirelik Yönetimi Dergisi*, *1*(1), 43–47.
33. Patrick, M. E., O'Malley, P. M., Kloska, D. D., Schulenberg, J. E., Johnston, L. D., Miech, R. A., & Bachman, J. G. (2016). Novel psychoactive substance use by US adolescents: Characteristics associated with use of synthetic cannabinoids and synthetic cathinones. *Drug and Alcohol Review*, *35*(5), 586–590.
34. Polat, G. (2014). Madde Bağımlılığı Tedavisinde Sosyal Hizmet Mesleği. *Okmeydanı Tıp Dergisi*, *30*(Ek Sayı), 143–148.

35. Reid, A. G. (2016). Substance use disorders. *Medicine*, *44*(12), 701–705.
36. Ritchie, H., & Roser, M. (2018). Substance Use. Erişim Tarihi: 15.01.2019, https://ourworldindata.org/substance-use
37. Schwinn, T. M., Schinke, S. P., Hopkins, J., & Thom, B. (2016). Risk and protective factors associated with adolescent girls' substance use: Data from a nationwide Facebook sample. *Substance Abuse*, *37*(4), 564–570.
38. Simonelli, A., Pasquali, C. E., & De Palo, F. (2014). Intimate partner violence and drug-addicted women: from explicative models to gender-oriented treatments. *European Journal of Psychotraumatology* , *5*.
39. Toker, E., & Aktaş, S. (2010). İngiltere' de Ebelik. *Maltepe Üniversitesi Hemşirelik Bilim ve Sanatı Dergisi*,*2*(3), 89–96.
40. Toker Uğurlu, T., Ceyhan Balcı, Ş., & Şengül, C. (2012). Bağımlılık Psikofarmakolojisi. *Psikiyatride Güncel Yaklaşımlar-Current Approaches in Psychiatry*,*4*(1), 37–50.
41. Trevillion, K., Feder, G., & Howard, L. M. (2011). Experiences of Domestic Violence and Mental Disorders: A Systematic Review and Meta-Analysis. *PLOS ONE*, *7*(12), 1–12.
42. TUBİM. (2018). *Türkiye Uyuşturucu Raporu - 2018*. Ankara. Erişim Tarihi:13.09.2019, http://www.narkotik.pol.tr/Duyurular/Documents/2018 TURKIYE UYUSTURUCU RAPORU.pdf
43. UNODC. (2018a). *Executive Summary Conclusions And Policy Implications-1*. *World Drug Report 2018*. Vienna: United Nations Office on Drugs and Crime. Erişim Tarihi:13.09.2019, https://www.unodc.org/wdr2018
44. UNODC. (2018b). *Global Overvıew Of Drug Demand And Supply, Latest trends, cross-cutting issues-2*. *World Drug Report 2018*. Vienna. Erişim Tarihi:15.09.2019, https://www.unodc.org/wdr2018
45. UNODC. (2018c). *Women and Drugs-Drug use, drug supply and their consequences 5*. *World Drug Report 2018*. United Nations: United Nations publication. Erişim Tarihi:15.09.2019, https://www.unodc.org/wdr2018
46. Uzbay, T. (2009). Madde Bağımlılığının Tarihçesi, Tanımı, Genel Bilgiler ve Bağımlılık Yapan Maddeler. *Meslek İçi Sürekli Eğitim Dergisi*, (21–22), 5–15.
47. Volkow, N. D. (2018). Drugs, Brains, and Behavior: The Science of Addiction. *Drug Misuse and Addiction*, 2–29. Erişim Tarihi:13.09.2019,https://d14rmgtrwzf5a.cloudfront.net/sites/default/files/soa.pdf
48. Wendell, A. (2013). Overview and epidemiology of substance abuse in pregnancy. *Clinical Obstetrics and Gynecology*, *56*(1), 91–96.
49. Yüncü, Z., Saatçıoğlu, H., Aydın, C., Özbaran, B. N., Altıntoprak, E., & Köse, S. (2014). Bir Şehir Efsanesi: Madde Kullanmaya Başlama Yaşı Düşüyor mu? *Sempozyum*, *1*(4), 43–50.

The Place And Importance Of Music Therapy In Schizophrenia Disorders: An Alternative Method

Şükran ERTEKİN PINAR[1]



[1] Asst. Prof. Dr. Cumhuriyet University Faculty of Health Sciences, Midwifery Department, Sivas

INTRODUCTION

Music is an instrument that enables people to express themselves by reflecting their feelings and thoughts. Individuals express their sorrow, joy, heroism, excitement, longing, love, sadness, and in short, all their feelings and thoughts about life by using the art of music. The positive effect of music on people has been used as an option in treatment. In fact, music does not have direct therapeutic properties. Music is therapeutic when used for individuals who are anxious, stressful, need help and cannot express themselves. Music exists in every aspect of life, in every age and culture, from birth to death. As a result of music therapy, it is aimed to reduce stress, pain, anxiety and isolation, to create positive mood, to improve quality of life, to help individuals to cope with their problems and to provide a positive environment. For this reason, music has been used as an alternative method in many diseases and application areas since the first ages. One of these areas is mental health and diseases. This section focuses on music therapy and the role and importance of music in schizophrenia disorders.

Music and Music Therapy

Music word derives from the word "musica" and passes from Greek as "mousa" (Muse). In Turkish, the word "Musiki" is mostly used (Güner, 2007). American Music Therapy Association (AMTA) (1980) described music therapy as "the clinical and evidence-based use of music interventions to accomplish individualized goals within a therapeutic relationship by a credentialed professional who has completed an approved music therapy program". World Federation of Music Therapy (WFMT) (1985) described music therapy as "the use of music and/or musical elements (sound, rhythm, melody and harmony) by a trained music therapist to develop and enhance communication, relationship, learning, expression, mobilization, organization and other relevant therapeutic elements that a person or group needs to meet their physical, emotional, social and cognitive needs" (https://www.musictherapyworld.net). In 1997, therapy with music was defined as "a branch of expertise that uses music and music activities in meeting the physical, psychological, social and mental needs of the individuals in need" (Erer & Atıcı, 2010). In addition, administering treatment under a regular method by adjusting the physiological and psychological effects of musical sounds and melodies according to various mental disorders is called "Therapy with Music" (Gençel, 2006).

History of Music Therapy

History of music therapy is as old as the history of medicine. Melodies and songs used to treat diseases, and to remove the evil spirits from the body until the antiquity formed the basis of music therapy. In ancient Greeks, music was the basis of all kinds of virtue and recognized as a great factor in the education and purification of the soul. In ancient Greece and Rome, music was believed to be effective in removal of human woes, it was used to give strength to patients in ancient Egypt (Erer & Atıcı, 2010). Confucius said that thanks to music, the interpersonal relations improve, the eyes shine, the ears are sharpened, the movement and circulation of the blood calm down (Karamızrak, 2014).

Pythagoras (585-500 BC) was one of the first scientific founders of music therapy. Pythagoras explored the possibility of treating patients who have hopelessness or quick temper with specific melodies. According to Pythagoras, music, which is the result of the harmony of sounds, is the most effective remedy in cases where the harmony in the body is disturbed. According to Hippocrates, the use of music is beneficial when medicine is insufficient. Socrates' student Platon stated that the music gave tolerance and comfort to the person by affecting the depths of the soul with the harmony and rhythm around 400 BC (Birkan, 2014).

Zekeriya Er-Razi (854-932), Farabi (870-950) and Avicenna (980-1037) used medication and music therapy methods in the treatment of mental disorders (Somakçı, 2003). Old Turkish physicians had their patients who had fear, excitement, suspicion, and mood swings listen various melodies. During these applications, they checked the pulses of the patients and found the appropriate melody and treated the patients with the same disease. In other words, they performed psychotherapy (Erer & Atıcı, 2010; Grebene, 1978).

In the Middle Ages, Turks tried to treat individuals with mental illnesses as accepting them patients and treated them as patients. For this reason, it is the Turks who applied the treatment of mental diseases with music in a systematic way and pioneered the treatment. The first music therapy studies about the healing power of music started during the Seljuks and Ottomans (Erer & Atıcı, 2010). Therapy in the Seljuks and Ottomans was carried out either individually or in the therapy houses (Uçan & Ovayolu, 2006).

The first therapy house (şifahane) was Nureddin Hospital, which was built in Damascus by the Seljuk Sultan Nureddin Zengi 900 years ago. Fatih Darüşşifası in Istanbul and Edirne Darüşşifası in Edirne are the most important institutions in which music therapy is applied (Gençel, 2006). It is stated that Edirne Darüşşifa is an establishment in which every mental illness is applied to a certain authority and mental patients are treated with sound of water and music (Karamızrak, 2014; Koç et al., 2016).

In the 20th century, this field of expertise was discovered as a result of the use of music in the health care institutions where wounded soldiers stayed after the World War II (Karamızrak, 2014). In the 1960s, it was started to train people in this field (Erer & Atıcı, 2010). In recent years, music therapy has been used in diseases such as schizophrenia, depression, Alzheimer's, mental retardation, autism, alcohol and drug addiction. Researchers began to study the effects of music on the neurological and nervous system experimentally (Uçan & Ovayolu, 2006). Thus, music therapy has become a systematic and scientifically applied discipline. In Turkey, Group for the Research and Promotion of Turkish Music (TUMATA) established by Dr. Rahmi Oruç Güvenç (1976) has been carrying out its studies on music therapy (www.tumata.com). The Turkish Treatment Music Practice Research Group (TUTEM), founded by Dr. Adnan Çoban, also carries out studies on music therapy.

The Effects of Music

Music allows the individual to relax physically and get rid of tension and create a high level of relaxation (Boşnak, Kurt, & Yaman, 2017; Karamızrak, 2014). It has an effect on hormones such as serotonin, norepinephrine, dopamine, melatonin, cortisol, adrenaline, testosterone. These hormones contribute to the formation of psychiatric diseases. However, it has a positive effect on physiological events such as blood pressure, respiration and pulse (Boşnak, Kurt, & Yaman, 2017). It balances the oxygen and blood build up in the brain (Boşnak, Kurt, & Yaman, 2017; Karamızrak, 2014). It strengthens the immune system, stimulates digestion, improves rough and fine motor movements, creates a relaxing effect in patients with acute and chronic pain, calms the hyperactivity. It decreases stress, pain, anxiety, and isolation, and creates behavioral change in individuals. It reduces feelings such as fear that aggravates pain (Arslan, 2007; Fındıkoğlu, 2015; Kemper & Danhauer, 2005). Music is also an important instrument that helps to distract attention in the individual, reduce nausea related to chemotherapy and improve the quality of life in the terminal period (Boşnak, Kurt, & Yaman, 2017; Karamızrak, 2014). Music helps to improve the fluency of speech, to focus and maintain the attention (Fındıkoğlu, 2015). It has got mitigating effects of insomnia (Bloch et al., 2010).

According to Farabi (870-950), the effects of the maqams of Turkish music on the soul

were classified as follows:

Rast maqam: brings people joy, peace, vitality, relaxation and comfort.

Rehavi maqam: brings people the idea of eternity.

Kuçek maqam: brings people sadness and anguish.

Büzürk maqam: brings people fear.

İsfahan maqam: brings people the capacity of action, the sense of security.

Neva maqam: brings people pleasure and contentment.

Uşşak maqam: brings people the feelings of laughter, happiness, strength and heroism.

Zirgüle maqam: brings people sleep.

Saba maqam: brings people bravery, power.

Buselik maqam: brings people strength.

Hüseyni maqam: brings people serenity, ease.

Hicaz maqam: brings people humility (Arslan, 2007; Koç et al., 2016; Somakçı, 2003).

Music also has some physiological and emotional effects on people:

Physiological: It is the limbic system in the brain where the effects of the emotions of music are collected and organized (Şengül, 2008). In this system, neural mechanisms of behavior and excitement, biological impulses, memory and learning structures are involved (Arslan, 2007; Kemper & Danhauer, 2005; Şahin Karadeniz, 2017; Şengül, 2008).

A music listened at low tempo affects the limbic system in the brain, the center of emotion and excitement, reducing the ability of neural transmission. It makes physiological and psychological changes by affecting neuroendocrine and autonomic nervous system. By activating the autonomic nervous system, it leads to a decrease in symptoms such as blood pressure, pulse, respiration. It increases endorphin secretion by stimulating the pituitary gland, thus decreasing the pain and anxiety of the individual, provides a positive effect on detection (Updike, 1990).

Emotional: The music allows the person to relax emotionally by moving away from the environment, increasing the coping power and social relations. It gives individuals a sense of trust and attachment, making it easier to deal with losses (Aslan, 2007).

Types of Music Therapy

Active Therapy: This therapy is the treatment of patients using musical instruments, their bodies and sounds. The purpose of active therapy is to protect and develop the body physically and spiritually. Active therapy can be individual's playing an instrument actively, dancing (music and movement therapy), performing a piece, doing sport in company with music and giving a concert (Gençel, 2006; Şahin Karadeniz, 2017; Peng, Koo, & Kuo, 2010). Psychotic patients who can not communicate through words can communicate more easily through dance (Grebene, 1978)

Passive Therapy: It is one of the most commonly used methods (Gençel, 2006). It is performed by the therapist by listening to live or recorded music. Appropriate selection of music to the person and his/her illness can affect the treatment positively. Persons participating in therapy are asked to concentrate fully on the music and to let the music flow to them (Şahin Karadeniz, 2017). Passive therapy is the individual's listening to music and rhythm, listening to a recorded music or listening to music in a concert. The only thing that the patient does here is to listen to the given music (Grebene, 1978; Peng, Koo, & Kuo, 2010).

Application of Music Therapy

Music therapy is not just a process in which music is used. Rhythm, melody and harmony in music are used for treatment purposes (Karamızrak). After the needs of the individuals are determined, the appropriate music, music type, rhythm and lyrics are selected by the specialist music therapist according to the condition of the disease and the individual. Music therapy can be carried out individually or by group, by the use of human voices or bodily movements, such as dance, under the supervision of a specialist therapist (Aydın, www.sanatpsikoterapileridernegi.org). The music therapist should be familiar with physical and mental illnesses, be competent enough to determine the needs of patients, have a good education and clinical experience, and know how to use music and music in therapy at an adequate level (Çoban, 2010).

Individual or group music therapy is used in the application of music therapy. ***Individual therapy*** is an option for individuals who do not have the necessary skills and social behaviors to participate in group work (Şahin Karadeniz, 2017). The patient and the music therapist conduct one-to-one work. This therapy allows the patient to allocate more time. The therapist observes and evaluates the patient individually (Fındıkoğlu, 2015; Özgenel, 2018). ***Group music therapy*** is done in a group with individuals with similar needs. It is more general and superficial than individual therapy (Şahin Karadeniz, 2017). It requires less detail and planning, is more flexible (Özgenel, 2018).

Use of Music in Schizophrenia

Schizophrenia is a chronic psychiatric disorder that lasts a lifetime, which significantly affects one's quality of life and daily life. In schizophrenia, deficiencies are seen in functions such as perception, emotion, thought, movement, speech and communication (Özgenel, 2018; Tseng et al., 2016).

Music therapy has been used for many years as an adjuvant therapy in schizophrenia (Tseng et al., 2016), and pharmacological treatments and treatment programs, in which patients are supported, have gained importance in recent years. Music therapy, which is one of these approaches, allows patients to socialize with each other and the therapist, and increases their adaptation to the environment outside the hospital (Ceccato, Caneva, & Lamonaca, 2006; Ulrich, Houtmans, & Gold, 2007). In particular, symptoms may cause loneliness, which may be useful for improving interpersonal relationships of patients. Music creates strong emotions by providing insight into the individual (Lundmark, www.sanatpsikoterapileridernegi.org). Music therapy may be useful in patients who do not wish to act because a person can passively listen to music without moving his body (Asano, 2013).

Therapies added to music therapy are reported to be more successful than standard treatments alone. However, the state of well-being seen in mental state, depression, anxiety, and cognitive functioning may vary according to the quality and number of music therapy (Birkan, 2014; Geretsegger et. al., 2017). A well planned and practiced music therapy can affect the feelings and thoughts positively by eliminating the problems of patients, and may contribute to the strengthening of social relations and ego, and the increase of self-esteem (Gençel, 2006). Music therapy can affect the psychological well-being and quality of life positively, increase cognitive functions and provide emotion expression. The aim of therapy is to help develop interpersonal relationships in people with mental disorders and to express themselves (Mössler et al., 2011).

A melody familiar to schizophrenic patients may lead them out of the imaginary world and return to the real world. There exists studies showing that music therapy is useful in dealing with symptoms in schizophrenic patients and that symptoms decrease after music therapy (Collins, Cull, & Sireling, 1989; Gallagher Dinan, & Baker, 1994; Hayashi, Tanabe,

& Nakagaw, 2002; Mohammadi et al., 2012; Peng, Koo, & Kuo, 2010; Tang, Yao, & Zheng, 1994; Wen-Ying et al., 1998; Yang et al., 1998). In addition, Silverman (2003) proved that music was effective in suppressing the psychosis symptoms and fighting with them as a result of a meta-analysis. Gold et al. (2005) showed that music therapy was more effective on negative symptoms and social functions than standard treatment alone. Talwar et al. (2006) found a significant improvement in the general symptoms of schizophrenia in the group listening to music, Grocke, Bloch and Castle (2009) found that listening to music increased the quality of life. De Sousa and De Sousa (2010) showed a decrease in positive and negative symptoms after one month in a group of schizophrenic patients who listened to music, Bloch et al. (2010) found that relaxing music had a positive effect on insomnia and mood. In another study, it was found that music added to the standard treatment decreased anxiety, depression and negative symptoms, improved the general situation and social functions of patients with schizophrenia (Mösler et al., 2011). In the study of Ertekin Pınar (2013) carried out with schizophrenic patients having auditory hallucinations, it was found that listening to music in Rast maqam had positive effects on positive symptoms and quality of life. Arlı (2015) determined that state-trait anxiety scores were lower after music therapy, Salur (2016) determined that music therapy had positive effects on the individual's functions, individual and social performance, depression, mood and coping with stress. In the study of Fındıkoğlu (2015) carried out with 22 patients with schizophrenia, patients were made to listen to music in Bûselik maqam and positive effects were observed on their mental status. In the same study, there was a significant decrease in negative and positive symptom scores of the patients. According to the results of the meta-analysis, Tseng et al. (2016) emphasized that music had a positive effect on mood, positive and negative symptoms of schizophrenic patients. In the study of Özgenel (2018), it was found that there was a difference in the level of positive syndrome and a decrease in the level of negative syndrome of the schizophrenic patient group who were applied music therapy, also the level of general psychopathology decreased and functional recovery increased. In addition, some studies have reported that music has a positive effect on auditory hallucinations in schizophrenic patients (Johnston et al., 2002; Na and Yang, 2009; Zarghami, Moonesi, & Khademloo, 2012).

CONCLUSION

Music therapy is used in schizophrenia to reduce symptoms, increase interpersonal relationships, and to cope with disease symptoms. Well-planned and practiced by an expert, music therapy can help patients with schizophrenia relax in a positive way. It can help improve self-esteem and self-confidence by making positive effects on social relationships, quality of life, positive and negative symptoms and functionality. In this context, nurses working with schizophrenic patients support listening to music, and include music therapy in therapy programs are important in terms of expressing patients' feelings and thoughts.

REFERENCES

1. Arlı, K. (2015). Stres ve Anksiyete İçin Alternatif ve Tamamlayıcı Bir Model Olarak Müzik Terapi. Üsküdar Üniversitesi Sosyal Bilimler Enstitüsü Yüksek Lisans Tezi, İstanbul.
2. Asano, M. (2013). Current status of music therapy for schizophrenic patients. *Acoust. Sci. & Tech, 34* (1), 13–18.
3. Arslan S. (2007). Dokunma, Müzik Terapi ve Aromaterapinin Yoğun Bakım Hastalarının Fizyolojik Durumlarına Etkisi. Atatürk Üniversitesi Sağlık Bilimleri Enstitüsü Doktora Tezi, Erzurum.

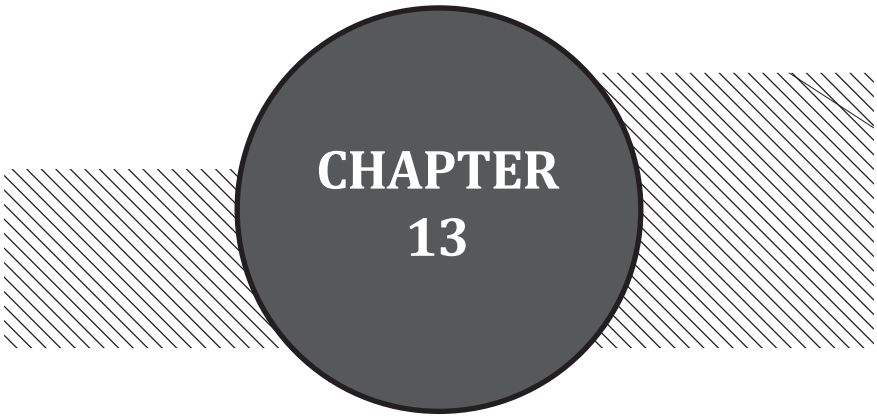
4. Aydın E. Müzik terapi: İşleyiş ve yaklaşımlar. www.sanatpsikoterapileridernegi.org.
5. Birkan, I. (2014). Müzikle tedavi, tarihi gelişimi ve uygulamaları. *Ankara Akupunktur ve Tamamlayıcı Tıp Dergisi,* 37–49.
6. Bloch, B., Reshef, A., Vadas, L., Haliba, Y., Ziv, N., Kremer, I., et al. (2010). The effects of music relaxation on sleep quality and emotional measures in people living with schizophrenia. *Joumal of Music Therapy*, *47* (1), 27–52.
7. Boşnak, M., Kurt, A.H., & Yaman, S. (2017). Beynimizin müzik fizyolojisi. *KSU Medical Journal*, *12*(1), 35–44.
8. Ceccato, E., Caneva, P., & Lamonaca, D. (2006). Music therapy and cognitive rehabilitation in schizophrenic patients: A controlled study. *Nordic Journal of Music Therapy, 15,* 111–120.
9. Collins, M.N., Cull, C.A., & Sireling, L. (1989). Pilot study of treatment of persistent of persistent auditory hallucinations by modified auditory input. *Br Med J, 299,* 431–432.
10. Çoban, A. (2010). Müzik ile terapi. 19. Anadolu Psikiyatri Günleri Eskişehir.
11. De Sousa, A., & De Sousa, J. (2010). Music therapy in chronic schizophrenia. *Journal of Pakistan Psychiatric Society, 7,* 13–17.
12. Erer S, & Atıcı, E. (2010). Selçuklu ve Osmanlılarda müzikle tedavi yapılan hastaneler. *Uludağ Üniversitesi Tıp Fakültesi Dergisi, 29*–32.
13. Ertekin Pınar, Ş. (2013). Rast Makamında Dinletilen Müziğin Şizofrenik Hastalarda İşitsel Halusinasyon ve Yasam Kalitesi Üzerine Etkisi. Erciyes Üniversitesi Sağlık Bilimleri Enstitüsü Doktora Tezi, Kayseri.
14. Fındıkoğlu, S. (2015). Şizofrenik Hastalarda Müzik Terapinin Ruhsal Durum Üzerine Etkileri. İstanbul Medipol Üniversitesi Sağlık Bilimleri Enstitüsü Yüksek Lisans Tezi, İstanbul.
15. Gallagher, A.G., Dinan, T.G., & Baker, L.J. (1994). The effects of varying auditory input on schizophrenic hallucinations: A replication. *Br J Med Psychol, 67,* 67–75.
16. Gençel, Ö. (2006). Müzikle tedavi. *Kastamonu Eğitim Dergisi, 14,* 697–706.
17. Grebene, B. (1978). Müzikle Tedavi, Güven Kitabevi Yayınları Sanem Matbaa, Ankara.
18. Geretsegger, M., Mössler, K.A., Bieleninik, Ł., Chen X.J., Heldal, T.O., & Gold, C. (2017). Music therapy for people with schizophre-nia and schizophrenia-like disorders. *Cochrane Database of Syst. Rev, 29* (5), CD004025.
19. Gold, C., Heldal, T.O., Dahle, T., & Wigram, T. (2005). Music therapy for schizophrenia or schizophrenia-like illness. *Cochrane Database Syst. Rev, 18* (2), CD004025.
20. Grocke, D., Bloch, S., & Castle, D. (2009). The effect of group music therapy on quality of life for participants living with a severe and enduring mental illness. *J Music Ther, 46,* 90–104.
21. Güner, S.S. (2007). Müziğin tedavideki yeri ve şekli. *Karadeniz Araştırmaları, 12*, 99–112.
22. Hayashi, N., Tanabe, Y., & Nakagawa, S. (2002). Effects of group musical therapy on inpatients with chronic psychoses: A controlled study. *Psychiatry and Clinical Neurosciences, 56,* 187–193.
23. Johnston, O., Gallagher, A.G., Mcmahon, P.J., & King, D.J. (2002). The efficacy of using a personal stereo to treat auditory hallucinations preliminary findings. *Behav Modif, 26,* 537–549.
24. Karamızrak, N. (2014). Healing effects of sound and music on the organs. *Koşuyolu Heart Journal 17*(1), 54–57.

25. Kemper, K.J., & Danhauer, S.C. (2005). Music as therapy. *Southern Medical Journal, 98*, 282–288.
26. Koç, E.M., Ayhan Başer, D., Kahveci, R., & Özkara, A. (2016). Ruhun ve bedenin gıdası: Geçmişten günümüze müzik ve tıp. *Konuralp Tıp Dergisi, 8*(1), 51–55.
27. Lundmark D. Müzikle tedavi ve psikoz. www.sanatpsikoterapileridernegi.org.
28. Mohammadi, A.Z., Minhas, L.S., Haidari, M.., & Panah, F.M. (2012). A Study of the effects of music therapy on negative and positive symptoms in schizophrenic patients. *German J Psychiatry, 15*, 56–62.
29. Mössler, K.1., Chen, X., Heldal, T.O., & Gold, C. (2011). Music therapy for people with schizophrenia and schizophrenia-like disorders. *Cochrane Database Syst Rev, 7*(12), CD004025.
30. Na, H.J., & Yang, S. (2009). Effects of listening to music on auditory hallucination and psychiatric symptoms in people with schizophrenia. *J Korean Acad Nurs, 39*, 62–71.
31. Özgenel, P.E. (2018). Şizofreni Hastalarında Müzik Terapinin Depresyon, İşlevsellik, Genel Psikopatoloji Klinik Parametreleri Üzerine Etkileri. Üsküdar Üniversitesi Sosyal Bilimler Enstitüsü Yüksek Lisans Tezi, İstanbul.
32. Peng, S.M., Koo, M., & Kuo, J.C. (2010). Effect of group music activity as an adjunctive therapy on psychotic symptoms in patients with acute schizophrenia. *Arch Psychiatr Nurs, 24*, 429–434.
33. Salur, Ö. (2016). Expressive fun: Emotional awareness activity over metaphors in a music therapy group with people diagnosed with schizophrenia. *VII. International Hisarlı Ahmet Symposium* s.284–295.
34. Silverman, M.J. (2003). The influence of music on the symptoms of psychosis: A meta-analysis. *Journal of Music Therapy, 40,* 27–40.
35. Somakçı, P. (2003). Türklerde müzikle tedavi. *Sosyal Bilimler Enstitüsü Dergisi, 15*,131–140.
36. Şahin Karadeniz, E. (2017). Demans - Alzheimer Hastalarında Farklı Müzik Terapi Uygulamalarının Zihinsel, Psikolojik, Anksiyete ve Ajitasyon Etkileri Üzerine Karşılaştırmalı Çalışma. Haliç Üniversitesi Sosyal Bilimler Enstitüsü Yüksek Lisans Tezi, İstanbul.
37. Şengül, E. (2008). Kültür Tarihi İçinde Müzikle Tedavi ve Edirne Sultan II. Bayezid Darüssifası. Trakya Üniversitesi Sosyal Bilimler Enstitüsü Yüksek Lisans Tezi, Edirne.
38. Talwar, N., Crawford, M.J., Maratos, A., Nur, U., McDermott, O., & Procter, S. (2006). Music therapy for in-patients with schizophrenia. *British Journal of Psychiatry, 189,* 405–409.
39. Tang, W., Yao, X., & Zheng, Z. (1994). Rehabilitative effect of music therapy for residual schizophrenia: A one-month randomised controlled trial in Shanghai. *Br. J. Psychiatry, 165*, 38–44.
40. Tseng, P-T., Chen, Y-W., Lin, P-Y., Tu, K-Y., Wang, H-Y., Cheng, Y-S., et al. (2016). Significant treatment effect of adjunct music therapy to standard treatment on the positive, negative, and mood symptoms of schizophrenic patients: A meta-analysis. *BMC Psychiatry*, *16* (16), 1–11.
41. Türk Musikisini Araştırma ve Tanıtma Grubu. www.tumata.com
42. Ulrich, G., Houtmans, T., & Gold, C. (2007). The additional therapeutic effect of group music therapy for schizophrenic patients: A randomized study. *Acta Psychiatr Scand*, *116*, 362–370.

43. Uçan, Ö., & Ovayolu, N. (2006). Müzik ve tıpta kullanımı. *Fırat Sağlık Hizmetleri Dergisi 1*, 14–22.
44. Updike, P. (1990). Music therapy results for ICU patients. *Dimensions of Critical Care Nursing, 9*, 39–45.
45. Wen-Ying, Y., Zheng, L., Yong-Zhen, W., Hong-Yı, Z., & Bio, M. (1998). Psychosocial rehabilitation effects of music therapy in chronic schizophrenia. *Hong Kong Journal of Psychiatry, 8,* 38–40.
46. World Federation of Music Therapy. https://www.musictherapyworld.net
47. Yang, W.-Y., Li, Z., Weng, Y.-Z., & Zhang, H.-Y. (1998). Psychosocial rehabilitation effects of music therapy in chronic schizophrenia. *Hong Kong J. Psychiatry, 8,* 38–40.
48. Zarghami, M., Moonesi, F.S., & Khademloo, M. (2012). Control of persistent auditory hallucinations through audiotape therapy. *European Review for Medical and Pharmacological Sciences, 16*, 64–65.

Evaluating Left-Censored Data Through Substitution, Parametric, Semi-Parametric, And Non-Parametric Methods: A Simulation Study

Mustafa AGAH TEKİNDAL[1]



[1] Assoc. Prof., Selçuk University Faculty of Veterinary Medicine Deparment of Biostatistics

INTRODUCTION

Most of the researches in applied sciences use censored measurement in study design. When deduce for system or process reliability, may not always be possible to observe all the components decay time that compose the system or the process. For instance; in the life test conducted to learn about the lifetime of an expensive electronic parts, observation of all of the parts breakdown, will increase the costs and testing time may not be preferred or it is hard to observe completely patient data that treated in a clinic. In those cases, censored data is acquired after observation or test. Censored data is using in the field of medicine, biology, nutriment, engineering, quality control or most of the other field.

Thus detection limit is defined by (Detection Limit) Strobel and Heineman (1989): Detection limit is simply the smallest analyze concentration of a variable can be defined statistically different. In order to define limit, two sets of measurements are required. A regular set, as well as many measurements (10-20 are recommended) must be performed under the same conditions on a draft. Equation is the same as LOD and equation can be defined like Miler and Miller (1993) did in, Equation 1:

$$y - y_B = ks_B \qquad \text{Equation (1)}$$

y, response produced from current analyze in detection limit concentration, y_B, 'draft response ' whereas s_B standard deviation of draft response (example). k degree, is the correction value of in order to provide acceptable level of false positive or false negative result in determining detection limit. In any real value k never be less than *+1 and as suggested by* International Union of Pure and Applied Chemistry *(* IUPAC) k ≡*+3 should be used.*

Because detection limit is certain positive amount, ın Equation 2 detection limit definition is reached.

$$\mathcal{L} \equiv (LOD) \equiv \frac{ksdraft}{|b|} \qquad \text{Equation (2)}$$

In this equation $|\beta|$ is size of the slope and s_{draft} is standard deviation of drafts co response.

Since Detection limit defined in equation 2 as test statistics of examples, equation 3 defined for population.

$$TL(\Lambda) \equiv trueLOD \equiv \frac{k\sigma_{draft}}{|\beta|} \qquad \text{Equation (3)}$$

In equation 3, $|\beta|$ is the underlying linear measurement models real size of the slope (analytical sensitivity of size) and σ_{draft} Population standard deviation of draft's response Actual detection limit defined in Equation 3 is accurate and is always more than zero. However, detection limit defined in equation 2 are functions of random variable: Calculation of draft measurement of uncertainty and measurement tool's analytical sensitivity. The resulting calculated as per one or more measured quantities, any measured result of random variable, are solid examples of some of the distribution of possible outcomes. As a result, detection limit that defined via examples, are random variable, It is characterized by fully determine its statistical properties of probability density functions. Especially, described in equation 2, detection limit defines probability density function of test statistics detection limit's (population mean) estimated value, the precision (for example, population standard deviation or significant figures) and confidence limits (95%) .

MATERIALS AND METHODS

Data sets are derived from different distribution (Log-normal, exponential and Weibull). Derived data sets are organized in different sample size (20-300) and in different censored proportions (%5-%65). While censored observations settlement change to be left in distributions, mean, standard deviation and performance via taking the difference of the median was evaluated. First uncensored data sets were derived in 3 different distributions these are Log-normal, exponential and Weibull distribution and then these data sets are left-censored in proportion of %5, %25, %45, and % 65. Censored data sets are estimated by using put in detection limit to left-censored datasets, detection limit of $/\sqrt{2}$(LOD ve LOD/$\sqrt{2}$), parametric method (MLE), Semi parametric method (ROS) and non-parametric method (KM).

In log-normal exponential and Weibull distribution coefficient of variation was taken 05 and 2 and data sets were derived for evaluating performance of left-censored data sets in exponential distribution, due to exponential distribution's probability distribution function, data was derived from 1 coefficient of variation. Data sets were derived from when coefficient of variation took 0.5 in log-normal distribution, mean was 1 and standard deviation was 0.473, in Weibull distributions shape parameter was 2,1, scale parameter was 1, when coefficient variation took 2, in log-normal distribution, mean was 1 and standard deviation was 1.27, in Weibull distributions shape parameter was 0.542, scale parameter was 1. In exponential distribution parameter was chosen 0.05.

Each of the specified distribution according to the above-mentioned parameters, firstly uncensored data was derived, and then these data sets are left-censored in proportion of %5, %25, %45, %65.After censoring, Censored data sets are estimated by using put in detection limit to left-censored datasets, detection limit of $/\sqrt{2}$ (LOD ve LOD/$\sqrt{2}$), parametric method (MLE), Semi parametric method (ROS) and non-parametric method (KM) in with help of updated on 2$^{nd\ of}$ July 2014 by Lopak Lee NADA (Nondetects And Data Analysis: Statistics for Censored Environmental Data) package of R (Version i386 3.0.2) program. The number of repetitions (Mod) for performed simulations is determined as 10000. Moreover, the assessment on sample scale was conducted by increasing 10 from 20 to 300.

In order to compare both uncensored data set and each other, abovementioned methods were used for analyzing left-censored data sets in different sample size, distribution and censored proportion. Firstly uncensored data sets were derived from 20 to 300 different sample size with different distribution, after that were ascending sorted and used censored. Censored data sets are estimated by using put in, parametric method, Semi parametric method and non-parametric method. Difference between mean, median and standard deviation were find between estimated this value and uncensored data sets and performance comparison were made.

Most of the research in applied sciences, censored measurement are using in the study design censored measurement is divided into two right censored and left censored. In left-censored study by using different methods estimated values are used instead of censored observations. In this type of data analyses there is foresight to use parametric or non-parametric tests when taking difference between mean, median and standard deviation Analysis method varies depending on the results obtained.

Gillom and Hensel (1986) compared put in method in mean and median and MLE method, when take the number of repetition 500 in log-normal distribution, in case of sample size changes 10 20 and 50 and when censored proportion was taken 20%, 40%, 60% and 80%. They determined that MLE gives the best performance Helsel and Cohn (1998) compare put in method in mean and median, ROS and MLE methods in their study

when they took the repetition number 500 and took the sample size stable 25 in case of censored proportion was 20%, 40%, 60% and 80%. They determined that ROS gives the best performance. However, in Shumway' study (2002), the repetition number was taken 20 and 50 and censor proportion was taken 50% and 80%, he compared ROS method in mean and median and MLE. He did not determine any superiority each other. In Hewett and Ganser's study (2007), when they determined the repetition number is 100 and censored proportion 10%, 20%, 40 % 60 and 80%, they compared put in method in mean and median, KM ROS and MLE. They determined that MLE and ROS is successful in all case, KM is not successful. According to the simulation study, it was determined that the number of repetitions was determined inadequate. In our study the repetition number is 10000, therefore, the following conclusions can be said to be more reliable.

Although in In deviation from the mean and the standard deviation LOD put in, KM, ROS and MLE's performance is too close to each other ROS showed the best performance for data set in log normal distribution that's mean is 1, Standard deviation is 0.473 with censored proportion is 5%, 25%, 45%, 65% In deviation from the median ROS method showed the best performance. In general, LOD, LOD/$\sqrt{2}$ put in methods shows largest deviation in deviation from mean, median and the standard deviation, ,in the sample scale of 20, 30, 70 and 110 In these methods, when sample scale increases, deviation from mean, median and the standard deviation is systematically decreasing. In this case, as can say that if sample scale is more than 110, the performance will be higher than in the case of sample scale is less than 110. When sample scale is 20, 30, 70, KM method has the largest deviation from mean, median and standard deviation. Also for KM method it can be said that it is not systematically true but if sample scale is less than 70, the deviation from mean, median and standard deviation will be largest. In MLE irrespective of sample size, it shows variation in different sample size. However, in MLE deviation from standard deviation is higher when sample scale increases. MLE showed largest deviation, especially when censored proportion increased, deviation was seriously increased

ROS showed the best performance for data set in log normal distribution that's mean is 1, Standard deviation is 1.27 with censored proportion is 5%, 25%, 45%, In deviation from the mean, the median and the standard deviation. Although, in 65% censored proportion, in deviation from mean and standard deviation. For deviation from the median, LOD/$\sqrt{2}$ showed the best performance LOD, LOD/$\sqrt{2}$, KM, MLE and ROS methods showed close largest deviation from the mean, the median and standard deviation, when sample size was below 50. However, for censored value, deviation from standard deviation increases while sample size increases. Especially for ROS and LOD/$\sqrt{2}$ methods while sample size is increasing, deviation from mean and standard deviation is decreasing. In all methods, the nature of log normal distribution when censored proportion increases, deviation started to seriously increase. However, deviation in MLE was worse than censored situation. In this case, the effect on the methods of censorship rate is seen clearly.

In Chowdhury and Gulshan (2012) study, they compared KM ROS and MLE for deviation from mean and median, when they took repetition number 1000, ın case of sample scale were 25, 35, 80 and 200, in case of 5%, 25%, 35% and 50% were decided for censored proportion. They discovered for 5% censored proportion best performance was shown by KM, for others by ROS. While taking into consideration that coefficient of variation of the exponential distribution was 1, the diversity of scenarios will be limited. In this case, taking consideration of in our study that includes more repetition number, it can be considered to be more reliable for following results.

KM For Data sets that derived from the exponential distribution with parameter 0.05, in censored proportion of 5%, 25% and 45%, while KM, LOD put in, LOD/$\sqrt{2}$ put in and ROS showed the closest performance each other for deviation from mean. LOD/$\sqrt{2}$ showed

the best performance. For deviation from median best performance was shown by ROS: for 65 % censored rate, best performance was shown for deviation from mean by ROS, for deviation from median by LOD/$\sqrt{2}$ method. LOD, LOD/$\sqrt{2}$ methods showed closely largest deviation from mean median and standard deviation close performance in sample scale of 20, 30, 70 and 110, in these methods while sample size was more than 70, deviation from mean, median and standard deviation started to decrease. In KM methods, deviation from mean median and standard deviation were largest in the sample scale of 20 30 and 70. When sample scale was 20, 70 and 110 in MLE method, deviation from mean and median started to increase. However, deviation from standard deviation can be seen more when sample scale increased. For KM when sample scale was less than 70, largest deviation can be seen. But these are not systematical changes. In ROS method, difference between censored and uncensored data sets for deviation from mean median and standard deviation was increasing while sample scale was more than 230. Especially for deviation from standard deviation was systematically increasing when sample case was more than 200.

According to Krol and Stedinger (1996) study,when it compared ROS and MLE for deviation from mean and median for Weibull distribution when they took the repetition number 500, sample scale held constant 25, and took 10% 20% 40% and 60 % censored proportion, they discovered MLE showed the best performance Schmoyer and friends (1997) study compared for deviation from mean and median for methods of put in KM, ROS and MLE for repetition number of 1000 in case of censored proportion was 20% 40% %60 and 80%. They discovered KM showed the best performance. According to Chowdhury and Gulshan (2012) study, when it compared KM, ROS and MLE for deviation from mean and median when they took the repetition number 1000, sample scale held t 25, 35, 80 and 200 and took 5% 25% 35% and 50 % censored proportion, they discovered ROS showed the best performance For different coefficient of variation in Weibull distribution, we can consider that the following comments were more reliable with in terms of sampling and repetition number variability.

LOD put in, KM, LOD/$\sqrt{2}$ put in; ROS were compared for data sets that derived in Weibull distribution with shape parameter of 0.542, scale parameter of 1, censored proportion of 5%, 25%, 45% and 65%. Although each of these methods performance was so close to each other in deviation from mean and standard deviation, LOD/$\sqrt{2}$ method showed the best performance. For deviation from the median, ROS showed the best performance. LOD, LOD/$\sqrt{2}$, KM, MLE methods show largest deviation in deviation from mean, median and standard deviation when sample scale was below 50 In these four methods, we can say that, when the sample scale started to increase, deviation systematically started to decrease. In that case we can say that, if the sample size is more than 50, these methods show better performance than in case of the sample size is less than 50. MLE shows largest deviation. In 5%, 25%, 45% and 65% censored rate MLE showed largest deviation for mean median and standard deviation. In high censored rate, ROS showed best performance for deviation from mean median standard deviation rather than low censored rate.

Data sets that derived from Weibull distribution in shape parameter was 1 scale parameter was 2.1 showed largest deviation from mean median and standard deviation in 5%, 25% and 65% censored proportion with without showing any increase or decrease in sample scale (30,70,110). LOD, LOD/$\sqrt{2}$, KM methods showed similar performance with largest deviation in case of sample scale was lower than 70.ın these three methods, while, sample scale is increasing, systematically deviation from mean median and standard deviation is decreasing. In MLE and ROS methods without showing any increase or decrease in sample scale with different sample scale (30, 160, 190, and 270) showed largest deviation in mean median and standard deviation. In deviation from mean and standard deviation LOD put in, KM, LOD/$\sqrt{2}$ put in and ROS showed similar performance, however, LOD/$\sqrt{2}$ showed lowest deviation. In median deviation, ROS showed best performance. In

45% censored rate for deviation from mean and standard deviation MLE LOD/$\sqrt{2}$ and ROS showed similar performance. However, MLE showed lowest deviation. In median deviation ROS showed best performance.

CONCLUSIONS

The main aim of this study is define deviation from mean median and standard deviation with different sample scale and censored proportion for data sample of censored or uncensored in Log-normal, Exponential and Weibull distributions. For that reason, firstly censored and censored types were explained, and then suggested for reviewing left-censored observations methods such as parametric (MLE), semi-parametric (ROS), and non-parametric (KM) methods were explained. In this study, in order to make a prediction in parametric life model, likelihood model is mostly used. It can be thought that in the field of health, when faced with left censored data sets, firstly distribution should be defined.

In execution section, at first uncensored data were derived from every distribution parameter. And then data sets were left-censored in rate of 5%, 25%, 45%, and 65%. Put in (LOD and LOD/$\sqrt{2}$) parametric (MLE), semi-parametric (ROS), and non-parametric (KM) methods used for estimating censored data also evaluation has been made by increasing ten by ten the sample size of data up to 20 to 300 used methods for analyzing left censored data used with uncensored data sets and different censored data sets in order to determine the performance. Performance comparison is made with censored and uncensored data sets by taking the deviation from mean median and standard deviation.

After the simulation study, best methods for different censored proportion and sample size in three distributions were shown in figure 1 and 3. This figure includes diagrams.

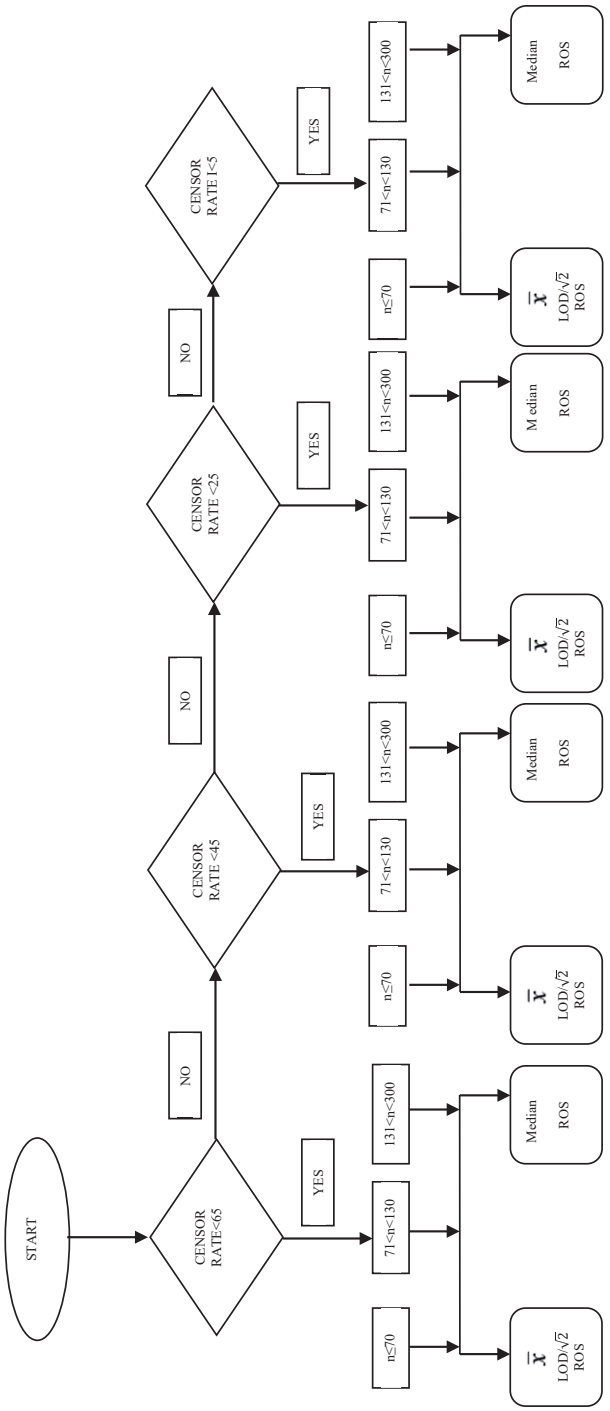


Figure 1. Suggested diagram of estimation methods for log normal distribution's summary statistic

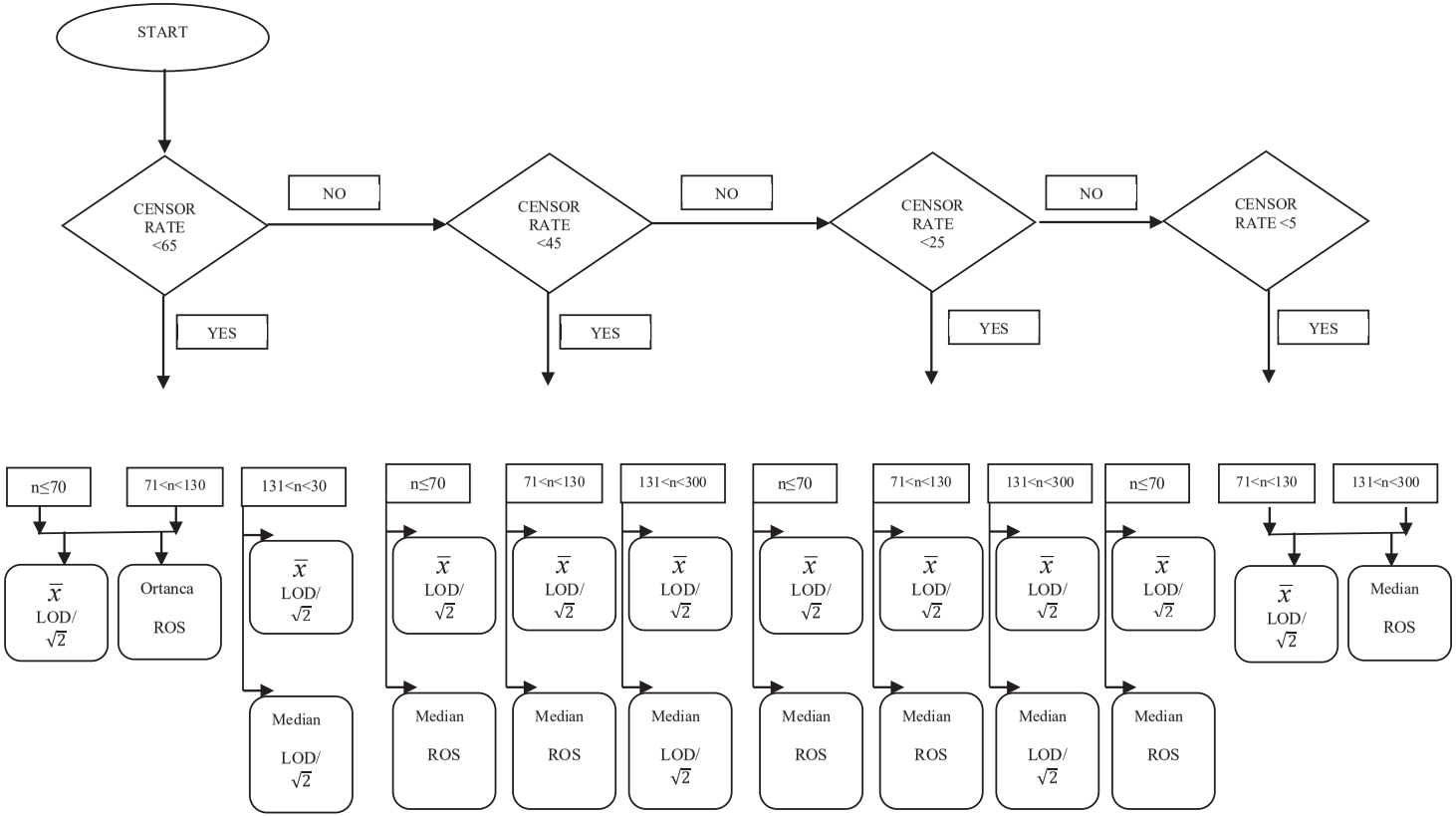


Figure 2. Suggested diagram of estimation methods for the exponential distribution's summary statistic

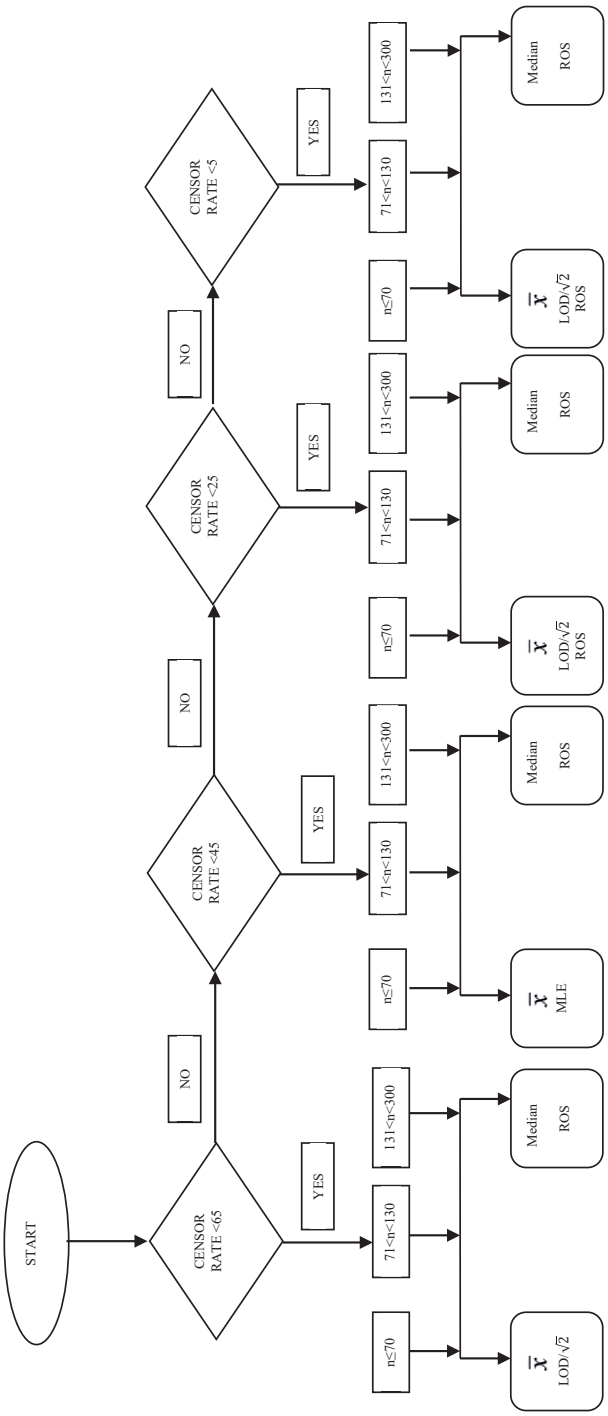


Figure 3. Suggested diagram of estimation methods for the Weibull distribution's summary statistic

Consequently, design of methods that has been explained in this dissertation has some advantages for execution. These are;

- Put in methods showed generally good performance
- When we look at the summary statistic, in cases where assumptions of parametric tests do not fulfilled, for the use of nonparametric methods ROS shows the best performance in all distribution methods and censored proportion with deviation from median. In this case when non-parametric tests are to be used, for censored observations for all condition ROS methods can be used for estimation.
- If data sets include some missing data, analyze can be done by put in methods.

On the other hand, to mention lack of this design, in cases of missing observations, although they can even analysis performed, it is expected to obtain some differences. In such cases, some deficiency can occur in interpretation of the results. Close scenarios generally preferred because it is not possible to do simulation study for all the possible scenarios. Over 65% censored rate, all methods have large deviation on the results. Therefore, according to this study can be considered it is not true to use data sets in case of censored rate are over 65%.

REFERENCES

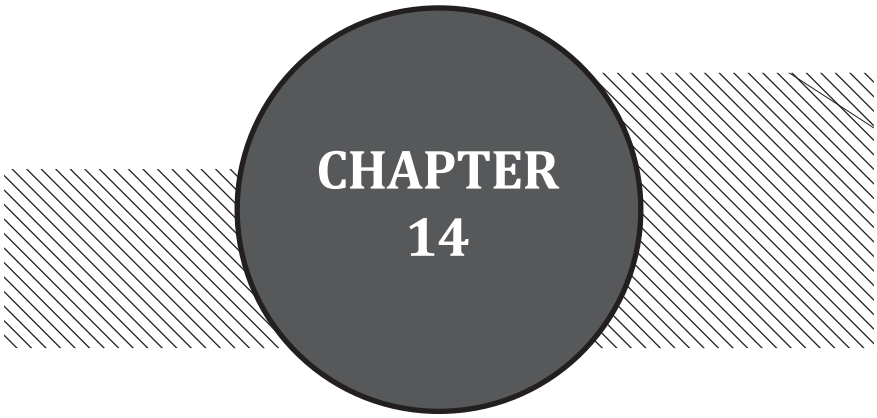
1. STROBEL, H. A., HEİNEMAN, W. R. (1989). Chemical Instrumentation, 3rd ed.; Wiley, New York.
2. HUSTON, C., JUAREZ-COLUNGA, E. (2009). Guidelines for computing summary statistics for data-sets containing non-detects, Written for the Bulkley Valley Research Center with assistance from the B.C. Ministry of Environment January 19
3. HORNUNG, R.W., REED, L. (1990). Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene*, 5, 46-51.
4. GLASS, D.C., GRAY, C.N. (2001). Estimating mean exposures from censored data: exposure to benzene in the Australian petroleum industry. *Annals of Occupational Hygiene*, 45, 275-282
5. HAWKINS, N.C., NORWOOD, S.K., ROCK, J.C. (1991). A strategy for occupational exposure assessment. Fairview, VA: American Industrial Hygiene
6. MULHAUSEN, J. DAMİANO, J. (1998). A strategy for assessing and managing occupational exposures. Second edition. Fairview, VA, American Industrial Hygiene Association, 3rd Edition, 349 pp.
7. LEE, L., HELSEL, D. R. (2005). Statistical analysis of water-quality data containing multiple detection limits: S-language software for regression on order statistics. *Computers & Geosciences*, 31, 1241-1248.
8. FINKELSTEIN, M.M., , VERMA, D.K. (2001). Exposure estimation in the presence of nondetectable values: another look. *American Industrial Hygiene Association Journal*, 62, 195–198.
9. SCHMOYER, R.L., BEAUCAMP, J.J., BRANDT, C.C. (1996). Difficulties with the log-normal model in mean estimation and testing. *Environmental and Ecological Statistics*, 3, 81–97.
10. SHE, N. (1997). Analyzing censored water quality data using a non-parametric approach. *Journal of the American Water Resources Association*, 33, 615–624.
11. HEWETT, P., GANSER, G.H. (2007). A comparison of several methods for analyzing censored data. *Annal of Occupational Hygiene*, 51, 611-632.

12. GILLIOM, R.J., HELSEL, D.R. (1986). Estimation of distributional parameters for censored trace level water quality data 1. Estimation techniques. *Water Resources Research*, 22, 135–146.
13. HELSEL, D.R., COHN, T.A.(1998). Estimation of descriptive statistics for multiply censored water quality data. Water Resources Research, 24.
14. KROLL, C.N., STEDİNGER, J. (1996). Estimation of moments and quantiles using censored data. *Water Resources Research*, 32, 1005–1012.
15. SHUMWAY, R.H., AZARI, R.S., KAYHANIAN, M. (2002). Statistical approaches to estimating mean water quality concentrations with detection limits. *Environmental Science and Technology*, 36, 3345–3353.
16. ANTWEILER, R.C., TAYLOR, H.E. (2008). Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. Summary statistics. *Environmental science & technology*, 42, 3732-3738.
17. El-SHAARAWI, A.H., ESTERBY, S.R. (1992). Replacement of censored observations by a constant: an evaluation. *Water Resources Research*, 26, 835–844.
18. FISHER, R.A. (1922). On the mathematical foundations of theoretical statistics. *Philosophical Transactions of the Royal Society, London Ser. A*, 222, 309-68
19. KAPLAN, E.L., MEIER, P. (1958). Non parametric estimation from incomplete observations. *Journal of the American Statistical Association*, 53, 457–81.
20. TRESSOU, J., LEBLANC, J.C.H., FEİNBERG, M., BERTAIL, P. (2004). Statistical methodology to evaluate food exposure to a contaminant and influence of sanitary limits: application to Ochratoxin A. *Regulatory Toxicology Pharmacology*, 40, 252-263.
21. POPOVIC, M., NIE, H., CHETTLE, D.R., MCNEILL, F.E. (2007). Random left censoring: a second look at bonelead concentration measurements, *Physics in Medicine and Biology*, 52, 5369-5378
22. HOSMER, D.W.,JR., LEMESHOW, S., MAY, S. (2008). Applied Survival Analysis: Regression Modeling of Time to Event Data 618. cilt/Wiley Series in Probability and Statistics, Wiley
23. WARE, J.H., DEMETS, D.L. (1976). Reanalysis of some baboon descent data. *Biometrics* 32:459-464.

Odontogenic Maxillary Sinusitis: Current Knowledge of Etiology, Clinical Presentation and Treatment

G. Neda HASANOĞLU ERBAŞAR[1]

Selen İNCE YUSUFOĞLU[2]



[1] Ankara Yıldırım Beyazıt Üniversitesi Diş Hekimliği Fakültesi Ağız ve Diş Çene Cerrahisi Anabilim Dalı

[2] Ankara Yıldırım Beyazıt Üniversitesi Diş Hekimliği Fakültesi Endodonti Anabilim Dalı

The maxillary sinus is a pyramid-shaped and air-filled cavity thatlies within the body of maxillary bone. Due to maxillary sinus is bordered by the nasalcavity and oral cavity, it is more susceptible to invasion by pathogenic microorganisms than other paranasal sinuses. Odontogenic sources are responsible for almost 10% to 12% of maxillary sinusitis cases (Mehra& Murad, 2014, Tucker, 2014, Yıldırım& et al., 2013).

Etiology of Odontogenic Sinusitis

In general, odontogenic sinusitisresults from dental pathologies including acute or chronic periapical diseases, endodontic problems, periodontal diseases and odontogenic cysts.This type ofmaxillary sinusitis may also occur iatrogenically(Little & et al., 2018, Mehra& Murad, 2014, Tucker, 2014).During the extraction of a lone standing maxillary molar tooth, there is a high risk of developing oro-antral communication due to increased resorption of alveolar bone around the tooth leading thinner bone between maxillary sinus and oral cavity (Mehra& Murad, 2014)**.** Similarly, traumatic displacement of maxillary roots can abrade maxillary sinus floor and penetrate the mucosa and may result inoro-antral communication and/or maxillary sinusitis (Figure1-2) (Kuan& Suh, 2017). Endodontic treatments in the posterior molar teeth have also been cited as causing odontogenic sinusitis by extrusion of root canal sealing and/or filling materials into the sinus cavity. Furthermore, some oral and maxillofacialsurgical procedures,such as alveolectomy/alveoloplasty, implant placement, sinus floor elevation, orthognathic surgery and cleft surgery areshown as potential causes of maxillary sinusitis(Kuan& Suh, 2017, Little & et al., 2018, Mehra& Murad, 2014, Tucker, 2014).Besides, maxillary bone trauma, neoplasms or other inflammatory processes are the other less common etiologic factors of odontogenic maxillary sinusitis (Little & et al., 2018).

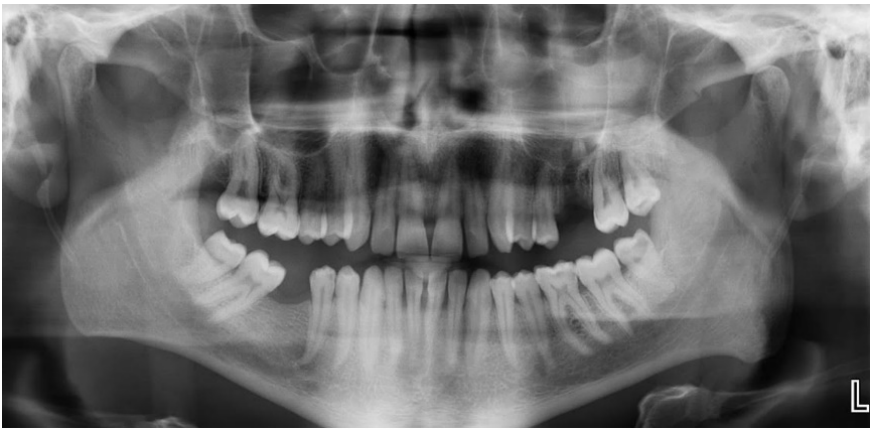


Figure 1. Orthopantogram shows the displacement of palatal root of tooth #26 into left maxillary sinus which resulted in oro-antral communication and maxillary sinusitis.

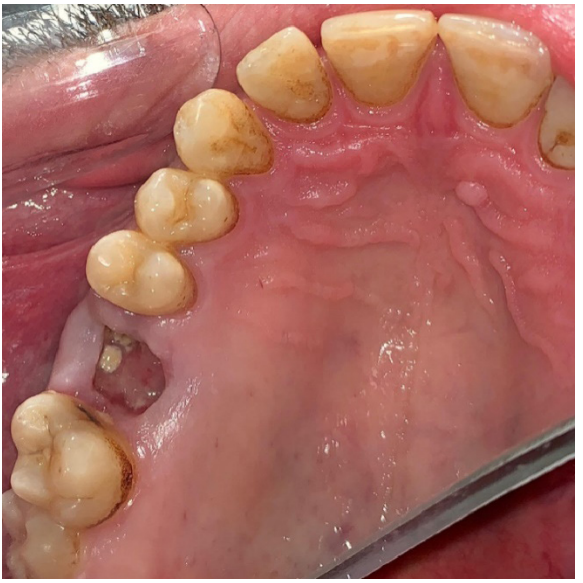


Figure 2. Intra-oral view of the patient with oro-antral fistula

In a retrospective meta-analysis of 770 maxillary odontogenic sinusitis cases, iatrogenia was found the most common etiology of this condition (55.97%) which was followed by periodontitis (40.38%) and odontogenic cyst (6.66%), respectively. The same study indicated that almost half of the iatrogenic sinusitis cases (47,56%) was occurred due to remaining roots and oro-antral fistulas.The extrusion of root canal materials into the maxillary sinusitis (22.27%) and dressing for closure of oro-antral communications and other foreign bodies represented (19.72%)were shown as other frequent iatrogenic sources. Also, implant placement and sinus lifting procedures were constituted almost 5% of the cases with iatrogenic causes (Arias-Irimia& et al., 2009). Similarly, in a recent systematic review of 674 chronic odontogenic sinusitis cases, iatrogenia was reported as the most frequent cause (65.7%) for this disease which were followed by apical periodontal pathologies (25.1%) including apical periodontitis, apical granulomas, and odontogenic cysts (Lechien& et al., 2014).Due to iatrogenic cause is constituted the most frequent aetiologia, when a patient presents unilateral sinusitis after recent dental procedures, odontogenic maxillary sinusitis should be considered by clinicians (Lechien& et al., 2014, Kuan& Suh, 2017).

Pathogenesis of Odontogenic Sinusitis

Expansion of an inflammatory process from maxillary molars and premolars (and sometimes from canine teeth) to the maxillary sinus can be explained by close anatomical relationships. Hence, indicates that the root ends can be reflected in the Schneiderian membrane, small elevations or sinus base (Oberli&et al.,2007). If pulpal infection progresses to sinuses, it is called endo-artral syndrome (Selden, 1989, 1999). Endo-artralsyndrom characterized by; pulpal inflamation in a tooth whose apex approximates the floor of the maxillary sinus, pulp-induced periapical lesions, radiographic loss of lamina dura, a light radio-opaque mass to the sinus cavity above the top of involved tooth, representing a localized swelling and thickening of the sinus mucosa and radio-opacity of the sinus cavity with varying degrees. However, the fact that clinical cases do not always exhibit these five characteristics has created difficulties in diagnosis and treatment.

Evoluation of the endodontic lesion, the acute phase is more invasive and can cause bacteria to spread directly in the surrounding tissues and trigger a reaction by the Schneiderian membrane (Taschieri&et al.,2015). If endodontic treatment does not eliminate microorganisms, it may cause recurrence of apical periodontitis and may cause secondary formation of periapical lesion (Nair, 2006, Haapasolo&et al.,2011).

Diagnosis and clinical evaluation

Chronic rhinosinusitis is inflamation of the nose and paranasal sinuses, characterized by two or more symptoms, one of them nasal congestion or facial pain and reduction of smell more than 12 weeks and chronic rhinosinusitis can be of dental origin (odontogenic rinosinusitis) (Fokkens&et al.,2012). Many authors agree that the odontogenic sinusitis is caused by the rise of the dentoalveolar unit to the Scheniderien membrane. These conditions are advanced dental disease such as; caries, apical periodontitis and iatrogenic causes and surgical procedure (Ferguson, 2014, Fokkens&et al.,2012, Mehra& Murad, 2014).

OS is similar to non-odontogenic sinusitis and usually unilateral. it also offers clinical features that are not associated with ostium obstruction (Patel & Matsumoto, 2012). According to a previous review, maxillary opacification was present in 57% of the patients with maxillary sinusitis and clinical symptoms were reported to be manifested from 1 month to 15 years (Longhini& Ferguson, 2011). The main symptoms of odontogenic sinusitis are as follows; facial pain; toothache; nasal pain; runny nose; nasal congestion; discomfort and odor in face and gums. however, the symptoms may vary and many cases may be asymptomatic (Matsumoto &et al.,2015). When odontogenic and non-odontogenic sinusitis were compared, there was no difference between the symptoms, and purulent nasal discharge was the most common symptom in both sinusitis (Lee, 2008).

In dentistry accurate detection and emergency treatment of odontogenic maxillary sinusitis is an essential issue. The microbiology of odontogenic sinusitis differs from that of other maxillary sinusitis and requires treatment plans based on the source of infection (Nash & Wald, 2001, Nord, 1995). Anaerobic bacteriological flora is the most common cause of chronic odontogenic sinusitis and in acute odontogenic maxillary sinusitis, Gram-negative bacilli, Peptostreptococcus and Fusobacterium spp are the most important bacterial flora in the literature (Akhlaghi&et al.,2015). When these infections are treated only with antibiotics, the infection usually decreases and subsequently recurrs. It is therefore important to determine whether the sinusitis is caused by an odontogenic infection to provide appropriate treatment (Legert&et al.,2004). Also 11% of patients with sinusitis report toothache, the correct diagnosis is very important to prevent unnecessary dental treatment (Ferguson, 2014).

Two-dimensional imaging techniques are limited in detecting periapical and sinus changes. Panoramic radiographs may show significant changes, such as MT or fluid accumulation, but cannot display sinuses perfectly. In general, dental films and clinical evaluations cannot detect maxillary tooth infection that may cause OS (Patel &Matsumoto, 2012).

The prevalence of odontogenic sinusitis was reported to be 10-15% in previous studies (Ericson, 1992, Maloney &Doku, 1968). Now, 2-dimensional intaroral and panoramic x-rays are used radiographically to assess teeth and odontogenic infections. And also use of three-dimensional radiologic techniques such as multislice computed tomography (MSCT) or cone beam computed tomography (CBCT), odontogenic sinusitis has been reported at higher frequencies of up to 40% of chronic sinusitis patients (Lechien&et al.,2014). Distinction between unilateral and bilateral sinusitis is also important.

CBCT is a three-dimensional computed tomography form in which rigid tissues such as bones and teeth can be visualized in detail; therefore, this imaging method can eas-

ily demonstrate the apical pathology of maxillary molars.Even small apical destruction can be determined by cbct (Lofthag-Hansen &et al.,2011, Fredriksson &et al.,2017). CT is considered the gold standard in the visualization of sinuses and is used in dentistry, because it allows clinicians to evaluate axial and coronal images, thus evaluating any change between the sinus base and soft tissue of sinus with periapical lesion (Guijarro-Martinez &Swennen, 2011, Vidal &et al., 2017). However, it has some disadvantages compared to CBCT, for example: higher exposure to radiation (making the technique more costly and time consuming and less comfortable for patients), and due to wider field of view is insufficient to visualize details such as endodontic and periodontal pathologies. Thus, ct should be used when cbct is not available (Lofthag-Hansen &et al.,2007, Guijarro-Martinez Swennen, 2011).

A cone-beam computed tomography (CBCT) based study showed that only mucosal thickness (MT)> 2 mm is considered pathological and cannot be diagnosed with sinusitis. MT can be found in young, asymptomatic individuals, but is more common in adults and the elderly and is closely related to dental infections (Guerra-Pereira &et al.,2015).

Unilateral maxillary sinus opacification is the most prominent feature, sometimes without the associated sinonasal symptom, and the osteomeatal complex is not obstructed (Pokorny &Tataryn, 2013). Furthermore, the size of opacification is directly related to the likelihood of dental diseases; more severe sinus disease is more likely to originate from an odontogenic source (Bomeli&et al.,2009). Especially for odontogenic sinusitis, periapical radiolucency is the most common evidence. The maxillary sinus base should be thoroughly checked for CT loss, separation, foreign bodies or mucosal thickening. If there is a direct communication between the oral cavity and the maxillary sinus, pathogens may induce direct inflammation without a radiographically distinct abscess around the apex of the root (Pokorny &Tataryn, 2013). It was seen that 16-19% of patients with odontogenic sinusitis were bilateral (Saibene&et al.,2014).

Odontogenic sinusitis, which cannot be properly diagnosed, may lead to ineffective treatments that allow the spread of pulp or implantological disease to adjacent sinuses, the periorbital area, and the cavernous sinus. Although acute morbidity is rare, it may occur. pansinusitis, osteomyelitis, meningitis, blindness and even infection lead to intracranial spread (Taschieri&et al.,2017, Ferguson, 2014). Accurate diagnosis of odontogenic sinusitis is important in preventing these possible complications of the disease and improving the quality of life of the patient. Patient symptoms in odontogenic sinusitis are generally similar to those of CRS, and the lack of distinctive features makes the diagnosis difficult (Workman &et al.,2017). In a retrospective analysis of cases of odontogenic sinusitis, facial pain (88%), postnasal discharge (64%), and congestion (45%) were the most common first complaint but all three of these symptoms are non-specific and do not suspect an odontogenic cause (Pokorny &Tataryn, 2013). Presence or absence of toothache makes it difficult to diagnose and only toothache and hypersensitivity are not determinative for a odontogenic sinusitis. Only one-third of patients with true odontogenic sinusitis has toothache (Arias&et al.,2010, Longhini& Ferguson, 2011). In patients with odontogenic sinusitis, the relative lack of dental pain is thought to be due to the lack of pressure in the area of infection. also upper tooth pain itself may be a symptom of true rhinogenic sinusitis, because ipsilateral sinus pain may be reflected in this region (Patel & Ferguson, 2012). Foul nasal drainage or halitozis probably has the highest rate for odontogenic sinusitis, however, only 15–48% of patients were observed (Lee & Lee, 2010, Pokorny &Tataryn, 2013).

Treatment Modalities for Maxillary Odontogenic Sinusitis

Treatment is complicated and consists of the removal of the etiological factor and surgical treatment of sinusitis. There is still no gold standard for the treatment of this disease. The literature suggests the following main treatment options for OMS: Caldwell-Luc (CL), Functional Endoscopic Sinus Surgery (FESS), tooth extraction (treatment) and combinations of these treatment options (Aukstakalnis&et al.,2018). OMS treatment consists of removal dental infection and sinus surgery. Dental treatment or oral surgery is necessary part of treatment (Mattos &et al.,2016). Depending on the case, the endodontic treatment of the infected tooth varies from its extraction to the closure of the oroantral fistula. If the odontogenic etiology is ignored, treatment fails because the source of infection is left untreated (Mattos et al 2016). After removal of the odontogenic infection, Caldwell-Luc or endoscopic sinus surgery is necessary for the treatment of the disease.

Caldwell-Luc Surgery

Caldwell-Luc surgery is a treatment option for sinus disease. In classic CL operation, maxillary sinus is entered intraoperatively from canine fossa and the inflamed sinus mucosa is cleared. A counter-opening is performed to ensure sinus drainage in the lateral wall of the inferior nasal meatus and is usually loaded with temporary meatal and astral packs. This antrostomy provides theoretically the drainage of the re-accumulating inflammation and provides the suction toilet after surgery (Huang &et al.,2011). Caldwell-Luc treatment is used for the treatment of various maxillary sinus pathologies until endoscopic sinus surgery (Albu&Baciut 2010, Aukstakalnis&et al.,2018). Today, despite the high revision surgery and complication rates, CL is currently being used (Sirecia&et al.,2017). According to studies conducted, 9 to 15 percent of patients had to be re-operated (Albu&Baciut, 2010). Because this method requires hospitalization and general anesthesia, this leads to greater risk, higher cost, harm to the patient and more contraindications due to general anesthesia. Also during the Caldwell-Luc operation eliminates the natural, physiological mucosa which providing sinus function and maintaining drainage (Hajiioannou et al 2010). On the other hand, this technique helps when the clinician needs better access to the sinus, perisinus, or pterygomaxiller fossa. Caldwell-Luc is usually operated to remove foreign bodies, teeth or roots, implants, as well as sinus cysts, tumors, maxillary osteonecrosis, epistaxis control, irreversible mucosal change, sinusitis, mycotic fungal balls and facial trauma (Huang&et al.,2011, Sirecia&et al.,2017).

Caldwell-Luc surgery complications; intraoperative complications; bleeding and infrorbital nerve damage. Immediate postoperative complications; facial swelling, cheek discomfort, pain, significant hemorrhage and temperature elevation. Long term complications; facial asymmetry, facial and teeth numbness or paresthesia, oroantral fistulas, gingivolabial wound dehiscences, dacryocystitis, facial pain, teeth devitalization, recurrent sinusitis, recurrent polyposis, antral wall sclerozis (Aukstakalnis&et al.,2018).

Endoscopic Sinus Surgery

Inflammatory sinus mucosis can be taken using endoscopic sinus surgery (ESS). This process is also performed under general anesthesia for the treatment of chronic, acute, fungal or bacterial sinusitis and for various other sinus pathologies. An endoscope is passed through the nose and provides an image of the infected sinus mucosa, osteomeatal complex (condition, polyps, etc.). The natural ostium is surgically opened and only the infected sinus mucosa is removed and the basement membrane remains intact. Thus, the natural sinus mucosa is preserved and its mucociliary clearance is not impaired. This procedure requires high experience and precision due to proximal contact to anatomical structures such as orbital nerve, internal carotid and eyes (Akhlaghi&et al.,2015). In a study conducted with more than 250 patients, endoscopic sinus surgery and simultaneous elimination of the odontogenic source showed a success rate of 99% (Felisati&et

al.,2013). This technique is effective and useful when the object is at the top of the sinus. However, access to the lower part of the sinus is complicated by the acute angle from the inferior meatus to the bottom of the sinus (Dundar&et al.,2017).

Although this technique is suggested to be safer than CL surgery, some possible complications can be seen (Chou et al 2016). The retrospective study performed by Chou *et al.* showed the main complications of endoscopic sinus surgery. Major complications such as cerebrospinal fluid rhinorrhea, medial rectus muscle damage and retrobulbar hematoma were seen in five patients. Minor complications such as blood loss of more than 15% of the perioperative estimated total body blood volume, lamina papirraci violation, orbital cellulite and postoperative bleeding were seen in 73 patients (Chou &et al.,2016).

Antibiotic treatments

The main treatment of odontogenic sinusitis is surgical treatment and odontogenic sinusitis is usually resistant to antibiotic treatment.However, antibiotics are effective when combined with other appropriate treatments.Patients with odontogenic sinusitis have a different microbiological burden than CRS, and antimicrobial therapy should address this difference (Saibene&et al.,2016). In 70% of OS, bacteria were susceptible to amoxicillin / clavulanate, whereas in all cases they were susceptible levofloxacin, teicoplanin and vancomycin (Saibena&et al.,2016). The broad scope of polymicrobial and anaerobic populations can often be obtained with or without a penicillin (amoxicillin) and a beta-lactamase inhibitor with or without metronidazole. Therefore, dentists should use a different antibiotic therapy protocol that targets b-lactamase producing bacteria. For individuals who are allergic to penicillin, doxycycline is the most appropriate treatment (Zirk&et al.,2017). In addition, surgical treatment is preferred to treat aspergillosis of the maxillary sinus, and adjunctive antifungal therapy may not be necessary (Costa &et al.,2008).

Other treatment modalities

The improvement of MT after endodontic treatment is generally accepted in many cases and it is generally accepted that an interdisciplinary approach is necessary (Patel & Matsumoto 2012, Saibena&et al.,2016) In a pilot study in 29 patients, it was observed that only 30% of the patients improved their sinus patozis (mucositis) after endodontic treatment, despite the decrease in pain scores (Nurbakhsh&et al.,2011). The correct identification and treatment of the underlying dental disease, especially in endodontic and periodontal infections, is essential to provide ideal conditions for sinus intervention (Maillet et al. 2011). In cases where the presence of foreign bodies leads to sinusitis, surgical removal of the object is indicated. However, there is a no-treatment consensus for cases without symptoms or symptoms of maxillary sinusitis (Kim&et al.,2016).

An oro-antral communication is a pathological communication between the oral cavity and maxillary sinus that ismainly iatrogenic and occurs after a dental procedure. If this advertent communication is not repaired, epithelialization process is initiated andfinally a chronic fistulous pathway(oro-antral fistula) isformed (Bravo Cordero, Minzer Ferrer & Fernández, 2016, Hajiioannou& et al., 2010).The oro-antral fistula causes maxillary sinus inflammation through contamination from oral microbial flora andcauses acute sinusitis in approximately half of the cases within the first 24 to 48 hours(Bravo Cordero, Minzer Ferrer & Fernández, 2016). According to Wassmund, sinusitis was occurred in 60% of cases on the fourth day following the sinus exposure (Wassmund, 1939). If any treatment is not performed,maxillary sinusitis will occur in almost every case (~90%) within the first two weeks.Although most of small oro-antral communications that smaller than 2 mm can close spontaneously in the absence of infection, surgical closure is indicated in the cases with larger oro-antral defects(>4-5 mm) and/or communications lasting for more than 3 weeks.The Rehrmann or Môczárbuccal flaps, full- or split-thickness palatal pedicle flaps,

and more recently buccal fat pad flaps (Figure 3-4) areused in the repair of oro-antral fistulas.Closure of the oro-antral fistula is of crucial importance to prevent food and saliva accumulation that leading sinus contamination and subsequent infection, impaired healing and chronic sinusitis(Bravo Cordero, Minzer Ferrer & Fernández, 2016, Khandelwal &Hajira, 2017).

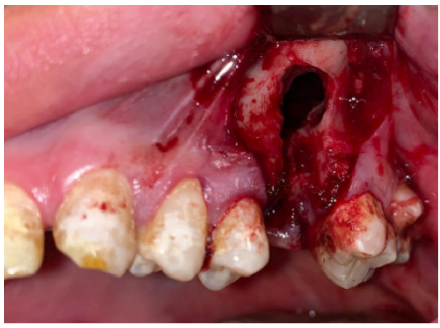


Figure 3. Intraoperative view shows large oro-antral defect following the removal of displaced root.

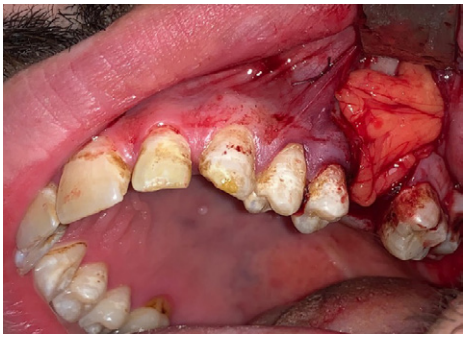


Figure 4. Intraoperative view of pedicled buccal fat pad in the closure of oro-antral communication.

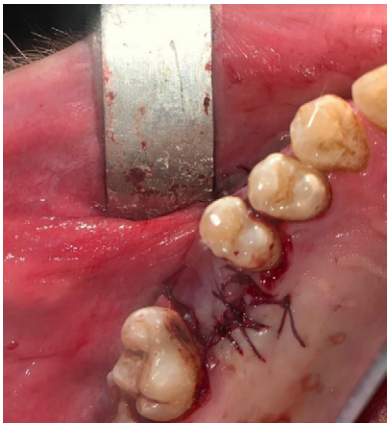


Figure 5. Primary closure of oroantral communication after the buccal fat pad secured with buccal advancement flap.

Literature suggests various options for odontogenic sinusitis treatment. The use of the Caldwell-Luc approach is nowadays limited and is recommended only when a better access to the sinuses is required, eg removal of large foreign bodies. Endoscopic sinus surgery is widely used today, while this technique removes the inflamed sinus mucosa, foreign bodies, and displaced teeth while maintaining the physiological function of the sinus. Furthermore, in order to obtain the perfect treatment result, the dental infection must be eliminated simultaneously or prior to sinus surgery (Aukstakalnis&et al., 2018).

REFERENCES

1. Akhlaghi F, Esmaeelinejad M, Safai P. Etiologiesand Treatments of Odontogenic Maxillary Sinusitis: A SystematicReview, *Iran Red Crescent Med* J. 2015; 17, e25536
2. Albu S, Baciut M. Failures in endoscopicsurgery of themaxillarysinus. *Otolaryngol Head Neck Surg. 2010;* 142: 196-201.
3. Arias-Irimia O, Barona-Dorado C, Santos-Marino J, Martinez-Rodriguez N, Martinez-Gonzalez J. (2009). Meta-analysis of the etiology of odontogenic maxillary sinusitis. *Medicina Oral Patología Oral y CirugiaBucal*, e70–e73. Doi:10.4317/medoral.15.e70
4. Aukstakalnis R, Simonaviciute R, Simuntis R. Treatment options for odontogenic maxillary sinusitis: a review. Stomatologija, *Baltic Dental and Maxillofacial Journal*, 2018:20;22-6.
5. Bomeli SR, Branstetter BFt, Ferguson BJ. Frequency of a dental source for acute maxillary sinusitis. *Laryngoscope* 2009; 119:580–584.
6. Bravo Cordero G, MinzerFerrer S, Fernández L. (2016). Odontogenic Sinusitis, Oro-antral Fistula and Surgical Repair by Bichat's Fat Pad: Literature Review. *ActaOtorrinolaringologica* (English Edition), 67, 107-113.
7. Chou TW, Chen PS, Lin HC, Lee KS, Tsai HT, Lee JC et al. Multiple analyses of factors related to complications in endoscopic sinus surgery. *J Chin Med Assoc* 2016; 79: 88-92
8. Costa F, Polini F, Zerman N, et al. Functional endoscopic sinus surgery for the treatment of Aspergillus mycetomas of the maxillary sinus. *Minerva Stomatol* 2008; 57:117–125
9. Dundar S, Karlidag T, Keles E. Endoscopic Removal of a Dental Implant From Maxillary Sinus. *J Oral Implantol*. 2017; 43: 228-231
10. Ericson S. Conventional and computerized imaging of maxillary sinus pathology related to dental problems. *Oral Maxillofac Surg Clin North Am* 1992;4(1):153–181
11. Felisati G, Chiapasco M, Lozza P, Borloni R. Sinonasal complications resulting from dental treatment: outcome oriented proposal of classification and surgical protocol. *Am J RhinolAllergy* 2013; 27: 101–106.
12. Ferguson M. Rhinosinusitis in oral medicine and dentistry. *Aust Dent J*. 2014;59:289–295.
13. Fokkens WJ, Lund VJ, Mullol J, et al. European position paper on rhinosinusitis and nasal polyps*. Rhinology Suppl*. 2012;23:1–298
14. Fredriksson MV, Öhman A, Flygare L, Tano K. When maxillary sinusitis does not heal: findings on cbct scans of the sinuses with a particular focus on the occurrence of odontogenic causes of maxillary sinusitis. *Laryngoscope Investigative Otolaryngology* 2017: 2;442-446.
15. Guerra-Pereira I, Vaz P, Faria-Almeida R, et al. CT maxillary sinus evaluation: a retrospective cohort study. *Med Oral Patol Oral CirBucal*. 2015;20:e419–e426

16. Guijarro-Martinez R, Swennen GR. Cone-beam computerized tomography imaging and analysis of the upper airway: A systematic review of the literature. *Int J Oral Maxillofac Surg.* 2011; 40:1227–1237.
17. Haapasalo M, Shen Y, Ricucci D. Reasons for persistent and emerging post-treatment endodontic disease. *Endod Topics* 2011; 18: 31–50.
18. Hajiioannou J, Koudounarakis E, Alexopoulos K, Kotsani A, Kyrmizakis DE. (2010). Maxillary sinusitis of dental origin due to oroantral fistula, treated by endoscopic sinus surgery and primary fistula closure. *The Journal of Laryngology & Otology*, 124(09), 986–989. Doi: 10.1017/s0022215110001027
19. Huang IY, Chen CM, Chuang FH. Caldwell-Luc procedure for retrieval of displaced root in the maxillary sinus. *Oral Surg Oral Med Oral Pathol Oral RadiolEndod*. 2011; 112: 59-63.
20. Khandelwal P, Hajira N. (2017). Management of Oro-antral Communication and Fistula: Various Surgical Options. *World Journal of Plastic Surgery*, 6(1), 3-8.
21. Kim SJ, Park JS, Kim HT, et al. Clinical features and treatment outcomes of dental implant-related paranasal sinusitis: a 2-year prospective observational study. *Clin Oral Implants Res*. 2016; 27:e100–e104.
22. Kuan EC, Suh JD. (2017). Systemic and Odontogenic Etiologies in Chronic Rhinosinusitis. *Otolaryngologic Clinics of North America*, 50(1), 95–111. Doi: 10.1016/j.otc.2016.08.008
23. Lechien JR, Filleul O, Costa de Araujo P, Hsieh JW, Chantrain G, Saussez S. (2014). Chronic Maxillary Rhinosinusitis of Dental Origin: A Systematic Review of 674 Patient Cases. *International Journal of Otolaryngology*, 2014, 1–9. Doi: 10.1155/2014/465173
24. Lee JY. Unilateral paranasal sinusd iseases: analysis of the clinical characteristics, diagnosis, pathology, and computed tomography findings. *Acta Otolaryngol.* 2008;128:621–626.
25. Lee KC, Lee SJ. Clinical features and treatments of odontogenic sinusitis. *Yonsei Med J* 2010; 51:932–937.
26. Legert KG, Zimmerman M, Stierna P. Sinusitis of odontogenic origin: Pathophysiological implications of early treatment. *Acta Otolaryngol* 2004;124(6):655–663.
27. Little RE, Long CM, Loehrl TA, Poetker DM. (2018). Odontogenic sinusitis: A review of the current literature. *Laryngoscope Investigative Otolaryngology*, 3(2), 110–114. Doi: 10.1002/lio2.147
28. Lofthag-Hansen S, Huumonen S, Gr€ondahl K, et al. Limited conebeam CT and intra oral radiography for the diagnosis of periapical pathology. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2007;103:114–119
29. Longhini AB, Ferguson BJ. Clinicalaspects of odontogenic maxillary sinusitis: a case series. *Int Forum Allergy Rhinol*. 2011;1:409–415.
30. Maillet M, Bowles WR, McClanahan SL, et al. Cone-beam computed tomography evaluation of maxillary sinusitis. *J Endod.* 2011;37:753–757
31. Maloney P, Doku H. Maxillary sinusitis of odontogenic origin. *J Can Dent Assoc* 1968;34(11):591–603.
32. Matsumoto Y, Ikeda T, Yokoi H, et al. Association between odontogenic infection sand unilateral sinus opacification. *Auris Nasus Larynx*. 2015;42:288–293.
33. Mattos JL, Ferguson BJ, Lee S. Predictive factors in patients undergoing endoscopic sinus surgery for odontogenic sinusitis. *Int Forum Allergy Rhinol*. 2016; 6: 697-700.

34. Mehra P, Murad H. (2004). Maxillary sinus disease of odontogenic origin. *Otolaryngologic Clinics of North America*, 37(2), 347–364. Doi: 10.1016/s0030-6665(03)00171-3

35. Mehra P, Murad H. Maxillary sinus disease of odontogenic origin. *Otolaryngol Clin North Am.* 2004;37:347–364.

36. Nair PNR. On the causes of persistent apical periodontitis: a review. *Int Endod J* 2006; 39: 249–81.

37. Nash D, Wald E. Sinusitis. *Pediatr Rev.* 2001;22(4):111–7. [PubMed: 11283323].

38. Nord CE. The role of anaerobic bacteria in recurrent episodes of sinusitis and tonsillitis. *ClinInfectDis.* 1995;**20**(6):1512–24. [PubMed: 7548501]

39. Nurbakhsh B, Friedman S, Kulkarni GV, et al. Resolution of maxillary sinus mucositis after endodontic treatment of maxillary teeth with apical periodontitis: a cone-beam computed tomography pilot study. *J Endod*. 2011;37:1504–1511.

40. Oberli K, Bornstein MM, vonArx T. Periapical surgery and the maxillary sinus: radiographic parameters for clinical outcome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; 103: 848–53

41. Patel NA, Ferguson BJ. Odontogenic sinusitis: an ancient but under appreciated cause of maxillary sinusitis. *Curr Opin Otolaryngol Head Neck Surg* 2012; 20:24–28

42. Pokorny A, Tataryn R. Clinical and radiologic findings in a case series of maxillary sinusitis of dental origin. *Int Forum AllergyRhinol* 2013; 3:973–979.

43. Saibene AM, Pipolo GC, Lozza P, et al. Redefining boundaries in odontogenic sinusitis: a retrospective evaluation of extramaxillary involvement in 315 patients. *Int Forum Allergy Rhinol* 2014; 4:1020–1023.

44. Saibene AM, Vassena C, Pipolo C, et al. Odontogenic and rhinogenic chronic sinusitis: a modern microbiological comparison. *Int Forum Allergy Rhinol* 2016; 6:41–45

45. Selden HS. Endo–antral syndrome and various endodontic complications. *J Endod* 1999; 25: 389–93.

46. Selden HS. The endo–antral syndrome: an endodontic complication. *J AmDentAssoc* 1989; 119: 397–402

47. Sirecia F, Nicolottib M, Battagliac P, Sorrentinob R, Castelnuovoc P, Canevari FR. Canine fossa puncture in endoscopic sinussurgery: report of two cases. *Braz J Otorhinolaryngol*. 2017; 83: 594-599.

48. Taschieri S, Torretta S, Corbella S, Del Fabro M, Francetti L, Lolato A, Capaccio P. Pathophysiology of sinusitis of odontogenic origin. *Journal of Investigative and Clinical Dentistry.* 2015:0;1-7

49. Tucker MR. (2014) Odontogenic Diseases of the Maxillary Sinus. Hupp JR, Tucker MR, Ellis E (Ed.), *InCurrent Oral and Maxillofacial Surgery* (p. 383-388). USA: Elsevier Publishing.

50. Vidal F, Coutinho TM, Ferreira DC, Souza RC, Gonçalves LS. Odontogenic sinusitis: a comprehensive review. *Acta Odontologica Scandinavia* 2017:8;623-633

51. Wassmund M. (1939).*Lehrbuch der praktischenChirurgiedesMundesund der Kiefer*. Leipzig, Germany: Ambrosius Barth.

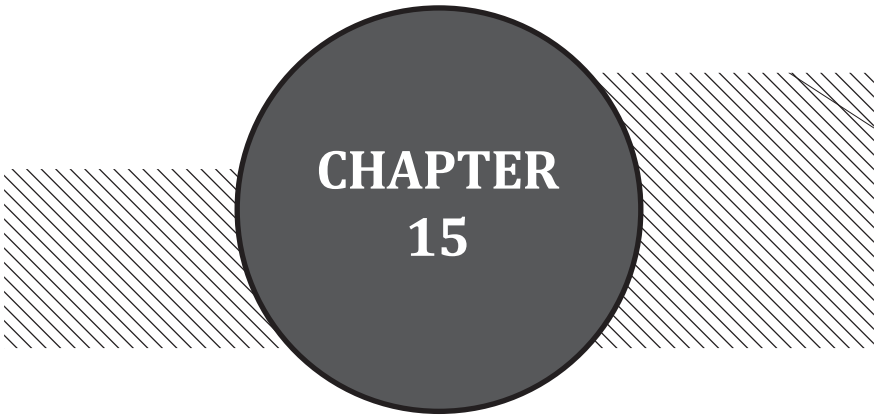
52. Workman AD, Granquist EJ, Adappa ND. Odontogenicsinusitis: developments in diagnosis, microbiology and treatment. *Curr Opin Otolaryngol Head Neck Surg* 2017;25:00-00.

53. Yildirim D, Eroglu M, Salihoglu M, Yildirim AO, Karagoz H, Erkan M. (2013) The Relationship between Dental Indentation and Maxillary Sinusitis. *Open Journal of Medical Imaging*, 03(02), 65–68. Doi: 10.4236/ojmi.2013.32009

54. Zirk M, Dreiseidler T, Pohl M, et al. Odontogenic sinusitis maxillaris: a retrospective study of 121 cases with surgical intervention. *J CraniomaxillofacSurg* 2017; 45:520–525.

Nutrigenetics: The Triangel Of The Gene-Nutrient-Disease

İbrahim KESER[1]



[1] Prof. Dr., Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, Antalya, Turkey

INTRODUCTION

At the beginning of 21st century, new sub-areas (omics) began to emerge with the completion of the Human Genome Project. The mean flow of genetic information and the sub-areas (ome and omics) of the Human Genome Project are given in Figure 1. One of these new areas is the concept of Nutritional Genomics, which tends to be replaced by the old nutrient-gene interactions concept (1). It has been shown that a large number of genetic polymorphisms may be effective on the basis of differences in the structure and function of proteins involved in nutritional metabolism. Nutritional genomic area consists of two parts: Nutrigenomics, which is the investigation of the interaction between the dietary components and the genome; the second is Nutrigenetics; it deals with genetic differences that determine the response to dietary components (2).

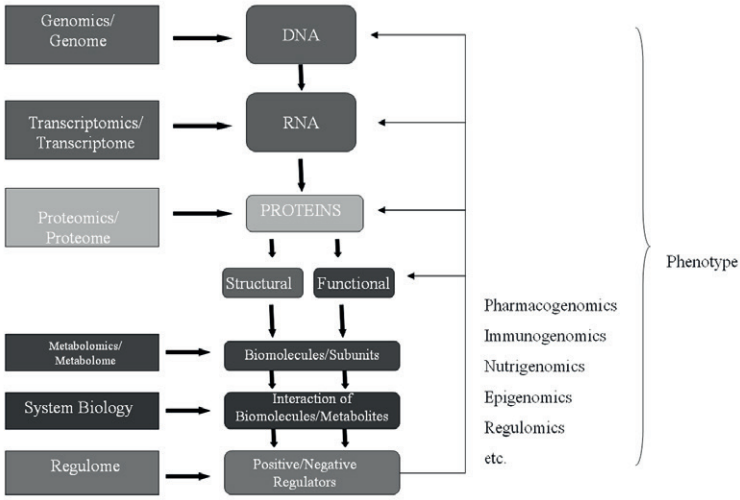


Figure 1. The mean flow of genetic information and the sub-areas (ome and omics) of the Human Genome Project

Nutrients can interact with the genetic material in the cell as environmental factors for each cell in our body. The content of a large part of the nutrients has been shown to play a role in the metabolism and repair of our genetic material, DNA. Nutrigenomics and nutrigenetics require an understanding of nutrition, genetics, biochemistry and a range of "omic" technologies to investigate the complex interaction between genetic and environmental factors relevant to metabolic health and disease. It is also known that there are many dietary factors that act as cofactors or substrates on metabolic pathways (3). Although nutrients are effective in the development of a particular phenotype, the fact that the individual genotype causes individual nutrition should also be taken into account (Figure 2).

The central role of genetic code in identifying human genome stability and health-related outcomes such as developmental disorders, degenerative diseases and cancer is well known (4). The etiology of multifactorial diseases, ie, complex chronic diseases, is clearly associated with both environmental and genetic factors (5). On the basis of diseases such as heart, diabetes and obesity seen in the adult period, it is suggested that many gene expression and nutrient-specific cells' structure and function during prenatal and early postnatal development of other environmental factors are affected. (6). To show that

nutrients have an effect on DNA stability, repair and different gene expression processes, nowadays turned all attention into nutritional science as nutrigenetics (7). As a result, genetic and epigenetic events can be affected by a large number of dietary components and therefore human health may be affected (8). Single nucleotide polymorphisms (SNPs) in the human genome are the most common genetic variants. SNPs occur normally throughout a person's DNA. They occur almost once in every 1,000 nucleotides on average, which means there are roughly 4 to 5 million SNPs in a person's genome. SNPs are normally found in at least 1% of the population (5). Many studies in humans have shown that there is interaction between SNPs in various genes in the metabolic response to diet. Moreover, SNP analysis contributes as a potential molecular tool to investigate the role of nutrition in human health, disease and identification of optimal diets (9). Some of these genetic differences, however, have proven to be very important in the study of human health.

Researchers have found SNPs that may help predict an individual's response to certain drugs, susceptibility to environmental factors such as toxins, and risk of developing particular diseases. SNPs can also be used to track the inheritance of disease genes within families. Future studies will work to identify SNPs associated with complex diseases such as heart disease, diabetes, and cancer. Nutrients entering our body and our genome interact in two ways. First, the nutrients may either induce or suppress gene expression. Thus, individual phenotype can change health and disease direction. Secondly, unlike the former, SNPs can alter the biological activity of important metabolic pathways and affect the ability of intermediary molecules and nutrients to interact with them (Figure 2).

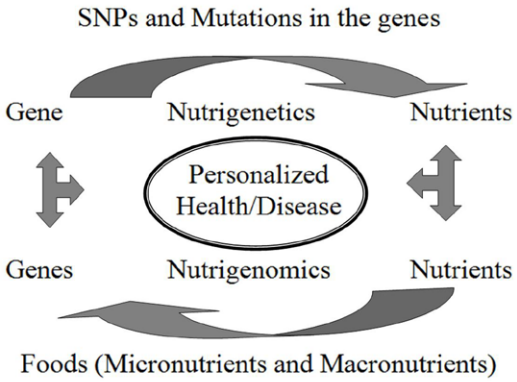


Figure 2. The relationship between the genes-nutrients (Nutrigenetics) and nutrients-genes (Nutrigenomics)

Nutrition and Genome Relationship

A new concept that expresses the interaction between nutrition and genome, the interaction between nutrients and the cellular and genetic structure, revealed nutritional genomics (10). In fact, nutritional genomics describes the understanding of biochemical genetic bases and reactions in human nutrition and the state of interactions at molecular genomic levels (11). Some conclusions can be drawn in these interactions. In nutritional genomics, general nutritional chemicals are effective on the human genome. This effect can be direct or indirect upon gene expression. In some circumstances, diet may be a serious risk factor for some diseases. Some genes, regulated by diet, with normal and

widespread variants, may play a role in the onset, progression and severity of chronic diseases. The relationship between the individual's genetic background and diet can affect the balance between health and disease. Individual nutritional requirements and nutrient intervention depending on the individual genotype may be needed to prevent, alleviate or ameliorate some chronic diseases.

Dr Richards O. Brennan has firstly used the term "nutrigenetics" in 1975 in his book Nutrigenetics (12). Nutrigenetics refers to understanding how the individual's genetic background affects diet (13). It is a new field of science developed in the context of the study of gene and nutrient interaction. The idea that negative diet/genome interaction may cause disease is not new. Unsuitable diet for any genotype may be a risk factor for monogenic and polygenic disease (10, 14). Genetic polymorphisms may affect the response to environmental elements such as changes in the circulating concentrations of chemicals and the enzymatic activity regulating the activity of their metabolites (5). In addition, some diseases such as PKU, long-term fatty acid oxidation and metabolic disorders associated with iron absorption (hemochromatosis), as examples of genetic variation, are genetic diseases that can be reasonably well managed with dietary restrictions (15).

As mentioned earlier, SNPs are found to be spread throughout the whole genome. In this context, considering the anatomy of a gene, SNPs can be found both in the encoded regions and in the regulatory sites of the gene. SNPs can reveal different clinical phenotypes depending on their position in the gene. Here, two groups are given according to the placement of the gene polymorphisms. (Figure 3)

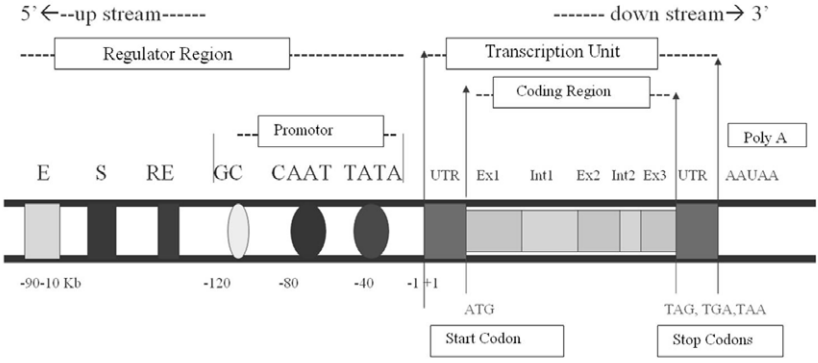


Figure 3. Possible target regions of SNPs and mutations in the human gene's anatomy. (E: Enhencer, S:Silencer, RE: Response element, UTR: Untranslated region, Ex:Exon, Int:Intron).

One example of SNP evaluated in nutrigenetic tests is rs762551 (−164 A>C) in CYP1A2. This gene encodes CYP1A2, a xenobiotic metabolizing enzyme responsible for ~13% of hepatic cytochrome P450 activity (16) and, among other compounds, for caffeine (1,3,7-trimethylxanthine) metabolism (17). The presence of C allele (also referred to as CYP1A2 *1F) associates with reduced enzyme metabolizing capacity compared to the presence of two allele A (also designed as CYP1A2 *1A/ *1A). Therefore, individuals carrying two major alleles (AA) are classified as "fast" metabolizers, while those carrying minor alleles (one or two) are "slow" metabolizers of caffeine. The main clinical relevance of being fast or slow metabolizer of caffeine is related to the risk of cardiovascular disease. In this context, slow metabolizers of caffeine showed higher risk of acute myocardial infarction with increased coffee consumption (>500 mL/day), which was not observed in fast metabolizers (18).

Another example of changes in enzyme activity with the variations in the encoded region of Glucose-6-phosphate dehydrogenized (G6PD) gene is given. Glucose 6-phosphate dehydrogenase (G6PD) deficiency is one of the most frequent red cell enzymopathies affecting some 400 million people globally (19). G6PD gene located on human chromosome Xq28 (20), is associated with at least 140 distinct disease producing mutations (21). These mutations are significant in causing hemolysis, favism, neonatal hyerpbilirubinemia(22). Nucleotide 1311 C>T polymorphism at exon 11 of G6PD gene is widely prevalent in various populations of the world.

The best known example, the Lactase gene (LCT) polymorphisms, which illustrate how SNPs responsible for lactase intolerance change the gene expression, as an example of polymorphisms in the regulator region located at the 5 'end of the enzyme gene. Lactose is the main carbohydrate in milk and is a major energy source for most young mammals. The enzyme responsible for hydrolysis of lactose into glucose and galactose is lactase. Without this enzyme, mammals are unable to break down and thus use lactose, and since milk is the essential component of young mammals' diet, lactase activity is fundamental to the early development of most mammals. After the weaning period is over, lactase production usually declines, although the mechanisms and evolutionary reasons for this downregulation are not fully understood. However, some humans continue to express lactase throughout adult life, and are thus able to digest the lactose found in fresh milk. This trait is called lactase persistence (LP). The LP trait frequency is found in around 35 per cent of adults living in the world today (23,24), but varies widely among human populations. In recent years, a number of single nucleotide polymorphisms (SNPs) have been found in association with the LP trait in different populations. The first to be identified, *–13910*T*, is found not in the *LCT* gene (the lactase gene) but within an intron of a neighbouring gene, *MCM6* (25). *In vitro* studies indicate that this nucleotide change affects lactase promoter activity (26,27) and thus is highly likely to cause LP.

Another important example of gene polymorphism is Glutathione peroxide. The relationship between selenium supplementation and human liver, colon, prostate and lung cancer incidence is shown. However, no individual can equally give equal answers. Glutathione peroxide is a selenium-dependent enzyme that acts as an antioxidant enzyme. The polymorphism in the codon 198 of human glutathione peroxides causes the proline to the leucine amino acid and is associated with an increased risk of lung cancer. Researchers (Pro / Lue) showed that individuals with genotypes were 80% greater for lung cancer and 130% higher risk of genotypes (Lue / Lue) than the genotype risk (Pro / Pro). The leucine-encoding allele was less responsive to increased activity due to selenium supplementation than the proline-containing allele (8).

Glutathione peroxidase 1 gene (GPX1,OMIM: 138320), encoding for an important antioxidant selenium-dependent enzyme that catalyses the breakdown of hydrogen peroxide and organic hydroperoxides, resulting in the oxidation of glutathione (GSH) to glutathione disulphide (GSSG) (28). GPX1 has been reported to be implicated in oncogenesis and progression of several cancer types (29), it's overexpression suppresses intracellular ROS which attenuates growth factor receptor activation mediated by oxidative stress, resulting in decreased cellular proliferation. GPX1 is located on chromosome position 3p21 and contains a genetic polymorphism (rs1050450) C>T that results in either a proline (Pro) or leucine (Leu) at codon 198, described to be a risk factor for the development of various cancers, including lung cancer (30), and prostate cancer, (31).

Another example for the metabolomics, the polymorphism of methylen tetrahydrofulate reductase (MTHFR) gene is Ala222Val that affects folate metabolism. It increases the conversion of dUMP to dTMP and leads to more folate-dependent thymidine biosynthesis and folate deficiency (33). This polymorphism is a risk factor for spontaneous abortions and decreased fetal viability, thus maternal folate supplementation can be useful for individuals with this polymorphism (34).

Nutrigenomics

Nutrigenomics is a new sub-field in the Human Genome Project that investigates the interaction of nutrients in the system biology with the whole genome (Figure 1). It aims to determine the effects of many nutrients on the genome (13) including macro nutrients and micronutrients. Investigate the interaction between genes and nutrients or nutrient bioactive and their effects on human health (35). In other words, the effect of nutrients on the transcriptional activity of genes, gene expression and the heterogeneous response of gene variants is also referred to as "Nutrigenomics". Nutrigenomics also describes the use of functional genomic tools to study a biological system to understand how nutritional molecules affect metabolic pathways and homeostatic control. This discipline will reveal the most appropriate form of diet in a number of nutritional changes. Whereas Nutrigenetics will provide clinicians with critical information to help determine the optimal diet for a particular individual, that is, personalized nutrition (13). Transcriptomics, proteomics and metabolomics are also technologies applied in Nutrigenomics studies. There are numerous studies that suggest that nutrients can alter the genes expression of through signal transduction, gene regulation, chromatin structure, and protein function (35).

Epidemiological studies in the world reveal a relationship between the incidence of food intake and the incidence of chronic diseases and the severity of the disease (36,37). Many nutritional diseases (cardiovascular diseases, metabolic syndromes, obesity, and type 2 diabetes) have been identified and are based on polygenic and multifactorial interaction. Several genes and gene variants, as well as various environmental factors (especially diet), have a role in the onset and progression of these diseases (38).

Chemicals in the diet can directly or indirectly affect gene expression. Nutrients at the cellular level can act as ligands for transcription factor receptors (39,40) or can be metabolized by primary or secondary metabolic pathways. Thus, they alter the substrate or intermediate concentrations and consequently affect the signaling pathways positively or negatively (41-43).

Nutrients, Trancription Factors and Gene Expression

Nutrients and transcription factors (TFs) can interact and alter the expression of the target gene. One of the most important groups of nutrient sensors is PPARs TFs with a lot of members in the human genome. Most receptors in this superfamily bind nutrients, their metabolites and affect the expression of specific genes involved in numerous metabolic processes in the liver, including fatty acid oxidation, ketogenesis, gluconeogenesis, amino acid metabolism, cellular proliferation, and acute phase response (44). Some fatty acids such as palmitic, oleic, and linoleic are ligands for PPAR-δ (45). In the specific interation of ligand and receptor, these nuclear receptors act as sensors for fatty acids. Lipid sensors usually heterodimerize with retinoid receptor, whose ligand is derived from another dietary chemical, vitamin A, and hyperforin, bind directly to nuclear receptors and influence gene expression.

Some chemicals taken together with the diet indirectly regulate some of the TFs involved in gene expression. Sterol regulatory element binding proteins (SREBPs) are activated by protease cleavage, which is regulated by low changes in insulin / glucose levels (46).

Also, alternative splicing or differential splicing, is a regulated process during gene expression that results in a single gene coding for multiple proteins. In this process, particular exons of a gene may be included within or excluded from the final, processed messenger RNA (mRNA) produced from that gene (47). Consequently, the proteins translated from alternatively spliced mRNAs will contain differences in their amino acid sequence and, often, in their biological functions. As a result, differences between individuals in terms of responses to nutrients will arise with different options of the same gene. (Figure 4).

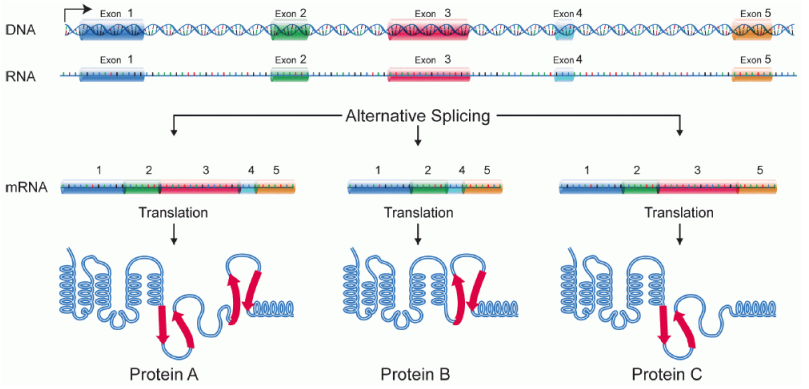


Figure 4. Alternative pre-messenger RNA splicing from a gene(47).

Carbohydrate-sensitive element binding protein (chREBP) is a large TF activated in response to high glucose. High glucose is activated by response, levels and reversible phosphorylation (48). This DNA binding protein acts as an effector of lipogenic gene expression (49). In addition, dietary chemicals can directly affect signal transduction pathways. For example, green tea contains the polyphenol, 11-epigallocatechin-3-gallate (EGCG) of EGCG. This polyphenol inhibits tyrosine phosphorylation of the Her-2 / neu receptor and the epidermal growth factor receptor via NF-kB. Activation of the NF-kB pathway is associated with some types of breast cancer (50,51). In addition, micronutrients, also called omega-3, and omega-6 fatty acids can also affect gene expression.

Approximately 40 micronutrients are needed in the human diet. The specific subnormal levels of micronutrients have been demonstrated the relation with vitamin B, E and carotenoids of CVD, folate and carotenoids of cancer, neural tube defects of folate and vitamin D of bone mass (52). Deficiencies of B6, B12 and folate, for example, are associated with increased serum homocysteine levels. Hyperhomocysteinemia is a risk factor and marker for coronary artery disease. Vit B12, folic acid, B6, niacin, C or E, iron or zinc deficiency, single and DNA in the case of double-helical breaks, seems to mimic the harmful radiation (53).

Blood glucose concentration is affected differently from simple and complex carbohydrates. Foods with a high glycemic index (GI) will increase insulin production and reduce the synthesis of insulin receptors. In addition, a high glucose concentration induces the transcription of several glycolytic and lipogenic pathway genes (54). Thus, dietary chemicals are regularly ingested and indirectly and directly involved in regulation gene expression, which follows that a subset of genes regulated by the diet should be included in the initiation, progression and severity of the disease (55,56).

Nutrition Epigenetics / Nutriepigenetics

The term “epigenetic” is used in gene expression. It occurs without alteration of the DNA sequence. The role of epigenetic regulation in the development of significant and

necessary to win stable expression or suppression of genes in specific cell types or defined developmental stages (57). Epigenetic changes may affect cell cycle, control, DNA damage, apoptosis, invasion, pressure and aging (8).

Most regulatory proteins that contain DNA are involved in methyltransferases, methyl-cytosine guanine dinucleotide binding proteins, histone modifying enzymes, chromatin remodeling factors and their multimolecular complexes in epigenetic processes (8). The best studied epigenetic modification is DNA methylation, and in mammals, the genome occurs in many cytosine residues followed by guanine cytosine islets (CpG islands), and in most cases methylation in these regions induces gene imprinting. However, this phenomenon may lead to the expression of neighboring genes (57). Research has found that DNA methylation depends on bioactive food components ranging from alcohol to zinc (8).

Nutrients and Continuity of the Genome

In human life, the whole genome needs to be healthy. Because very minor damage (such as a single point mutation in a gene) may have very important clinical effects. This improvement is based on various dietary factors from functionality such as DNA metabolism and repair, non-nutritional cofactors or substrates.

Some nutrients (vitamin E and C, carotenoids) play a role in the prevention of DNA oxidation. Folate is involved in the prevention of uracil addition to DNA. Methionine, cholin, folate and vitamin B12 are needed for the continuation of CpG methylation in DNA. In order to regulate telomere length, niacin and folate are important molecules (58-60).

Many chronic diseases occur with polygenic interactions. This group is a result of interactions of diseases, genes and environmental factors. The individual genotype lies on the basis of the dietary requirement and use. Due to this, the tad individual nutrition Buna situation arises. This detection show us that individual genotype-individual dietary can be used for the control or treatment of many chronic diseases such as cardiovascular diseases, cancer and metabolic syndromes (44).

The Effect of Nutritional Deficiency on Genome Damage

As mentioned earlier, the nutritional status affects the stability of the genome and the lack of certain micronutrients can cause damage to the genome that has a vital effect. Studies have shown that some of the micronutrients (retinol, nicotinic acid, vitamin E, folate, Ca, β-carotene, riboflavin, biotin and pantothenic acid) affect genome stability in humans in vivo (4). Folate and vitamin B12 are two essential factors for DNA replication, repair and maintenance of DNA methylation patterns.

The effect of nutrient deficiency on genomic structure has been investigated. Studies in human cells, both in vivo and in vitro, clearly show that folate and vitamin B12 deficiencies and high plasma homocysteine, chromosomal fragile regions, chromosomal breaks, DNA hypomethylation, and excessive uracil expression in DNA are associated. Nicotinic acid (niacin) also plays a fundamental role in chromosome integrity and in reducing the risk of cancer(61).

Some of the most important factors contributing to DNA damage are reactive oxygen species (ROS). In contrast, enzymes such as antioxidants (Vit C and E), superoxide dismutase, catalase, and glutathione peroxidase can control ROS-induced lipid and protein oxidation. Developmental, many degenerative diseases and aging are partly due to DNA damage. Therefore, it is important to determine and use the optimal requirements of essential minerals and vitamins to prevent nuclear and mitochondrial DNA damage (61).

Another consequence of genome damage on human health is infertility. Specific micronutrient deficiencies cause genome damage. This may lead to developmental

disorders in the fetus during prenatal period or to increase the risk of cancer in children. For example, inadequate Vit C uptake results in oxidation of sperm DNA; folate deficiency increases the risk of neural tube defect (NTD) and genomic damage. It has been shown that the risk of childhood leukemia increases in children of mothers who do not receive enough folic acid support during pregnancy. In addition, zinc deficiency causes oxidative damage in DNA. As a result, teratogenic effects occur with disrupt DNA repair (61, 62).

One of the most important units of a functional chromosomal structure is telomeres. Telomeres are form nucleoprotein structures, close the ends of the chromosomes and maintain chromosome stability. Degeneration of telomeres leads to whole chromosomal instability and chromosomal fusion and thus gene amplification, which is an important risk factor for cancer. In addition, oxidative stress plays a role for telomere shortening, too. Studies have shown that telomere shortening has been observed in many conditions such as obesity, immune dysfunction, cancer, psychological stress and cardiovascular diseases. In vitro studies have found that antioxidant treatment approaches prevent telomere damage (3).

CONCLUSIONS

Genomics related with nutrients illuminates the interaction between nutrients, metabolic intermediates and mammalian genome. The response to bioactive food components is dependent on the genetic background "nutrigenetics effects" that may affect absorption and metabolism targets or impact regions. Similarly, DNA methylation and other epigenetic events depend on the response to food components. The ability of bioactive food components to affect gene expression patterns "nutrigenomics effects" is also a factor determining the overall response. Finally, bioactive food components can affect protein synthesis, degradation, and post-translational modification. An understanding of the relationships between human genetics diversity, genome function and dietary components will ensure precise manipulation of genome function and stability throughout the life cycle for optimal human health and disease prevention. In addition to learning more about gene-nutrient interaction, changes in diet and single nutrient interventions can help protect against cancer, reduce the occurrence of cardiovascular and other chronic diseases, and perhaps increase human life.

REFERENCES

1. Sharanova NE, Vasil'ev AV. Postgenomic Properties of Natural Micronutrients. Bull Exp Biol Med. 2018 Nov;166(1):107-117
2. Subbiah MT (2007). Nutrigenetics and nutraceuticals: the next wave riding on personalized medicine. Transl Res, 149(2): 55–61.
3. Bull C, Fenech M (2008). Genome health nutrigenomics and nutrigenetics: nutritional requirements for chromosomal stability and telomere maintenance at the individual level. Proc Nutr Soc, 67(2):146-56.
4. Fenech M (2008). Genome health nutrigenomics and nutrigenetics-diagnosis and nutritional treatment of genome damage on an individual basis. Food Chem Toxicol, 46(4): 1365-70.
5. El-Sohemy A (2007). Nutrigenetics. Forum Nutr, 60: 25-30.
6. Dolinoy DC, Jirtle RL (2008). Environmental epigenetics in human health. Environ Mol Mutagen, 49(1): 4-8.
7. Paoloni-Giacobino A, Grimble R, Pichard C (2003). Genetics and nutrition .Clin Nutr, 22(5): 429-35.
8. Trujillo E, Davis C, Milner J (2006). Nutrigenomics, Proteomics, Metabolomics, and the Practice of Dietetics. J Am Diet Assoc, 106(3): 403-13.

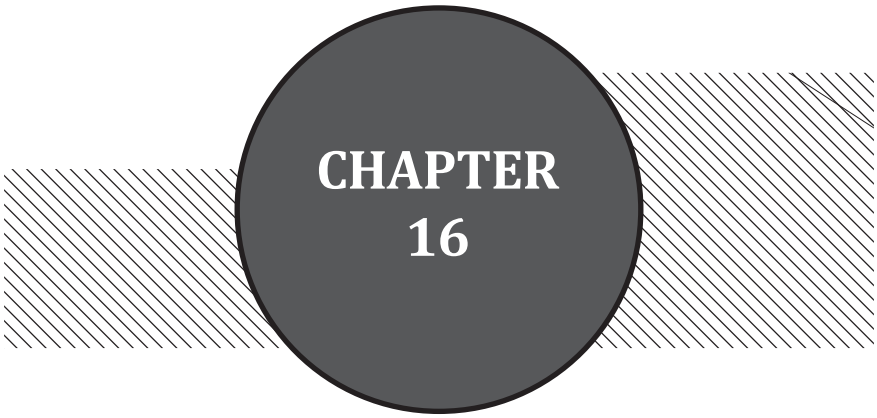
9. Ferguson LR. (2006). Nutrigenomics: integrating genomics approaches into nutrition research. Mol Diag Ther, 10(2):101-8.
10. Kaptur J, Raymond LR (2004). Nutritional genomics: the next frontier in the Post genomic era. Physiol Genomics, 16: 167-77.
11. Milner J. A (2004). Molecular Targets for Bioactive Food Components. J Nutr, 134(9): 2492S -2498S.
12. Simopoulos AP (2010). Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk Experi Biol Med, 235:785-95.
13. David M (2005). Mutch, Walter Wahli, Gary Williamson. Nutrigenomics and nutrigenetics: the emerging faces of Nutrition. FASEB J, 19(12): 1602-16.
14. Ames BN, Elson-Schwab I, Silver EA (2002). High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased Km): relevance to genetic disease and polymorphisms. Am J clin Nutr, 75(4): 616-58.
15. Palou A (2007). From nutrigenomics to personalized nutrition. Genes Nutr, 2(1): 5–7.
16. Zhou SF, Chan E, Zhou ZW, Xue CC, Lai X, Duan W. Insights into the structure, function, and regulation of human cytochrome P450 1A2. Curr Drug Metab. 2009;10:713–29.
17. Guessous I, Dobrinas M, Kutalik Z, Pruijm M, Ehret G, Maillard M, Bergmann S, Beckmann JS, Cusi D, Rizzi F, Cappuccio F, Cornuz J, Paccaud F, Mooser V, Gaspoz JM, Waeber G, Burnier M, Vollenweider P, Eap CB, Bochud M. Caffeine intake and CYP1A2 variants associated with high caffeine intake protect nonsmokers from hypertension. Hum Mol Genet. 2012;21(14):3283–92.
18. El-Sohemy A, Cornelis MC, Kabagambe EK, Campos H. Coffee, CYP1A2 genotype and risk of myocardial infarction. Genes Nutr. 2007;2(1):155–6
19. Beutler E: The genetics of glucose-6-phosphate dehydrogenase deficiency. Semin Hematol 1990, 27(2):137-164.
20. Pai GS, Sprenkle JA, Do TT, Mareni CE, Migeon BR: Localization of loci for hypoxanthine phosphoribosyltransferase and glucose-6-phosphate dehydrogenase and biochemical evidence of nonrandom X chromosome expression from studies of a human X-autosome translocation. Proc Natl Acad Sci USA 1980, 77(5):2810-2813.
21. Beutler E, Vulliamy TJ: Hematologically important mutations: glucose-6-phosphate dehydrogenase. Blood Cells Mol Dis 2002, 28(2):93-103.
22. Beutler E: G6PD deficiency. Blood 1994, 84(11):3613-3636.
23. Ingram C. J., Mulcare C. A., Itan Y., Thomas M. G., Swallow D. M. Lactose digestion and the evolutionary genetics of lactase persistence. Hum. Genet. 124, 579–591, 2009.
24. Itan Y., Jones B. L., Ingram C. J., Swallow D. M., Thomas M. G. A worldwide correlation of lactase persistence phenotype and genotypes. BMC Evol. Biol. 10, 36.10.1186/1471-2148-10-36, 2010.
25. Enattah N. S., Sahi T., Savilahti E., Terwilliger J. D., Peltonen L., Jarvela I. Identification of a variant associated with adult-type hypolactasia. Nat. Genet. 30, 233–237, 2002.
26. Lewinsky R. H., Jensen T. G., Moller J., Stensballe A., Olsen J., Troelsen J. T. T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity *in vitro*. Hum. Mol. Genet. 14, 3945–3953, 2005.
27. Olds L. C., Sibley E. Lactase persistence DNA variant enhances lactase promoter activity *in vitro*: functional role as a *cis* regulatory element. Hum. Mol. Genet. 12, 2333–2340, 2003.

28. Brigelius-Flohe, R. (1999). Tissue-specific functions of individual glutathione peroxidases. Free Radical Biology and Medicine, 27(9/ 10), 951–965.
29. Diwadkar-Navsariwala, V., & Diamond, A. M. (2004). The link between selenium and chemoprevention: A case for selenoproteins. Journal of Nutrition, 134(11), 2899–2902.
30. Lee, C.-H., Lee, K. Y., Choe, K.-H., Hong, Y.-C., Noh, S.-I., & Eom, S.-Y. (2006). Effects of oxidative DNA damage and genetic polymorphism of the glutathione peroxidase 1 (GPX1) and 8-oxoguanine glycosylase1 (hOGG1) on lung cancer. Journal of Preventive Medicine & Public Health, 39(2), 130–134.
31. Liwei, L., Wei, Z., & Ruifa, H. (2012). Association between genetic variants in glutathione peroxidase 1 gene and risk of prostate cancer: A meta-analysis. Molecular Biology Reports, 39, 8615–8619.
32. Parlaktas, B. S., Atilgan, D., Gencten, Y., Benli, I., & Ozyurt, H. (2015). A pilot study of the association of manganese superoxide dismutase and glutathione peroxidase 1 single gene polymorphisms with prostate cancer and serum prostate specific antigen levels. Archives of Medical Science, 11(5), 994–1000.
33. Stover PJ. Influence of human genetic variation on nutritional requirements. *Am J Clin Nutr,* 83(2): 436- 442. (2006)
34. Stover PJ. Nutritional genomics. *Physiol Genomics*, 15; 16(2):161-165. (2004)
35. El-Sohemy A (2008). The Science of Nutrigenomics. *Health Law Review,* 16(3): 5-8.
36. Willett W (2002). Isocaloric diets are of primary interest in experimental and epidemiological Studies. *Int J EPidemiol*, 31(3): 694-5.
37. Jenkins D JA, Kendall ccw, Ransom TPP (1998). Dietary fiber, the evolution of the human diet and Coronary heart disease. *Nutr Res,* 18: 633-52.
38. Iacoviello L, Santimone I, Latella MC, de Gaetano G, Donati MB (2008). Nutrigenomics: a case for the common soil between cardiovascular disease and cancer. *Genes Nutr*, 3(1):19-24.
39. Dauncey MJ, White P, Burton KA, Katsumata M (2001). Nutrition hormone receptor-gene interactions: implications for development and disease. *Proc Nutr Soc,* 60(1): 63 -72.
40. Jacobs MN, Lewis DF (2002). Steroid hormone receptors and dietary ligands: a selected review. *Proc Nutr Soc,* 61(1): 105-22.
41. Clarke SD (1999). Nutrient regulation of gene and protein expression. *Curr opin Clin Nutr Metab care,* 2(4): 287-9.
42. Eastwood MA (2001). A molecular biological basis for the nutritional and Pharmacological benefits of dietary plants. *QJM,* 94(1): 45-8.
43. Lin SJ, Guarentel (2003). Nicotinamide adenine dinucleotid, a metabolic regulator of transcription, longevity and disease. *Curr opin, cell Biol* 15(2): 241-6.
44. Afman L, Müller M (2006). Nutrigenomics: From Molecular Nutrition to Prevention of Disease. J Am Diet Assoc, 106(4): 569-76.
45. Kliewer SA, Xu HE, lambert MH, Wilson TM (2001). Peroxisome Proliferator-activated receptors: from genes to Physiology. *Recent Prog Horm Res,* 56: 239-63.

46. Masuda M, Suzui M, Weinstein IB (2001).Effects of epigallocatechin - 3 -gallate on growth, epidermal growth factor receptor signaling pathways, gene expression, and chemosensitivity in human head and neck squamous cell carcinoma cell lines. *Clin cancer Res,* 7(12): 4220 -29.
47. Black, Douglas L. (2003). "Mechanisms of alternative pre-messenger RNA splicing". *Annual Review of Biochemistry*. 72 (1): 291–336.
48. Uyeda K, Yamashita H, Kawaguchi T (2002). Carbohydrate responsive elementbinding protein (chREBP): a key regulator of glucose metabolism and fat storage. *Biochem Pharmacol,* 63(12): 2075-80.
49. Kosaku Uyeda1 and Joyce J. Repa (2006). Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metabolism*, 4: 107-10.
50. Edwards PA, Tabor D, Kast HR, Venkateswaran A (2000). Regulation of gene expression by SREBP and SCAP. *Biochim Biophys Acta,* 1529(1-3): 103 -13.
51. Nobel S, Abrahmsen L, Oppermann U (2001). Metabolic conversion as a Pre-receptor control Mechanism for lipophilic hormones. *Eur J Biochem,* 268(15): 4113-25.
52. Fairfield KM, Fletcher RH (2002). Vitamins For chronic disease Prevention in adults: Scientific review. *JAMA,* 287(23): 3116-26.
53. Ames BN (2001). DNA damage from micronutrient deficiencies is likely to be a major cause of cancer .*Mutat Re,* 475(1-2): 7-20.
54. Kaput J, Swartz D, paisley E, Mangian H, Daniel WL, Visek WJ (1994). Diet- disease interactions at the molecular level:an experimental paradigm. *J Nutr*, 124: (8 Suppl):1296 S-305S.
55. Park EI, paisley EA, Mangian HJ, Swartz DA, Wu MX, O'Morchoe PJ, Behr SR, Visek WJ, Kaput J (1997). Lipid level and type alter stearoyl coA desaturase mRNA abundance differently in mice with distinct susceptibilities todiet–influenced diseases. *J Nutr*, 127(4): 566-73.
56. Ross SA (2007). Nutritional genomic approaches to cancer prevention research. *Experimental oncology,* 29(4): 250-6.
57. Stover PJ, Caudill MA (2008). Genetic and Epigenetic Contributions to Human Nutrition and Health: Managing Genome-Diet Interactions.
58. Fenech M (2007). Nutrition and genome health. *Forum Nutr,* 60:49-65.
59. Fenech M (2005). The Genome Health Clinic and Genome Health Nutrigenomics concepts: diagnosis and nutritional treatment of genome and epigenome damage on an individual basis. *Mutagenesis*, 20(4): 255-69.
60. Fairfield KM, Fletcher RH (2002). Vitamins For chronic disease Prevention in adults: Scientific review. *JAMA,* 287(23): 3116-26.
61. Fairfield KM, Fletcher RH (2002). Vitamins For chronic disease Prevention in adults: Scientific review. *JAMA,* 287(23): 3116-26.
62. Fenech M, R.Ferguson L (2001). Vitamins/minerals and genomic stability in humans. *Mut Res*, 475: 1-6.

Orthorexia Nervosa And Nursing Care

Burcu ARKAN[1]



[1] Assist. Prof. Dr. Bursa Uludag University, Health Science Faculty Psychiatric Nursing Department, Bursa, Turkey

INTRODUCTION

Orthorexia nervosa is defined as a healthy diet obsession affecting a person's lifestyle (Donini et al. 2004). The word orthorexia is formed by the combination of two Greek words, *orthos* (true, appropriate) and *orexia* (appetite, hunger, nutrition). Orthorexia nervosa was first described by Steven Bratman in 1997 in order to differentiate it from anorexia nervosa (Bratman & Knight, 2000).

Orthorexic individuals are obsessed with the use of pure, additive-free, herbicide-free, pesticide-free and synthetic matter-free healthy foods (Bartrina, 2007; Mathieu, 2005). In orthorexia nervosa, the aim is the consumption of healthy and pure foods. Orthorexic individuals are not only obsessed with healthy foods but also obsessed with the way food is prepared, the materials used during preparation, and some methods of cooking (such as the use of an aluminum cap). Most individuals diagnosed with orthorexia nervosa are in vegetarians. Because of their fear of harmful substances, pesticides and food preparation techniques, they may remove some foods from their diets and, ultimately, onlyeata limited number of food groups (Mathieu, 2005; Kazkondu, 2010).

Orthorexic individuals avoid consuming foods which they do not consider healthy; the amount of nutrients taken in thus decreases and the energy gained from these nutrients is limited. Individuals limit their food consumption in order to remain healthy and fit, which leads to malnutrition. Malnutrition is accompaniedby osteoporosis, hypotension, the disruption of the menstrual cycle and cardiological problems (Bratman & Knight, 2000; Mathieu, 2005).

Etiology

Eating disorders have a mixture oforigins, are long-lasting serious diseases and are generally not related to one factor alone. Genetic factors, biochemical factors, environmental factors, psychological factors and sociocultural factors all affect eating disorders (Satman, Yılmaz & Sargın, 2002).

Eating behaviors are influenced by individuals' perceptions, previous experiences with foods and nutritional status as much as by their social, demographic and cultural conditions (Köster, 2009).

Theimportance attached to "being slim" by a given community, and pressures from family members, friends, the social environment and media channels play an important role in the development of eating disorders. Women have astronger desire to be slimthan men and therefore have a greater tendency to diet anda higher risk of eating disorders (Davidson & Neale, 2004; Satman, Yılmaz & Sargın, 2002). In addition, the dietary habits of individual families and the number of meals eaten per day are important factors in the formation of eating disorders (Sztainer, Wall, Story & Fulkerson, 2004).

Clinical Characteristics

Although the clinical features of orthorexia nervosa are not yet well known, orthorexic individuals focus excessively on the selection of foods, the size of the servingand their attitudes to eating, and allocate most of their time to planning what they will eat (Bosi, Çamur & Güler, 2007; Aydın, 2010).

Orthorexic individuals examine the packages of products for a long time before buying them and examine product contents to see whether there are dyes and other additives in their food. They often prefer to consume raw foods because they place extra emphasis onfood which is "pure" and does not have anyadditives. Over time, they give up consuming numerous foods and begin to lose weight, as in anorexia nervosa (Brytek-Matera, 2012; Donini et al. 2004).

The life of an orthorexic person exhibits behaviors such as the struggle against eating attractive foods, negative self-judgment due to minor episodes of binge-eating, and not consuming food prepared by others. They may also thinkthemselves superior to others with unhealthy diets. The desire to eat healthily turns into an obsessive pattern over time. It may progress to a point at which there is no room for other personal activities, their personal relationships and daily functioning become disrupted, and serious physical risks emerge. They start to live an isolated life (Strand, 2004; Aydın, 2010).

These patients spend most of their time dealing with strict rules. These rules are established to maintain and improve health. This situation is similar in this aspect to obsessive-compulsive disorder. However, it has been suggested that thesense of extreme preoccupation is not as significant as in eating disorders, therefore it cannot be defined as a separate category and is only related to the nature of the food (Ergin, 2014).

There are differences and similarities betweenorthorexia nervosa and other eating disorders. Orthorexia nervosa is similar to other eating disorders in terms of the weight loss, amenorrhea and the strictness of the diet. On the other hand, there is an obsession with consuming healthy and "pure" foods instead of the obsession with the amounts of food and weight found in anorexia nervosa and bulimia nervosa. In orthorexia nervosa, the healthiness of foods is a major concern; however, excessive control of food intake causes weight loss in individuals over time (Brytek-Matera, 2012; Bosi, Çamur & Güler, 2007; Strand, 2004; Bratman, & Knight, 2000). Orthorexic individuals think aboutconsuming healthy foods and rejecting unhealthy foods, which is an additional aspect not covered by a diagnosis of anorexia nervosa (Bosi, Çamur, Güler, 2007). In summary, the content and quality of a food is more important in orthorexia nervosa, while the amount of food is more important in the other two nutritional disorders (Mathieu, 2005). If healthy eating habits adversely affect the daily life of an individual and take up an excessive amount of time, the situation becomes pathological (Donini et al. 2004).

Diagnostic Criteria

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), there are defined and undefined eating and nutritional disorders such as pica, regurgitation disorder, avoidant/restrictive food intake disorder, anorexia nervosa, bulimia nervosa and binge-eating disorder. These undefined nutritional and eating disorders include all eating problems other than anorexia nervosa and bulimia nervosa, but there are no specific definitions of them (Rome, 2012; Wheeler, Greiner, Boulton, 2005). Orthorexia nervosa was not included in DSM-V. Studies on orthorexia nervosa as an undefined eating disorder continue (Arusoğlu et al. 2008; Ergin, 2014).

The Studies on Orthorexia Nervosa

The studies on orthorexia neurosis are very limited. Data obtained from the available studies are given below.

Batıgün and Utku (2006) and Ünalan et al. (2009) assessed the relationship of attitudes to eating disorders of young people in Turkey. It was reported that females had a higher number of eating disorders than males (Batıgün & Utku, 2006; Ünalan et al. 2009).

Arusoğlu et al. (2008) studied the symptoms of orthorexia nervosa and found that orthorexic symptoms increased as the level of education increased. Married people may have a higher tendency towards suffering from orthorexia nervosa than unmarried individuals. Having children increased the orthorexic tendency and an increase in the number of children may also cause an increase in orthorexic symptoms. It was determined that the tendency of males to be orthorexic was lower than that of females. Individuals who dieted had a lower tendency to be orthorexic than those who did not diet (Arusoğlu et al. 2008).

In the study conducted with 327 students aged 18-25 in Poland, it was determined that the female students tended to have more orthorexia nervosa than the male students (Brytek-Matera et al. 2015).

Özenoğlu and Dege investigated the effect of nutrition education and self-esteem on the development of orthorexia nervosa in university students who were studying health. It was found that female students were more orthorexic than the male students. A lack of awareness about nutrition and low self-esteem may contribute to the development of obsessive symptoms and orthorexia nervosa (Özenoğlu, Dege, 2015).

Oğur, Aksoy and Güngör conducted a study to determine the tendency to suffer from orthorexia nervosa in 474 university students in 2015; it was found that there was a high likelihood of orthorexia nervosain the students. The risk of being orthorexic was higher in the female students than the male students. Thin students had a higher tendency to have orthorexia nervosa (Oğur, Aksoy & Güngör, 2015).

In contrast to these studies, Fidan et al. (2010) conducted a study with 878 medical students to determine the prevalence of orthorexia nervosa. The prevalence of orthorexia nervosa was determined to be 43.6%. In the same study, it was found that the male students had a higher risk of orthorexia nervosa than the female students (Fidan et al. 2010).

In a study conducted with 404 participants in 2004, Donini et al. found a higher tendency to have orthorexia nervosa in the males with low levels of education (Donini et al. 2004).

Bağcı et al. (2007) found that 45.5% of individuals were hypersensitive about their eating habits; this ratio was higher in the males than in the females. The difference was thought to be caused by the fact that the sample group consisted of medical doctors.

Similarly, in a study by Kazkondu et al.it was found that the male students had a higher tendency to be orthorexic than female students. Those students living at home had a higher tendency to be orthorexic than students staying in dormitories (Kazkondu, 2010).

In 2007, Erikson et al. conducted a study with 251 people (166 women and 85 men) in a fitness center to determine whether individuals were orthorexic. It was reported that eating disorders such as orthorexia nervosa were more common in individuals doing physical exercises that required specific weights and body shape. In particular, the male bodybuilders felt themselves under pressure to develop a muscular physique (Erikson et al. 2008).

In a study conducted by Gezer and Kabaran in 2013 with 106 female university students, it was found that the risk of eating disorders was higher in obese students, while the risk of orthorexia nervosa was higher in the weak students.Furthermore, an increase in the risk of orthorexia nervosa was associated with an increase in the risk of obsessive-compulsive behavior and a decrease in the risk of eating disorders. These changes in eating behavior did not significantly affect the quality of the diet (Gezer & Kabaran, 2013).

In 2017, Arslantaş et al. investigated the relationship between eating behaviors and orthorexia nervosa in nursing students. Their study found that approximately three-quarters of the students were at risk in terms of eating attitudes while half of them were at risk of orthorexia nervosa. The fear of obesity fond to increase the tendency to have an eating disorder and orthorexia nervosa (Arslantaş et al. 2017).

Orthorexia Nervosa Treatment

Because the factors underlying orthorexia nervosa vary, its treatment is difficult, and as orthorexic people are afraid of consuming some nutrients, it is difficult to change their diet. Investigating the factors underlying the disorder may facilitate the transition to

normal nutrition (Shah, 2012; Ergin, 2014). Orthorexic individuals may need professional help to overcome all-or-nothing thinking and commit to switch to normal eating (Kazkondu, 2010).

The goals of the treatment of eating disorders are listed below:

- Restoration of the person's normal and healthy weight
- Treatment of physical complications
- Treatment of co-morbidities
- Prevention of relapse after recovery
- Removal of improper food restrictions
- Helping the patient develop healthy eating habits (Yager et al., 2006)

Individual psychotherapy techniques, family therapy, group psychotherapy and pharmacological treatments are applied in the treatment of eating disorders. In general, many treatment approaches are used simultaneously.

Individual Psychotherapy: Psychotherapy is an indispensable part of the treatment of eating disorders. In this therapy, it is thought that patients misinterpret environmental signals, and that disruptive emotions and then abnormal behaviorsemerge as a result. Thoughts, attitudes and related logical errors can be identified and questioned by using cognitive restructuring methods. Problem-solving training is useful, and role-playing is particularly helpful when preparing for discharge from a clinicso as to be able toconfront schooland work environments, as well as family and friends (Maner, 2001).

Family Therapy: There is no single family model that makes individuals prone to eating disorders. In family therapy, the characteristics of the disease are reviewed, and the family's efforts are supported. Any areas concerning the family as a wholewhich need improvement should be clearly defined. These may involve communication problems and concerns about how to rear a child healthily. If the father is distant and unattainable, the mother usually handleschild-rearing and may feel a sense of "defeat" and depression during this period. Spousal problems between parents are identified and discussed in separate sessions. Group therapy can be performed with families in order that support can be given, received and shared (Maner, 2001).

Pharmacological Treatment: Selective Serotonin Reuptake inhibitors (SSRIs) can be useful. However, the individual may refuse medication as they tend to only consume natural, pure and additive-free products. Therefore, pharmacological treatment combined with cognitive behavioral therapy may be more effective (Mathieu, 2005).

Treatment requires proper teamwork. This requires cooperation between the doctor, psychologist, dietitian and psychiatric nurse.

Nursing Assessment

Patient History: The patient's daily nutritional intake, weight and recent changes in weight, health problems such as inappetence and food tolerance, liked and disliked foods, dysphagia, anorexia, vomiting, diarrhea or constipation, surgical and/or chronic diseases and medications are discussed and recorded.

Physical Examination: The individual's height, weight, skin thickness, body mass index, vital signs, general appearance, muscle weakness, mobility, skin and mucous membranes, intestinal sounds, laboratory results are assessed and recorded (Dal, 2007).

Nursing Diagnosis and Interventions

Malnutrition and Subnutrition

Collaboration is established between the physician and dietitian and a balanced diet rich in calories, protein and vitamin C is prepared. Daily weight and calorie intake are monitored. The patient is encouraged to eat. Before each meal, care is taken to ensure the patient's oral hygiene is good and to remove any unpleasant smells from the room. The patient is provided with small and frequent meals. If necessary, total parenteral nutrition, nutritional supplements and tube feeding are administered intravenously.

- Eating requires energy and the physical strength and energy of malnourished patients is decreased. The patient is therefore allowed to rest before and after meals.
- The patient's level of knowledge is evaluated, and the patient is given any necessary training/education. Lack of information may lead to malnutrition. The training given helps the patient to choose healthy foods (Dal, 2007).

Risk of inadequate or excess volume of liquids

- Oral mucous membranes, urinary density, level of consciousness and laboratory findings are monitored every four hours. Drying of mucous membranes, increased urine density, changes in level of consciousness, electrolyte disturbances and dehydration may be observed.
- Daily weight gain and weight loss are followed to monitor fluid imbalance.

If there is no problem in the oral feeding of the patient, fluids are given in small amounts, taking the patient's preferences into consideration. Frequent and small amounts helpthe patient to maintain adequate fluid intake and be able to tolerate the fluids taken in (Dal, 2007).

Risk of Infection

- Signs of infection in the patient are followed up every four hours. A fever may indicate an infection. Feeling unwell, experiencing chills and shivering, as well as erythema and leukocytosis are signs and symptoms of infection. Monitoring the patient in terms of these signs and symptoms helps to detect infections early and prevent complications.
- Attention is paid to the risk of medical and surgical asepsis. Handwashing is the best practice to prevent the spread of pathogenic microorganisms. An aseptic technique should be used during the replacement of central catheters or dressings.
- The signs and symptoms of infection are taught to the patients and their families. To reduce the risk of infection, the correct handwashing technique is taught. Informing the patient of theseallows them to participate in their own care and reduces the rate of infection (Dal, 2007).

Risk of deterioration of skin integrity

- The skin is assessed every 4 hours. Regular assessments are important in early diagnosis of the signs of a deterioration in the integrity of the skin integrity.
- The patient's position is changed every two hours. The patient should be encouraged to perform active and passive exercises as these reduce pressure and ensure the oxygenation of cells.
- The patient's skin is kept clean and dry. Regular maintenance of IV catheters should be performed. Pneumatic bearings can be used. These measures ensure patient comfort and reduce the risk of a deterioration of skin integrity (Dal, 2007).

Conclusion and Suggestions

Orthorexia nervosa is a group of psychiatric disorders with biological, psychological and sociocultural dimensions. In addition to the limitations placed on nutrition, the methods and materials used in food preparation also cause concern to the patient. As a result of these kinds of obsessions, orthorexic individuals do not "eat out" and their social relations deteriorate. Although orthorexia nervosa is not yet included in DSM-V, orthorexia nervosa is an eating disorder that is increasingly prevalent. As it is a relatively new concept and not included in the diagnostic criteria, the number of studies on the subject is limited. This type of eating disorder is new, and new and effective treatment methods should therefore be developed.

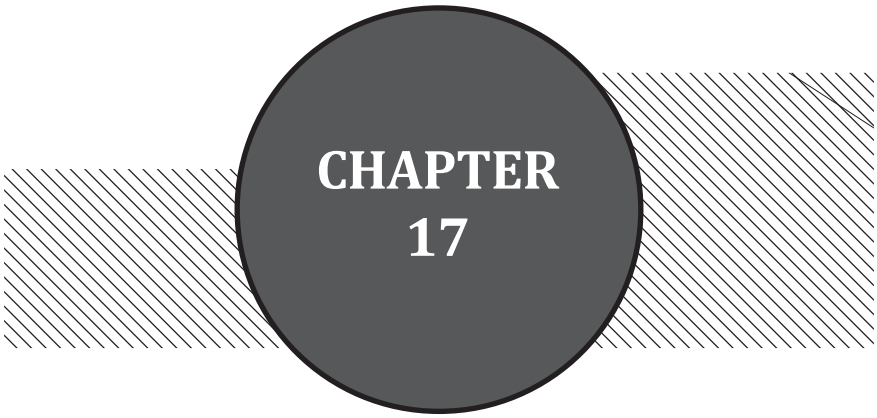
REFERENCES

1. Arusoğlu, G., Kabakçı, E., Köksal, G., Merdol, T. K. (2008). Orthorexia Nervosa and Adaption of Orto- 11 into Turkish. *Turkish Journal of Psychiatry, 19 (3)*, 283-291.
2. Aydın, C. (2010). Yeme Tutumu, Ortorektik Belirtiler ve Ana Babaya Bağlanma Arasındaki İlişkiler. Hacettepe Üniversitesi Sosyal Bilimler Enstitüsü Psikoloji Anabilim Dalı, Ankara.
3. Arslantaş, H., Adana, F., Öğüt, S., Ayakdaş, D., Korkmaz, A. (2017). Hemşirelik Öğrencilerinin Yeme Davranışları ve Ortoreksiya Nervoza (Sağlıklı Beslenme Takıntısı) İlişkisi: Kesitsel Bir Çalışma. *Psikiyatri Hemşireliği Dergisi, 8 (3),*137–144.
4. Bağci, AT., Camur, D., Güler, C. (2007). Prevalence of orthorexia nervosa in resident medical doctors in the faculty of medicine (Ankara, Turkey). *Appetite, 49,*661–6.
5. Bartrina, J. A. (2007). Orthorexia or when a healthy diet becomes an obsession. *Arch Latinoam Nutr, 57 (4),* 313-315.
6. Batıgün, A. D., Utku, Ç. (2006). Bir Grup Gençte Yeme Tutumu ve Öfke Arasındaki İlişkinin İncelenmesi. *Türk Psikoloji Dergisi, 21 (57),* 65-82.
7. Bosi, T.B., Çamur, D., Güler, Ç. (2007). Prevalence of orthorexia nervosa in resident medical doctors in the faculty of medicine in Ankara. *Appetite, 49,* 661-666.
8. Bratman, S., Knight, D. (2000). Health food junkies: overcoming the obsession with healthful eating. Broadway Books, New York.
9. Brytek-Matera, A. (2012). Orthorexia nervosa–an eating disorder, obsessive-compulsive disorder or disturbed eating habit. *Archives of Psychiatry and psychotherapy, 1 (14),* 55-60.
10. Brytek-Matera, A., Donini, L. M., Krupa, M., Poggiogalle, E., Hay, P. (2015). Orthorexia Nervosa and Self-Attitudinal Aspects of Body Image in Female and Male University Students. *Journal of Eating Disorders, 3,* 2-2.
11. Dal Ü. (2007). Malnutrisyonu Olan Hastanın Hemşirelik Bakımı, Gazi Üniversitesi Hemşirelik Yüksekokulu, ANKARA.
12. Davidson, G.C., Neale, J. M. (2004). Anormal Psikolojisi (çev .Dağ İ).Türk Psikologlar Derneği Yayınları, no: 29, 256 s.İstanbul.
13. Donini, L. M., Marsili, D., Graziani, M. P., Imbriale Canella, C. (2004). Orthorexia nervosa: a preliminary study with a proposal for diagnosis and an attempt to measure the dimension og the phenomenon, *Eating and Weight Disorders*, *9*, 151-157.
14. Ergin, G. (2014). Sağlık personeli olan ve olmayan bireylerde ortoreksiya nervoza sıklığı araştırması. Başkent Üniversitesi Sağlık Bilimleri Enstitüsü. Beslenme ve Diyetetik Anabilim Dalı, Doktora Tezi, Ankara.

15. Eriksson, L., Baigi, A., Marklund, B., Lindgren, E. C. (2008). Social physique anxiety and sociocultural attitudes toward appearance impact on orthorexia test in fitness participants. *Scandinavian Journal of Medicine and Science in Sports, 18(3),* 389-394.
16. Fidan, T., Ertekin, V., Işıkay, S., Kırkpınar, I. (2010). Prevalence of orthorexia among medical students in Erzurum, Turkey. *Comprehensive Psychiatry, 51 (1),* 49-54.
17. Gezer, C., Kabaran, S. (2013). Beslenme ve diyetetik bölümü kız öğrencileri arasında görülen ortoreksiya nervosa riski. *S.D.Ü Sağlık Bilimleri Dergisi, 4 (1),* 14-22.
18. Kazkondu, İ. (2010). Üniversite Öğrencilerinde Ortoreksiya Nervoza (Sağlıklı Beslenme Takıntısı) Belirtilerinin İncelenmesi. Gazi Üniversitesi, Eğitim Bilimleri Enstitüsü, Aile Ekonomisi ve Beslenme Eğitimi Anabilim Dalı, Yüsek lisans tezi, Ankara.
19. Köster, E. P. (2009). Diversity in the determinants of food choice: A psychological perspective. *Food Quality and Preference,* 70–82.
20. Maner, F. (2001). Yeme Bozuklukları. *Psikiyatri Dünyası, 5,* 130-139.
21. Mathieu, J. (2005). What is orthorexia? *J Am Diet Assoc, 105 (10),* 1510-1512.
22. Oğur, S., Aksoy, A., Güngör, Ş. (2015). Üniversite Öğrencilerinde Ortoreksiya Nervoza Eğiliminin Belirlenmesi. *BEÜ Fen Bilimleri Dergisi, 4 (2),* 93-102.
23. Özenoğlu, A., Dege, G. (2015). Üniversite Gençliğinde Yeme Bozukluğunun Yordayıcıları Olarak Benlik Saygısı Ve Beslenme Eğitiminin Ortoreksiya Nervoza Gelişmesi Üzerine Etkisi. *Bozok Tıp Dergisi, 5 (3),* 5-14.
24. Rome, E.S. (2012). Eating Disorders in Children and Adolescents. Curr Probl Pediatr Adolesc Health Care. http://www.cppah.com/article/S1538-5442(11)00168-4/abstract (erişim tarihi :13.05.2016).
25. Satman, İ., Yılmaz, T., Sargın, M. (2002). Population-Based Study of Diabetes and Risk Characteristics in Turkey. *Diabetes Care, 25 (9),* 1551-1556.
26. Shah, S. M. (2012). Orthorexia nervosa: Healthy eating or eating disorder?.
27. Strand, E. (2004). A new eating disorder? *Psychol Today, 37 (5),* 1-3
28. Sztainer, D.N., Wall, M., Story, M., Fulkerson, J.A. (2004). Are family meal patterns associated with disordered eating behaviors among adolescents. *Journal of Adolescents Health, 35 (5),* 350–359.
29. Ünalan, D., Öztop, B. D., Elmalı, F., Öztürk, A., Konak, D., Pırlak, B., Güneş, D. (2009). Bir Grup Sağlık Yüksekokulu Öğrencisinin Yeme Tutumları İle Sağlıklı Yaşam Biçimi Davranışları Arasındaki İlişki. *İnönü Üniversitesi Tıp Fakültesi Dergisi, 16 (2),* 75-81.
30. Wheeler, K., Greiner, P., Boulton, M. (2005). Exploring alexithymia, depression, and binge eating in self-reported eating disorders in women. *Perspect psychiatr Care, 41(3),* 114-123.
31. Yager, J., Devlin, M. J., Halmi, K. A. et al. (2006). Practice guideline for the treatment of patients with eating disorders, 3rd ed. Washington: American Psychiatric Association: 29-33.

Cancer Epigenetics: Focus On DNA Methylation

Berna DEMİRCAN[1]



[1] İstanbul Medeniyet University, Medical School, Department of Medical Biology

INTRODUCTION

Epigenetics is defined as the study of mitotically and/or meiotically heritable changes in gene expression that occur independent of alterations in the DNA sequence itself (1). Epigenetic mechanisms help to coordinate changes in gene expression that accompany the transition from undifferentiated embryonic cells to terminally differentiated tissue to ensure cellular homeostasis (2,3). DNA methylation, covalent histone modifications, chromatin remodeling and microRNAs (miRNA) are the main epigenetic mechanisms that are essential for normal mammalian development and regulation of gene expression. Failure of the suitable maintenance of these mechanisms can lead to improper activation or inhibition of various signalling pathways and result in diseases such as cancer (1,2,4-10). Historically, research has focused on the genetic aspect of cancer since genetic mutations occur in cancer cells and these events induce disease-associated changes in gene expression and/or function. However, since the 1990's, growing data of the research have demonstrated that the complexity of human carcinogenesis cannot be accounted for by genetic alterations alone but also involves epigenetic changes which regulate heritable changes in all human cancer types (4). Aberrations in DNA methylation, histone modifications, chromatin remodeling and miRNAs patterns are associated with tumorigenesis. These epigenetic mechanisms are disrupted during the initiation and progression of cancer (4-10).

Unlike genetic mutations, epigenetic alterations are reversible and this feature offers a potential for cancer therapy (11).

This chapter will first describe the epigenetic modifications, focusing on especially DNA methylation in normal and cancer cells and then discuss the opportunities and challanges of epigenetic therapy.

DNA Methylation

DNA methylation is the most extensively studied epigenetic modification in cancer. It involves addition of a methyl group to the 5' position of cytosines at the 'CpG' dinucleotides and is catalyzed by three seperate DNA methyltransferases (DNMT1, DNMT3a, DNMT3b). This enzymatic reaction uses S-adenosyl methionine (SAM) as a methyl donor. DNMT1 acts during replication and methylates hemimethylated DNA (2,3,6). Thus, it is viewed as the DNA methyltransferase principally responsible for continuation of DNA methylation patterns in daughter cell (*maintenance* methylation). In contrast, DNMT3a and DNMT3b are responsible for the methylation of previously unmodified cytosines (*de novo* methylation) (1,4,7,12). It has been reported that DNMT1 has higher catalytic activity than DNMT3a and DNMT3b, and all of these three enzymes are involved in crucial cellular functions such as differentiation (2). A fourth methyltransferase,DNMT2, is found in mammalian cells but it has unknown biological function. Another methyltransferase, DNMT3L, is not directly involved in DNA methylation because of its lack of the ability to bind SAM. However, it plays a role for increasing the binding of DNMT3a to SAM (13).

CpG dinucleotides are distributed asymmetrically in the human genome. In certain areas of the genome, a high concentration of the CpG dinucleotides is found and these are referred to as 'CpG islands'. These islands are usually located within upstream promoter regions or gene transcription start sites and occupy ~ 60% of human gene promoters (9). In normal somatic cells, most of the CpG sites throughout the genome are usually methylated, whereas promoter CpG islands of the genes are unmethylated and associated with active gene transcription. Exceptions of this trend are imprinted genes which require DNA hypermethylation of the promoter at one of the two parental alleles to establish monoallelic expression. Other exceptions include hypermethylation and silencing of genes found on the inactive X-chromosome of females and germ-line genes in all tissues.

Methylation of CpG dinucleotides also occurs at repetitive genomic sequences in normal cells, which prevents the reactivation of transposable elements by silencing non-coding DNA and thus, protect chromosomal integrity (2,5,14,15).

Global DNA hypomethylation and gene-specific promoter DNA hypermethylation are two major altered methylation patterns seen in almost all cancers (8).

It is well documented that tumor cells display an overall loss of methylated cytosines compared to normal tissues despite the fact that many CpG islands are subject to hypermethylation in cancer cells (1,2,4,8,9,11). This tumor-associated global DNA hypomethylation is the first epigenetic alteration noted in cancer cells and occurs predominantly in repetitive DNA sequences, retrotransposons, coding regions and introns in human genome (14,16,17). While the molecular mechanisms that responsible for this loss of DNA methylation are not yet fully understood, studies indicate that DNA hypomethylation levels increase through the transformation of a tumor from a benign lesion to an invasive cancer (16). It has been known that most of the CpGs in the genome, except for CpG islands, are 80% methylated but the average CpG methylation levels are 40%-60% in cancer due to global DNA hypomethylation (4).

One of the potential consequences of DNA hypomethylation is genomic instability which may lead to mutations, deletions, amplifications, inversions and translocations in cancer patients (2,18). This occurs through increased mobility of retrotransposon sequences of the LINE (long interspersed nuclear element) and SINE (short interspersed nuclear element) classes as well as human endogenous retroviruses due to DNA hypomethylation. In normal cells, mobility of these dormant mobile elements is kept under control via dense methylation of CpG dinucleotides within their genomic structure. However, hypomethylation of these elements can result in their activation and translocation to other genomic regions, thus increasing genomic instability.

Another important consequence of DNA hypomethylation is the reactivation of normaly methylated and silenced proto-oncogenes (2,8,9,14).

In addition to the hypomethylation of CpG dinucleotides within repetitive DNA elements and growth-promoting genes, cancer-associated hypomethylation also occurs in regions of the genome encoding single-copy genes. Dysregulation of allele-specific methylation will result in the loss of imprinting and cause pathological biallelic gene expression as in the case of insulin-like growth factor-2 (*IGF-2*) in Wilms's tumor. Loss of imprinting of *IGF-2* has also been linked with an increased risk of colorectal cancer. It has been reported that loss of gene imprinting is one of the most important mechanisms contributing to the very early stages of cancer development. Experiments in mouse models suggest that loss of imprinting events alone may be sufficient to initiate the tumorigenesis process (1,9).

In contrast to hypomethylation, which causes increased genomic instability and activates proto-oncogenes, gene-specific DNA hypermethylation contributes to tumorigenesis by silencing tumor suppressor genes. DNA hypermethylation typically occurs at CpG islands in the promoter region of the genes (19-25). However, the exact mechanism responsible for the DNA methylation in a given promoter is not fully understood. DNA hypermethylation is believed to be an early event in tumorigenesis, and play a major role in tumor initiation and progression. In fact, this epigenetic event affects more genes than do mutations (4). Epigenetic silencing of the genes via promoter hypermethylation may also contribute to tumor development by promoting clonal selection of cells with growth advantage (7-9). There have been a number of studies showing that hundreds of genes are subjected to epigenetic silencing by CpG island promoter hypermethylation in human cancers (3,4). Tumor suppressor and other cancer-associated genes silenced by DNA hypermethylation are involved in cell cycle regulation, DNA repair, apoptosis, angiogenesis, invasion and adhesion, which are crucial in key cellular processes and disrupted during the evolution

of cancer (4,5,9). Some examples of these genes identified from various cancers are listed in Table 1.

An increase in DNA methylation also occurs with ageing. Consequently, the increased cancer predisposition observed with ageing may be partially attributable to the age-dependent aberrations in genome methylation (25).

Both global DNA hypomethylation and promoter DNA hypermethylation of different regions of the genome are very important two mechanisms in cancer development. In almost every cancer type, same cancer cells harbor simultaneously genome-wide DNA hypomethylation and hundreds of transcriptionally inactivated genes by DNA hypermethylation (3,4).

Since genetic mutations or epigenetic silencing of the tumor suppressor genes are recessive in many human cancers, transcriptional silencing of a tumor suppressor gene requires disruptive events in both allelic copies. This idea is known as the 'two-hit' hypothesis and it was proposed by Alfred Knudson in 1971. Based on this hypothesis, promoter DNA methylation may act as a first or second hit for inactivation of the tumor suppressor gene, in which the other allele may be inactivated by a mutation or eliminated through a loss of heterozygosity event. In familial cancers, the first hit is the germ-line mutations and both genetic and epigenetic changes can cause the second hit. However, the loss of one or both alleles of a tumor suppressor gene by DNA methylation has been observed in sporadic cancers (26-28).

Gene	Function	Tumor types
BRCA1	DNA damage repair	Breast, ovarian
CDKN2A (p16)	Cell cycle regulation	Lung, brain, breast, colon, bladder, melanoma
PTEN	Regulation of cell growth and apoptosis	Prostat, endometrial, melanoma, brain
APC	Regulation β-catenine, cell adhesion	Colorectal, gastrointestinal
CDH1(E-cadherin)	Homotypic epithelial cell-cell adhesion	Bladder, breast, colon, liver
MGMT	DNA repair	Brain, colon, lung, lymphomas
MLH1	DNA repair	Colorectal, endometrial, ovarian
RB	Respression of transcription factor	Retinoblastoma
GST-Pi	Cellular detoxification	Prostate
SFRP1,-2,-4,-5	Regulation of Wnt pathway	Breast, prostat, ovarian, gastric
HIC1	Zinc finger protein	Brain, breast, colon, renal
GATA4/5	Zinc finger protein	Colorectal, breast, lung

Table 1. Hypermethylated genes in cancer

It has been reported that there is a strong cooperation between genetic and epigentic abnormalities to drive tumorigenesis. According to the recent studies, epigenetic silencing of the tumor associated genes via DNA hypermethylation may predispose cells to further mutations. For example, DNA repiar gene, *MGMT,* removes carcinogen-induced O6-methylguanine adducts from DNA, which result in G to A transition mutations. Cancers with hypermethylated *MGMT* are susceptible to genetic mutation in critical genes such as *p53* or *KRAS* (2,10).

Silencing of other DNA repair genes such as *MLH1* and *BRCA1* leads to new mutations and rapid progression of cancer due to a lack of efficient DNA repair (29). Conversely, most cancers have been suggested to harbor mutations in genes which are involved in epigenetic modifications including aberrant DNA methylation, histone modifications and nucleosome positioning. These epigenetic alterations can lead to abnormal gene expression and genomic instability(30).

DNMTs are often overexpressed in cancer cells (10,14). Compared to normal tissues, the expression of DNMT1 is almost always increased in tumors. However, since DNMT1 is regulated in parallel with DNA synthesis in normal cells, much of this increased expression may simply reflect increased cell proliferation within the tumor. It has been still unresolved whether high expression of DNMT1 is responsible for aberrant mehylation in cancer cells. In contrast, increased expression of DNMT3a and DNMT3b in some tumors is likely important because these enzymes are normally expressed at low levels in somatic cells. However, it is still unknown to what extent over expression of these enzymes is responsible for cancer-associated DNA hypermethylation especially when one considers that cancer cells exhibit genome-wide hypomethylation (14).

Although DNA methylation is the most comprehensively analyzed epigenetic modification in human tumors, there are other very important epigenetic mechanisms capable of altering gene expression during carcinogenesis such as histone modifications which is discussed in below.

Histone Modifications

Eukaryotic chromatins are composed of nucleosomes which consist of an octamer of basic proteins called histones (two each of H2A, H2B, H3 and H4), around which approximately 146 bp of DNA winds (7). Chromatin is organized in two different states, open and closed configuration. The closed chromatin (termed heterochromatin) is tightly compacted and associated with transcriptionally inactive genes, wheras open chromatin is less compacted and more likely to be transcribed (termed euchromatin). Each histone posseses an amino-terminal tail rich in the amino acid lysine. These lysine residues are subject to many post-translational modifications such as phosphorylation, ubiquitination, sumoylation, acetylation and methylation. The most studied post-translational modification of histones is acetylation and methylation of specific lysine residues on histones H3 and H4. Histone acetylation of lysine residues neutralizes the positive charge of the histones and loosens their interaction with negatively charged DNA backbone, resulting in a more open chromatin configuration accessible to being successfully transcribed. For this reason, histone acetylation is strongly correlated with active gene expression.

Unlike acetylation, histone methylation does not change the charge of the histone tails. Histone methylation is associated with transcriptional repression or activation depending on the specific amino acid affected. For example, methylation of histone 3 (H3) lysines 4 (K4) is associated with transcriptionally active chromatin, while methylation of H3 at K9 or 27 and H4 at K20 is associated with transcriptionally silent chromatin.

These histone modification patterns are regulated by enzymes including histone acetyltransferases (HATs) and deacetylases (HDACs), which introduce and remove acetyl groups, respectively. Histone methyltransferases (HMTs) and demethylases (HDMs) on the other hand, introduce and remove methyl groups (2,3,7,8).

In addition to performing their individual roles in the genome, DNA methylation and histone modifications are proposed to act synergistically in the progressive silencing of genes (1-4). One mechanism that accounts for silencing of tumor suppressor genes is that some transcription factors may not bind to promoter sequences of methylated gene.

However, a number of studies revealed that alteration of chromatin structure through the repressive histone modifications mentioned above is another mechansim involved in transcriptional silencing of methylated genes. Several proteins that recognize and bind specifically methylated DNA can block the formation of transcription initiation complex. These proteins are methyl-CpG-binding domain (MBD) family proteins including MeCP2, MBD1, MBD2, MBD3 and MBD4. They recruit HDACs to the site of methylation. All three biologically active DNMTs also bind HDACs to repress transcription. In addition, MeCP2 recruits several other proteins including co-repressor molecules such as Sin3A that promotes localized histone deacetylation, thus leading to transcription repression. The NuRD is a nucleosomal remodeling complex involved in methylation-mediated gene silencing. This repressor complex contains MBD3, HDACs and a chromatin remodeling ATPase (8,14). However, silencing of the tumor-suppressor genes may occur independent of promoter methylation in some situations. At this point, Polycomb group (PcG) complexes which are chromatin modifiers play an importan role in human cancers as well as development. They are negative regulators of gene expression and are crucial in maintaining the repression of their target genes. For example, a PcG protein EZH2, a histone methyltransferase, catalyze H3K27 trimethylation and is involved in the initiation of gene silencing. Many studies suggest that there is a strong link of EZH2 to oncogenesis and to cancer-related gene silencing. Its overexpression has been shown to promote tumor growth both in vivo and in vitro such as melonomas, lymphomas, prostate and breast cancers. Although EZH2-mediated gene silencing usually occurs in the absence of DNA methylation, EZH2 has been indicated to interact with all three functional DNMTs (2,3).

MiRNAs

MiRNAs are small, 20- to 22- nucleotides long, non-coding RNA molecules that inhibit gene expression at the posttranscriptional level. They are generally transcribed by RNA polymerase II into the primary miRNAs (pri-miRNAs) and subsequently cleaved by Drosha in the nucleus into the precursor miRNAs (pre-miRNAs) and transported to the cytoplasm by Exportin-5. Pre-miRNAs are further processed in the cytoplasm by Dicer into their final configuration of double stranded miRNAs. One strand of the double-strand is incorporated into the RNA-induced silencing complex (RISC), which delivers mature miRNAs to their mRNA targets. miRNAs bind to complementary sequences of mRNAs and induce either degradation or translational silencing of the target mRNAs. It is estimated that about 30% of human genes are targets of miRNAs.

miRNAs have been indicated to be linked to cancer and can act as tumor suppressor or oncogenes in tumorigenesis. The expression of miRNAs is regulated by epigenetic mechanisms. Furthermore, miRNAs can modify epigenetic mechanisms by targeting enzymes involved in DNA methylation and histone modifications. Experimental studies have indicated that global alterations in miRNA expression occur in cancer cells, and numerous miRNAs are down-regulated while some are up-regulated by genetic or epigenetic mechanisms (31-34).

Epigenetic Therapy of Cancer

Epigenetic modifications, unlike genetic mutations, can be reversed through epigenetic therapy in which drugs that can modify chromatin or DNA methylation patterns are used alone or in combination to obtain best therapeutic results. The aim of this therapy is to target the chromatin in rapidly dividing tumor cells and return it to a normal state while only slightly irritating the epigenome of healthy cells (35).

i. DNMT Inhibitors

There are two classes of epigenetic drugs: DNA methylation inhibitors and HDAC inhibitors. DNMT inhibitors are the most advanced epigenetic therapeutics currently available for the cancer treatment. They are divided into nucleoside and non-nucleoside analogues. The Nucleoside analogues incorporate into newly synthesized DNA where they form a covalent bond with DNMTs, which ultimately inhibit DNA methylation in subsequent rounds of DNA synthesis (36). The nucleoside analogues 5-azacytidine (Azacitidine or Vidaza) and 5-aza-2'deoxcytidine (Decitabine or Dacogen) are approved by the US Food and Drug administration (FDA) for the treatment of patients with myelodysplastic syndrome (MDS) in 2004 and 2006, respectively (2,37). In fact, these drugs were synthesized and introduced in the clinic as cytotoxic agents in the late 1960's. However, they were observed to be very toxic at relatively high doses without great antitumor activity. Reduced doses in the 1990's proved promising therapeutic outcomes in MDS patients, leading to FDA approval for this purpose (38-40).

Azacitidine induced 60% response rate and improved survival rate of the patients with MDS. Decitabine, on the other hand, has been demonstrated to induce 70% response rate in these patients (7). In addition to MDS, promising results have also emerged from the treatment of other hematological malignancies such as acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) by using these drugs (41). Nevertheless, some negative effects of these DNMT inhibitors have been reported in studies, which are their short half-life, instability in aqeous solutions, toxic effects and lack of specificity in the mechanism of action. Zebularine, a next generation DNA methylation inhibitor, may overcome these challenges since it is more specific, more stable in aqueous solution and less toxic compared to azacitidine (42). Additionally, the most advanced novel DNMT inhibitory drug has been SGI-110 (guadecitabine) which has showed increased half-life and stability in clinical experiments (43). SG-110 is now in phase II-III clinical trials in MDS and AML.

The fact that the nucleoside analogues are incorporated into DNA raises concerns regarding their potential toxic effects on healthy cells. However, therapy related toxicities have not been documented, and it has been argued that these inhibitory drugs mainly act on rapidly dividing tumor cells, leaving slowly dividing normal cells mostly unaffected (7).

Non-nucleoside analogues are not integrated into DNA but instead bind to the catalytic site of DNMTs and inhibit DNA methylation (9,44). RG108, MG98 are the examples of non-nucleoside analogue drugs. These drugs may be less toxic and more specific than nucleoside analogues, however their weak inhibitory potentials requires the development of more potent inhibitory drugs in the future (9,12).

DNMT inhibitors may also increase the expession of miRNAs which modify the epigenome. For example, in response to the tratment with DNMT inhibitry drugs, miR-101 has been reported to repress the translation of EZH2 and thereby reduce H3K27 methylation. Another example showing miRNAs play roles in epigenetic therapy is that reactivation of mir-127 following treatment with decitabine and 4-phenylbutyric acid cause downregulation of the oncogene BCL6 (9).

Utilizing of DNMT inhibitory compounds for the treatment of solid tumors remains a challenge in epigenetic therapy since solid tumors have lower drug penetrance and mostly proliferate at slower rate than hematological cancers, which are the impediments to the treatment of solid tumors because DNMT inhibitors are effective on rapidly dividing cells and also unstable in aqeous solution (45). Combination of DNMT inhibitory compounds with HDAC inhibitors for the treatment of solid cancers indicated some objective responses on non-small-cell lung cancer patients. There are ongoing clinical trials to understand the effects of DNMT inhbitory drugs on solid tumors in combination with HDAC inhibitors and/or other agents (46,47).

ii. HDAC inhibitors

HDAC inhibitors have been proven to be effective in the treatment of cancer by leading re-establisment of acetylation to reactivate silenced genes. The main antiproliferative effects of HDAC inhibitors are growth arrest, induction of differentiation and apoptosis but they can also inhibits angiogenesis and metastasis (16). Most HDAC inhibitors affect zinc-dependent HDACs and are divided into several classes of depending on their chemical nature: short-chain fatty acids (such as sodium phenylbutyrate, sodium butyrate and valproic acid), hydroxamic acids (such as suberoylanilide hydroxamic acid-SAHA or vorinostat and panobinostat), cyclic tetrapeptides (such as romidepsin) and benzamides (such as entinostat) (5,7). Two of these inhibitors (SAHA or Vorinostat) and depsipeptide (Romidepsin) have been approved by FDA for the treatment of cutaneous T-cell lymphoma (8).

Valproic acid has been first approved by FDA for the treatment of patients with epilepsy but it has also been used as an epigenetic drug for the treatment of cervical cancer and leukemia (5).

Depsipeptide, an example of cyclic peptide HDAC inhibitor, has been showed to lower DNA methylation and increase acetylation of histones in lung, pancreatic and colon cancer cell lines. However, the mechanism is not well understood (7).

Similar to DNMT inhibitors, HDAC inhibitors have the lack of specificity in the mechanism of action. It has been proposed that restoration of histone acetylation patterns through HDAC inhibitors may result in open chromatin configuration and transcriptional activation. This hypothesis, however, needs to be supported by further research experiments (48). One exciting improvement associated with HDAC inhibitors is that they reverse drug resistance which may derive via epigenetic mechanisms (49). Thus, epigenetic therapy may be used for overcoming the resistance to standart chemotherapy in the cancer management, where drug resistance has been a major concern.

iii. Combination Epigenetic Therapy

The concept of the combination of the drugs in epigenetic therapy exploits synergistic effects of DNMT and HDAC inhibitors in cancer treatment. Furthermore, a synergistic effect has also been found when combining epigenetic drugs with conventional chemotherapy. Such combination treatment strategies have been observed to be more effective than individual treatment approaches (4).

Clinical trials are currently ongoing for testing these combinations for several tumor types. The first study used Vidaza and the older HDAC inhibitor sodium phenyl butyrate on patients with MDS and AML, and the patients achieved clinical response (50).

Subsequently, a study of Vidaza and Valproic acid also suggested high efficacy in MDS (51).

A number of HDAC inhibitors such as trichostatin A, belinostat and varinostat have been reported to act as synergists with a number of conventional chemotherapeutic drugs such as paclitaxel, gemcitabine, cisplatin, doxorubicin (16). Additionally, some mutated enzymes such as EZH2 in the PcG system and the JARID histone demethylases could be therapeutic targets for specific DNA methylation and histone deacetylation changes (34).

One of the major challanges of epigenetic therapy is the possibility of reactivation of normally silenced sequences (repetitive sequences or imprinted genes) in response to inhibitory drugs. Although there is no report supporting clinically increased tumorigencity associated with this, it might arise in near future. Therefore, new epigenetic compounds that selectively targeting specific genes are needed to obtain the best therapeutic outcome. Another challenge of epigenetic therapy is the presence of primary or acquired resistance

to these drugs both in vitro and in vivo. It has been reported that only 50% of MDS or AML patients treated with DNMT inhibitors achieve clinical response (12). Thus, much research remains in finding predictive biomarkers to DNMT inhibitors and designing new compunds for epigentic therapy.

In summary, the potential for reversing epigenetic abnormalities for the goal of cancer prevention and treatment is the revolution in the field of cancer biology. However, there is much work ahead to exploit all the epigenetic knowledge for the efficient translational purposes. The rising interest in epigenetic research may lead to improvement in cancer therapy in future.

REFERENCES

1. Rodríguez-Paredes M, Esteller M. (2011). Cancer epigenetics reaches mainstream oncology. *Nat Med.* Mar;17(3):330-9.
2. Taby R, Issa JP. (2010). Cancer epigenetics. *CA Cancer J Clin.* Nov-Dec;60(6):376-92.
3. Ahuja N, Sharma AR, Baylin SB. (2016). Epigenetic Therapeutics: A new weapon in the war against cancer. *Annu Rev Med.* 67:73-89.
4. Baylin SB, Jones PA. (2016). Epigenetic Determinants of Cancer. *Cold Spring Harb Perspect Biol.* Sep 1;8(9).
5. Mulero-Navarro S, Esteller M. (2008). Epigenetic biomarkers for human cancer: the time is now. *Crit Rev Oncol Hematol.* Oct;68(1):1-11.
6. Huang YW, Kuo CT, Stoner K, Huang TH, Wang LS. (2011). An overview of epigenetics and chemoprevention. *FEBS Lett.* Jul 7;585(13):2129-36.
7. Cortez CC, Jones PA. (2008). Chromatin, cancer and drug therapies. *Mutat Res.* Dec 1;647(1-2):44-51.
8. Tsai HC, Baylin SB. (2011). Cancer epigenetics: linking basic biology to clinical medicine. *Cell Res.* Mar;21(3):502-17.
9. Sharma S, Kelly TK, Jones PA. (2010). Epigenetics in cancer. *Carcinogenesis.* Jan;31(1):27-36.
10. You JS, Jones PA. (2012). Cancer genetics and epigenetics: two sides of the same coin? *Cancer Cell.* Jul 10;22(1):9-20.
11. Sinčić N, Herceg Z. (2011). DNA methylation and cancer: ghosts and angels above the genes. *Curr Opin Oncol.* Jan;23(1):69-76.
12. Sato T, Issa JJ, Kropf P. (2017). DNA Hypomethylating Drugs in Cancer Therapy. *Cold Spring Harb Perspect Med.* May 1;7(5).
13. Lo PK, Sukumar S. (2008). Epigenomics and breast cancer. *Pharmacogenomics.* Dec;9(12):1879-902.
14. Brown D, Demircan B. (2009). "Cancer Epigenetics", in: Epigenetics Mechanisms, Functions and Human Effects. , Pinter B, Meszaros Z, Eds., *Nova Science Publishers*, Inc, pp.273-280.
15. Toyota M, Suzuki H, Yamashita T, Hirata K, Imai K, Tokino T, Shinomura Y. (2009). Cancer Epigenomics: Implications of DNA methylation in personalized cancer therapy. *Cancer Sci,* 100:5, 787-791.
16. Kristensen LS, Nielsen HM, Hansen LL. (2009). Epigenetics and cancer treatment. *Eur J Pharmacol.* Dec 25;625(1-3):131-42.
17. Pfeifer GP. (2018). Defining Driver DNA Methylation Changes in Human Cancer. *Int J Mol Sci.* Apr 12;19(4).

18. Gagliardi M, Strazzullo M, Matarazzo MR. (2018). DNMT3B Functions: Novel Insights From Human Disease. *Front Cell Dev Biol.* Oct 22;6:140.
19. Drake TM, Søreide K. (2019). Cancer epigenetics in solid organ tumours: A primer for surgical oncologists. *Eur J Surg Oncol.* Feb 5. pii: S0748-7983(19)30274-4.
20. Jones PA, Ohtani H, Chakravarthy A, De Carvalho DD. (2019). Epigenetic therapy in immune-oncology. *Nat Rev Cancer.* Feb 5.
21. Xue B, Zhao J, Feng P, Xing J, Wu H, Li Y. (2019). Epigenetic mechanism and target therapy of UHRF1 protein complex in malignancies. *Onco Targets Ther*. Jan 11;12:549-559.
22. Fardi M, Solali S, Farshdousti Hagh M. (2018). Epigenetic mechanisms as a new approach in cancer treatment: An updated review *Genes Dis.* Jun 18;5(4):304-311.
23. Vacante M, Borzì AM, Basile F, Biondi A. (2018). Biomarkers in colorectal cancer: Current clinical utility and future perspectives. *World J Clin Cases.* Dec 6;6(15):869-881.
24. Montellier E, Gaucher J,. (2019). Targeting the interplay between metabolism and epigenetics in cancer. *Curr Opin Oncol.* Mar;31(2):92-99.
25. Lei Y, Huang YH, Goodell MA. (2018). DNA methylation and de-methylation using hybrid site-targeting proteins. *Genome Biol.* Nov 6;19(1):187.
26. Garinis GA, Patrinos GP, Spanakis NE, Menounos PG. (2002). DNA hypermethylation: when tumour suppressor genes go silent. *Hum Genet.* Aug;111(2):115-27.
27. Knudson A. (2001). Alfred Knudson and his two-hit hypothesis. (Interview by Ezzie Hutchinson). *Lancet Oncol.* Oct;2(10):642-5.
28. Jones PA, Laird PW. (1999). Cancer epigenetics comes of age. *Nat Genet.* Feb;21(2):163-7.
29. Baylin SB. (2005). DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol.* Dec;2 Suppl 1:S4-11.
30. Shen H, Laird PW. (2013). Interplay between the cancer genome and epigenome. *Cell.* Mar 28;153(1):38-55.
31. Davalos V, Esteller M. (2010). MicroRNAs and cancer epigenetics: a macrorevolution. *Curr Opin Oncol.* Jan;22(1):35-45.
32. Chuang JC, Jones PA. (2007). Epigenetics and microRNAs. *Pediatr Res.* May;61(5 Pt 2):24R-29R.
33. Vasudevan D, Bovee RC, Thomas DD. (2016). Nitric oxide, the new architect of epigenetic landscapes. *Nitric Oxide.* Sep 30;59:54-62.
34. Baylin SB, Jones PA. (2011). A decade of exploring the cancer epigenome-biological and translational implications. *Nat Rev Cancer*. Sep 23;11(10):726-34.
35. Dawson MA, Kouzarides T. (2012). Cancer Epigenetics: From mechanism to therapy. *Cell.* Jul 6;150(1):12-27.
36. Momparler RL. (2005). Epigenetic therapy of cancer with 5-aza-2'-deoxycytidine (decitabine). *Semin Oncol.* Oct;32(5):443-51.
37. Jabbour E, Issa JP, Garcia-Manero G, Kantarjian H. (2008). Evolution of decitabine development: accomplishments, ongoing investigations, and future strategies. *Cancer.* Jun;112(11):2341-51.
38. Ahuja N, Easwaran H, Baylin SB. (2014). Harnessing the potential of epigenetic therapy to target solid tumors. *J Clin Invest.* Jan;124(1):56-63.

39. Kaminskas E, Farrell AT, Wang YC, Sridhara R, Pazdur R. (2005). FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. *Oncologist.* Mar;10(3):176-82.

40. Lübbert M. (2000). DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action. *Curr Top Microbiol Immunol.,* 249:135-64.

41. Plimack ER, Kantarjian HM, Issa JP. (2007). Decitabine and its role in the treatment of hematopoietic malignancies. *Leuk Lymphoma.* Aug;48(8):1472-81.

42. Gnyszka A, Jastrzebski Z, Flis S. (2013). DNA methyltransferase inhibitors and their emerging role in epigenetic therapy of cancer. *Anticancer Res.* Aug;33(8):2989-96.

43. Chuang JC, Warner SL, Vollmer D, Vankayalapati H, Redkar S, Bearss DJ, Qiu X, Yoo CB, Jones PA. (2010). S110, a 5-Aza-2'-deoxycytidine-containing dinucleotide, is an effective DNA methylation inhibitor in vivo and can reduce tumor growth. *Mol Cancer Ther.* May;9(5):1443-50.

44. Brueckner B, Garcia Boy R, Siedlecki P, Musch T, Kliem HC, Zielenkiewicz P, Suhai S, Wiessler M, Lyko F. (2005). Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res.* Jul 15;65(14):6305-11.

45. Issa JP, Kantarjian HM. (2009). Targeting DNA methylation. *Clin Cancer Res.* Jun 15;15(12):3938-46.

46. Stewart DJ, Issa JP, Kurzrock R, Nunez MI, Jelinek J, Hong D, Oki Y, Guo Z, Gupta S, Wistuba II. (2009). Decitabine effect on tumor global DNA methylation and other parameters in a phase I trial in refractory solid tumors and lymphomas. *Clin Cancer Res.* Jun 1;15(11):3881-8.

47. Juergens RA, Wrangle J, Vendetti FP, Murphy SC, Zhao M, Coleman B, Sebree R, Rodgers K, Hooker CM, Franco N, et al. (2011). Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer. *Cancer Discov.* Dec;1(7):598-607.

48. Xu WS, Parmigiani RB, Marks PA. (2007). Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene.* Aug 13;26(37):5541-52.

49. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, McDermott U, Azizian N, Zou L, Fischbach MA, et al. (2010). A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell.* Apr 2;141(1):69-80.

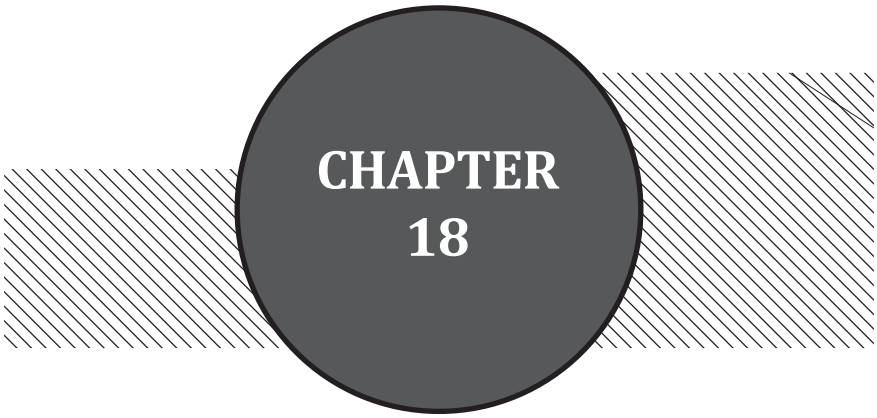
50. Gore SD, Baylin S, Sugar E, Carraway H, Miller CB, Carducci M, Grever M, Galm O, Dauses T, Karp JE, et al. (2006). Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res.* Jun 15;66(12):6361-9.

51. Voso MT, Santini V, Finelli C, Musto P, Pogliani E, Angelucci E, Fioritoni G, Alimena G, Maurillo L, Cortelezzi A, et al. (2009). Valproic acid at therapeutic plasma levels may increase 5-azacytidine efficacy in higher risk myelodysplastic syndromes. *Clin Cancer Res.* Aug 1;15(15):5002-7.

Brucellosis in Small Ruminants

Elçin GÜNAYDIN[1]

Gülşen GONCAGÜL[2]



[1] University of Hitit, Alaca Avni Çelik Vocational School, Çorum

[2] University of Bursa Uludag, Mennan Pasinli Equine Vocational School, Bursa

Except sporadic infections caused by *Brucella abortus* (B.abortus) or *Brucella suis* (*B. suis*) which have been seen rarely in small ruminants, Brucellosis in sheep and goats (excluding *Brucella ovis* infection) is primarily caused by one of the three biovars of *Brucella melitensis* (OIE, 2016, Kelkay et al., 2017). The disease is also caused by *Brucella ovis* (*B. ovis*) which severely affects sheep (OIE, 2015).

Infection with *Brucella* in sheep and goats is endemic in the Mediterranean region, but infection is widespread world-wide. However, North America (except Mexico), northern and central Europe, south-east Asia, Australia and New Zealand are believed to be free from the agent. Pathologically and epidemiologically, *Brucella melitensis* (*B.mellitensis*) infection in sheep and goats is very similar to *B. abortus* infection in cattle. In most circumstances, the primary routes of transmission of *Brucella* are the placenta, fetal fluids and vaginal discharges expelled by infected ewes and goats when they abort or have full-term parturition. In addition to this, Brucella is also shed in udder secretions and semen. *Brucella* can be isolated from various tissues, such as lymph nodes from the head, spleen and organs associated with uterus, epididymides and testes, and from arthritic lesions (Alton et al., 1988).

B. melitensis is the most important cause of brucellosis which primarily affects sheep and goats and also very pathogenic for human beings. The species *B. melitensis* is the causal organism of brucellosis in small ruminants and undulating or "Malta fever" in humans. Although *B. melitensis* infects mainly sheep and goats and its zoonotic importance, plays a significant role in the national economy and the public health of many developing countries (OIE, 2016)

The disease caused by the infection of sheep with *B. ovis* is characterized by infertility in rams due to epididymitis, and also abortion and neonatal mortality are the other clinical signs caused by the infection (OIE, 2015).

B. melitensis infection in wild ruminants may occur when these species are in close contact with sheep and goats in enzootic areas. The manifestations of brucellosis in these animals are similar to those in cattle or sheep and goats. However, in several wild ruminant species considered as dead-end carriers (e.g. chamois [Rupicapra rupicapra], Alpine ibex [Capra ibex] and the Iberian wild goat [Capra pyrenaica], Brucellosis have been reported with the clinical manifestations such as purulent or calcified arthritis and orchitis as well as uveitis and neurological troubles (OIE, 2016).

AGENT PROPERTIES OF *B. MELITENSIS* AND *B. OVIS*

Since the microbiological and serological properties of *B. melitensis* and *B. ovis* were given in *Office des International Epizootica* (OIE, 2015, OIE, 2016) in detail, in this section some general features of *Brucella* spp. are emphasized briefly.

Brucellae are coccobacilli or short rods 0.6 to 1.5 μm long by 0.5 to 0.7 μm in width. *Brucella* are non-motile. They do not form spores, flagella, or pili. True capsules are not produced. Brucellae are Gram-negative and usually do not show bipolar staining. They are not truly acid-fast but resist decolouration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method. *Brucella* members are aerobic, but some strains require an atmosphere containing 5-10% carbon dioxide (CO2) added for growth, especially on primary isolation. Contrary to the situation with several *B. abortus* biovars as well as *B. ovis*, the growth of *B. melitensis* or *B. suis* is not dependent on an incubating atmosphere containing 5–10% CO2 but such a CO2 enriched-atmosphere is optimal for the culture of all *Brucella* (OIE, 2016). The optimum pH for growth varies from 6.6 to 7.4, and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C, but most strains can grow between 20°C and 40°C. Brucella require biotin, thiamin and nicotinamide.

Smooth *Brucella* cultures, especially *B. melitensis* cultures, have a tendency to undergo variation during growth, especially with subcultures, and dissociate to rough (R) forms, and sometimes mucoid (M) forms (OIE, 2016).

B. ovis is one of the two *Brucella* species naturally in the rough phase. *B. ovis* is similar to the other *Brucella* spp. in its morphology, staining properties and cultural characteristics, However it gives negative reactions to the oxidase and urease tests (OIE, 2015).

EPIDEMIOLOGY OF *B. MELITENSIS* AND *B. OVIS*

B. melitensis is the most virulent species of the *Brucella* genus and has three biovars, with biovars 1 and 3 being the ones isolated most frequently in small ruminants in the Mediterranean, the Middle East and Latin America (Blasco & Molina-Flores, 2011, Lucero et al., 2008). Brucellosis is a barrier to trade in animals and animal products and causes significant losses from abortion, as well as being a serious zoonosis (Banai, 2007, Benkirane, 2006).

In the European Union the following Member States and regions have been recognised as being free from *B. melitensis*: Belgium, Denmark, Finland, Germany, Ireland, Luxembourg, the Netherlands, Sweden, the United Kingdom (OIE, 2016).

In most circumstances, the primary route of dissemination *Brucella* is the placenta, foetal fluids and vaginal discharges expelled by infected ewes after abortion or full-term parturition. Shedding of *Brucella* is also common in udder secretions and semen, (Alton et al., 1988).The persistent infection of the mammary glands and supramammary lymph nodes leads to a constant or intermittent shedding of the organisms providing an important source of infection for man and young animals. While orchitis and epididymitis are uncommon in rams and billy goats, they do occur. *B. melitensis* biovar 3 has been isolated from a testicular hygroma of a ram (Musa& Jahans, 1990).

Transmission to man is as a result of contact with infected animal carcasses, aborted fetus, placenta, consumption of unpasteurized milk and cheese. It is common to observe human cases that are in contact with sheep and goats in an area where active brucellosis outbreak occurs. Raw vegetable and water contaminated with the extra of infected animals can also serve as a source ofinfection. *Brucella* organisms can remain viable in milk,water, and damp soil for up to four months (Radostits et al., 2007).

PATHOGENESIS

Infection is on the basis of host's ability to prevent invading organism. Invading *B. melitensis* is usually localized in the lymph nodes, draining the invasion site. This situation results with hyperplasia of lymphoid and reticuloendothelial tissue and the infiltration of inflammatory cells. If the bacteria survives local infection initiates. During the bacteremic case, which may last 2-8 weeks, bones, joints, eyes, and brain can be infected. Super mammary lymph nodes, milk, iliac lymph nodes, spleen and uterus are the places where bacteria are mostly isolated. There is preferential localization to the reproductive tract of the pregnant animals. Allantoic fluid factors, stimulate the growth of *Brucella*. Erythritol, is considered to be one of these factors. Abortion is associated replication of the brucellae within the chorioallantoic trophoblasts. This massive intracellular replication ruptures the infected trophoblasts and allows the bacteria direct access to the fetus. Following broken down placental integrity, fetal infection lead to termination of the pregnancy or the premature birth of a weak and infected lamb (Hotez et al., 2012). Subsequently, development of placentitis is observed. The uterine infection persists for months, and mammary gland may remain infected (Mariane et al., 2010).

In *B.ovis* infections, deteriorated sperm quality and extravasations with a subsequent immunological reaction seen in the tail and causing a spermatocele and therefore reduced fertility. Testicular and epididymal lesions which may be unilaterally, or occasionally, bilaterally can be palpated at about nine weeks after infection but may occur earlier in some rams. A significant proportion of infected rams have no palpable lesions but still excrete the organism (Mariane et al., 2010, Lawrence, 1961).

CLINICAL SYMPTOMS

Brucellosis in sheep and goats are mainly caused by *B. melitensis* and *B. ovis*. However, other types of *Brucella-B. abortus*, *B. suis*- can infect sheep and goats (Luchsinger & Anderson, 1979, Paolicchi et al., 1993). Brucella infection produces a chronic disease on sexually mature animals genital tract is the main target of the bacteria. While *B. melitensis* affects both sheep and goats with the main clinical sign, abortion in females, whereas *B. ovis* affects only sheep. *B. ovis* is the causative agent of contagious epididymitis of rams [Alton, 1985, FAO/OMS, 1986].

The primary clinical signs of brucellosis are related to the reproductive tract. The incubation period is uncertain. It may vary between 15 days to month and years depending on the invasion site, infective dose, and others, (FAO, 2010). Natural *Brucella* infection is identified as an abortion in females. Abortion usually occurs at 3-4 month of pregnancy in infected goats. Goats exhibits the clinical manifestations such as mastitis, with reduced milk production. Unlike sheep, goats that have aborted once are not likely to occur the second time. Second abortion is mostly observed in sheep. Fetal membrane retention may or may not occur in both goats and shepp (FAO, 2010). Goats shade *Brucella* in milk for years, however sheep may shade the bacteria for a short time ie: during one or more lactation period. After abortion execration of bacteria via vaginal fluid and urine may last for the 4-6 month (FAO, 2010).

B. melitensis locates lymphatic ganglia of females and males in the reproductive tract. In addition to this, the agent produces focal granulomatous lesions in the central nervous system, bone marrow, mammary glands, bones, renal cortex and synovial membranes (Enright, 1990, Jubb et al., 1993). The infection of sheep and goats occur mainly through the nasopharynx route. *B. melitensis* can also be transmitted from the mother to the lamb "in uterus" or via the colostrum or milk (Grillo et al., 1997). Before the agent reach the regional lymph nodes, it travells via lymphatic vessels. if local defenses is insufficient in controlling the infection, the uterus will become infected, following a bacteremic phase. After *B. melitensis* can colonize the udder of lactating goat and sheep females, resulting in acute mastitis with the production of clotted and watery milk and reducing milk yields (Enright, 1990). The main clinical signs of *B. melitensis* infection in the female are abortion in the last 2 months of gestation, placenta retention, and giving birth to weak offspring. Grey necrotic cotyledons and edema are seen in the placenta of an aborted female (Aldomy et al., 1992). After abortion females shed large numbers of *Brucella* to the environment, contaminating pastures, soil, and water. Through the vaginal fluid shedding of bacteria can be extended to 2 or 3 months after the abortion or parturition in goats. In sheep shedding of bacteria extends to about 3-4 weeks. In future parturition, Altough the infected females having normal delivery, they continue to shed bacteria through the placenta, vaginal fluids and milk (Alton, 1990). Bronchopneumonia, hemorrhagic fluid in the thoracic cavity, and enlarged lymph nodes, liver and spleen can be seen in aborted fetuses (Alton, 1990). In sheep and goat males, the infection can be located in genital organs with an inflamation. The main output of the disease in males is semen of bad quality and a consequent fertility loss. In the acute phase, orchitis with inflammation of tunica vaginalis, and the scrotal sac can be seen with an either hemorrhagic or fibrino-purulent exudate. In a chronic stage, hygromas and joints' inflammation can be observed in male goats. (Leon, 1994, Enright,

1990, Jubb et al., 1993).

B. ovis is responsible for a disease in rams known as Contagious Epididymitis. Ovine brucellosis due to *B. ovis* occurs worldwide affecting most of the countries where sheep is raised (Burges et al., 1982, Haughes et al., 1968). The initial clinical signs on rams are pyrexia, lassitude and increased respiratory rate associated with swollen testis and epididymis, pain and accumulation of exudate in the scrotal sac (Simmons & Hall, 1983). In the chronic stage, increased size up to four or fivefold of epididymis can be observed. At palpation the affected epididymis is firm, even hard, testicles are usually atrophic. Between the epididymis, the parietal tunica vaginalis and the testes, adhesions can be detected (Simmons & Hall, 1983, Kennedy et al., 1956, Plant et al., 1986). It is necessary to have in mind that some infected rams showing palpable lesions at one examination may be clinically normal a few weeks later (Plant et al., 1986) and that not all the *B. ovis* infected rams are going to develop lesions in their external genital organs (Kennedy et al., 1956). While normal libido is seen in rams, semen quality is variable. Spermatozoa concentration and motility are often reduced (Bulgin, 1990). Clinically, prediagnose can be done by the palpation of external genital organs, if any suspicion of epididmytis in rams exist. Both testicles and epididymides are palpated simultaneously, comparing their size, shape, consistency, and symmetry (McGowan & Shultz, 1967, Van Tonder, 1977).

DIAGNOSIS OF *B. MELITENSIS* AND *B. OVIS*

A) Culture

The gold standard method for the diagnosis of brucellosis in small ruminats is isolation of bacteria (Alton et al., 1988). For the presumptive diagnosis of *B. melitensis*, microscopic examination of stained smears from tissues, secretions and exudates (e.g., placenta, reproductive discharges or the contents of the fetal stomach), using modified ZiehlNeelsen (Stamp) staining may be used. However, morphologically related micro-organisms Brucellae are not truly acid-fast, but they are resistant to decolorization by weak acids and stain red. They appear as coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. Organisms such as *Chlamydia* spp. and *Coxiella burnetii* can resemble *Brucella*. Vaginal swabs and milk samples are the best samples to isolate *B. melitensis* from sheep and goats. In necropsied animals, spleen and lymph nodes are the preferrable samples for the isolation (Marín et al., 1996a). *B. melitensis* does not require serum or CO2 for growth and can be isolated on ordinary solid media under aerobic conditions at 37°C, but selective media should be used. Inorder to increase the sensitivity of bacteriological diagnosis simultaneous use of both the Farrell's and the modified Thayer-Martin's media is adviced. (Marín et al. , 1996b). The Rev-1 vaccine strain can be distinguished from field strains by its growth characteristics and sensitivity to antibiotics and other additives, as well as by genetic tests. Species identification is often done at reference laboratories, as it is complicated by the high genetic similarity between brucellae and the possibility of ambiguous phenotypic tests (OIE, 2016).

Clinical lesions (epididymitis and orchi-epididymitis) in rams may be indicative of the existence of infection, but laboratory examinations are required to confirm the disease. Direct diagnosis is made by bacteriological isolation of B. ovis from semen samples or tissues of rams, or vaginal discharges, milk and tissues of ewes, on adequate selective media (OIE, 2015).

B) Molecular Tests

Definitive diagnosis requires culture and/or the detection of nucleic acids by PCR or other genetic techniques. Most PCR tests only identify Brucella to the genus level, but a few *B. melitensis*-specific PCRs have been published (Kaden et al, 2017) . Multiplex

PCR assays that can identify more than one species of *Brucella* (e.g., the Brucella ladder assay or the older AMOS test) are also used (OIE, 2016, Bricker et al., 1995)Techniques such as multiple-locus variable number tandem repeat analysis (MLVA) can be used in epidemiological investigations of outbreaks (OIE, 2016). In the case of tissues or fluids contaminated with non viable or a low number of *B. melitensis*, PCR could be a potentially useful method for the diagnosis of brucellosis. Several authors reported a good sensitivity of PCR for detecting of *Brucella* DNA on pure cultures (Fekete et al., 1990, Baily et al., 1992, Da Costa et al., 1996). PCR is an useful tool when used alone (PCR, AP-PCR, rep-PCR, ERIC-PCR) or in combination with labelled probes to differentiate some *Brucella* species and biovars (Fekete et al., 1992b, Mercier et al., 1996, Ouahrani-Bettache et al., 1996). Especially PCR techniques overcome isolation problem in heavily contaminated samples with other micro-organisms, and even detect the dead DNA, help to increase the rate of detecting infected animals with *Brucella* spp. (Fekete et al.,1992a; Romero et al., 1995b; Matar et al., 1996).

Molecular methods have been developed for complementary identification of *B. ovis* based on specific genomic sequences (OIE, 2015). Polymerase chain reaction (PCR) based methods can provide additional means of detection (Xavier et al., 2010, OIE, 2016)

C) Serology

Serology can help diagnose clinical cases or monitor herds. Commonly used assays in small ruminants include the buffered *Brucella* agglutination tests (e.g., serum agglutination test and rose bengal tests) the fluorescence polarization assay, complement fixation, and indirect or competitive ELISAs (Nielsen et al., 2004, Marin et al.,1994, Delgado et al., 1995, Debbarh et al., 1996, OIE, 2016) . For the purpose of monitoring the herd Rose Bengal test and serum agglutination test are usually used (OIE, 2016). To decide the herd is positive, complement fixation test is used as a confirmatory test. Combinations of serological tests are often used to improve sensitivity and specificity (Blasco et al., 1994). Antibodies to *Brucella* in sheep and goat milk can be detected with ELISAs . Although serological tests are used widespread, there are some disadvantages about the tests. Serological tests can cross-react with organisms such as *Francisella tularensis*, *Escherichia coli* O:157, *Escherichia coli* O:116 and *Yersinia enterocolitica* O:9 (OIE, 2016). Especially, cross-reactivity with *Yersinia enterocolitica* O:9 can be very difficult to distinguish from reactivity to Brucella (OIE, 2016). Morever distinguishing vaccine-induced antibodies and infection antibodies against Brucella is impossible (OIE, 2016) . A brucellin skin test has also been used to test unvaccinated sheep and goats for exposure to *B. melitensis* (OIE, 2016) Skin tests can be useful as herd tests, but they are not sensitive enough to be detect infections in individual animals (Blasco et al., 1994b)

Serological tests such as CFT, AGID, iELISA is preferred for routine diagnosis of *B. ovis*. CFT is the confirmatory test whereas iELISA is sometimes reported as a less specific method (Estein et al., 2002, Nielsen et al., 2004).

VACCINATION

For the significant decrease in prevalance of *B. melitensis* infections, whole flock vaccination, repeated at regular intervals is the practical method controlling infection in small ruminants in areas where high prevalence of Brucellosis reported. Mass vaccination programs have been described as the unique and first basic strategy to be applied in countries with high brucellosis prevalence in order to control the disease (Blasco & Molina-Flores, 2011). To decide which strategy to combat against Brucellosis is appropriate for all conditions and countries, degree of *Brucella* prevalance should be determined. In addition to the strategy selected, all the nonspecific sanitary measures

for preventing spread infection must be applied in a systematic way (Garin-Bastuji et al., 1998) .

The initial control programmes against *B. melitensis* infection in small ruminants in many countries were based on the compulsory vaccination exclusively of young female replacements with the Rev 1 vaccine using the recommended subcutaneous standard dose (1 X 109 colony forming units) (Alton & S. Elberg 1967, Elberg, 1981). For 5-7 years vaccination, the whole population would have been life-long immunised. However, subcutaneous route has some adverse affects such as vaccine induced abortions, a long lasting serological response which hampers to distinguish the vaccine response during test-and-slaughter during the eradication programmes operated (Mar'in et al., 1999). Due to residual virulence it may induce abortions and also lead to persistent immune responses which could interfere with classical methods of serological diagnostic tests. With the general aim to minimise these adverse effects, different procedures for the administration of the vaccine (conjunctival route instead of subcutaneous route and/or reduction of the dose of vaccine) have been studied in the recent decades. A reduction of the vaccine dose was the second application to overcome the disadvantages emphasized above. A shorter and less intense antibody response induced following vaccination were demonstrated (Gasca et al., 1985; Sales-Henriques et al., 1992, Delgado et al., 1995). Accordingly, a reduced dose (10 3- 10 6 CFU) has been used subcutaneously in field trials and it was proven that reduced dose Rev-1 vaccine relatively safe in pregnant sheep and goats (Gasca et al., 1985; Sales-Henriques et al., 1992, Delgado et al., 1995). However, it was failed to prevent abortion in pregnant sheep and goats, and vaginal excretion of the vaccine strain was demonstrated by the studies (Jiménez de Bagués et al., 1989). Morever, level of protection was found to be poor in goats which received a dose of 104 CFU (Alton, 1970).

Those disadvantages were overcomed by applying REV-1 vaccine in young animals by conjunctival route at full dose (0.5 x109 cfu) which ensures aproximatelly equal protection compared to subcutaneous route Rev-1 besides short-term serological response which is compatible with eradication programmes based on test-and-slaughter principles (Fensterbank et al., 1985).

Anyway, conjunctival vaccine-induced abortions occur 40-60 days after vaccination depending on the stage of pregnancy at the time of vaccination. It was reported that when animals are vaccinated at approximately before first 2 months of pregnancy, the percentage of abortions obtained higher when sheep are vaccinated during the last months of pregnancy (Jiménez de Bagués et al., 1989). Inorder to avoid abortions, it is proposed to vaccinate adult animals at the last months of pregnancy, 2 months before mating or during lactation period, because excretion of vaccine strain with milk is considered to be negligible in terms of potential risk of public health (Fensterbank et al., 1985, Elberg, 1981).

For the prevention from ovine epididymitis, a single standard dose (109 colony-forming units) of the live *B. melitensis* Rev.1 vaccine, administered subcutaneously or, better, conjunctivally, can be used safely and effectively in ram (OIE, 2015).

REFERENCES

1. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.04_BRUCELLOSIS.pdf
2. Kelkay, M. Z., Gugsa,G., Hagos, Y., Taddelle, H. (2017). Sero-prevalence and associated risk factors for Brucella sero-positivity among small ruminants in Tselemti districts, Northern Ethiopia. Journal of Veterinary Medicine and Animal Health, 9 (11), 320-326.

3. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.07.08_OVINE_EPID.pdf
4. Alton, G.G., Jones, L.M., Angus, R.D., Verger, J.M. (1988).Techniques for the brucellosis laboratory, INRA, Paris.
5. Blasco, J.M. and Molina-Flores, B., (2011).Control and eradication of *Brucella melitensis* infection in sheep and goats. Veterinary Clinics of North America: Food Animal Practice, 27 (1), 95–104.
6. Lucero,N.E., Ayala, S.M., Escobar, G.I., Jacob, N.R. (2008). Brucella isolated in humans and animals in Latin America from 1968 to 2006. Epidemiology and Infection, 136 (4), 496–503.
7. Banai, M. (2007). Control of *Brucella melitensis*. Memorias del IV Foro Nacional de Brucelosis, Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México (FMVZ-UNAM), 26–27 November, Mexico, DF.
8. Benkirane, A. (2006). Ovine and caprine brucellosis: world distribution and control/ eradication strategies in West Asia/ North Africa region. Small Ruminant Research, 62 (1–2), 19–25.
9. Musa, M.T. and Jahans K.L. (1990).The isolation of *Brucella melitensis* biovar 3 from a testicular hygroma of a ram in a nomadic flock of sheep and goats in western Sudan'', Journal of Comparative Pathology, 103(4):467-70.
10. Radostits,O.M., Gay, C.C., Hinchcliff, K.W. & Constable, P.D. (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats. Saunders Elsevier, Edinburgh, pp. 963-984.
11. Hotez, P.J., Savioli, L., Fenwick, A. (2012). Neglected tropical diseases of the Middle East and North Africa: Review of their prevalence, distribution, and opportunities for control. PLoSNegl. Tropical Diseases, 6, e1475, 2012.
12. Mariana Xavier, N., T.A. Paixão, den Hartigh A.B., Tsolis, R.M., Santos,R.L.(2010).The Open Pathogenesis of *Brucella* spp. Veterinary Science Journal, 4, 109-118, 2010.
13. Lawrence W.E., (1961). Ovine brucellosis. A review of the disease in sheep manifested by epididymitis and abortion, British Veterinary Journal, 117, 435-46.
14. Luchsinger, D.W., Anderson, R.K. (1979). Longitudinal studies of naturally acquired *Brucella abortus* infection in sheep, American Journal of Veterinary Research, 40, 1307-1312.
15. Paolicchi, F.A., Terzolo, H.R., Campero, C.M. (1993). Isolation of *Brucella suis* from the semen of a ram. Veterinary Record, 132(3), 67.
16. Alton, G.G.(1985).The epidemiology of B. melitensis infection in sheep and goats:187-196. *B. melitensis*. In: Verger JM, Plommet M, Eds. Martinus Nijhoff Publishers: The Netherlands.
17. FAO/OMS (1986). Comité Mixto de Expertos en Brucelosis. Sexto Informe. Organización Mundial de la Salud, Ginebra: Suiza. p. 149.
18. Food and Agriculture Organization (2010). Brucella melitensis in Eurasia and the Middle East. FAO Animal Production and Health Proceedings. No 10. Rome.
19. Enright, F.M. (1990). The pathogenesis and pathobiology of infection in domestic animals. Animal brucellosis. In: Nielsen & Duncan, Eds. CRC Press, Boca Ratón: Florida, USA, pp. 301-320.
20. Jubb,K.V.F., Kennedy, P.C., Palmer, N.(1993). Pathology of domestic animals. 4th ed. Academic Press: San Diego, California, USA vol. 3: p. 653.

21. Grilló, M.J., Barberán, M., Blasco, J.M.(1997).Transmission of *B. melitensis* from sheep to lambs. Veterinary Record, 140, 602-95.
22. Aldomy,F.M., Jahans,K.L., ltarazi,Y.H. (1992). Isolation of *B. melitensis* from aborting ruminants in Jordan. Journal of Comparative Pathology, 107, 239-42.
23. Alton,G.G.(1990). *B. melitensis*. Animal Brucellosis. In: Nielsen &Duncan, Eds. CRC Press: Boca Raton Florida, USA. vol. 17: pp. 383-409.
24. Leon, C.F. (1994). *Brucelosis ovina* y caprina. Ed. Office International dês Epizooties – OIE. Paris: Francia. p. 451.
25. Burgess, G.W., McDonald, J.W. Norris, M.J.(1982). Epidemiological studies on ovine brucellosis in selected ram flocks. Australian Veterinary Journal, 59,45-47.
26. Haughey,K.G., Hughes,K.L., Hartley, W.J. (1968). *Brucella ovis* infection. 2. The infection status in breeding flocks as measured by examination of rams and the perinatal lamb mortality. Australian Veterinary Journal, 44(12):531-5.
27. Simmons, G.C., Hall, W.T.(1953). Epididymitis of rams. Australian Veterinary Journal, 29, 33-40.
28. Kennedy, P.C., Frazier,L.M., McGowan, B. (1956). Epididymitis in rams, Pathology and bacteriology. Cornell Veterinary, 46, 303-19.
29. Plant, J.W., Eamens, G.J., Seaman,J.T. (1986).Serological, bacteriological and pathological changes in rams following different routes of exposure to *Bucella ovis.* Australian Veterinary Journal, 63, 409-12.
30. Bulgin,M.S. (1990). *B. ovis* excretion in semen of seronegative, clinically normal breeding rams. Journal of American Medicine Association, 196, 313-315.
31. McGowan, B., Shultz, G. (1956). Epididymitis of rams: Clinical description and field aspects. Cornell Veteterinary, 46, 277-81.
32. Van Tonder, E.M.(1977). Examination of rams for genital soundness. Journal of South African Veterinary Medicine Association, 48, 267-72.
33. Marín, C.M., Alabart, J.L., Blasco,J.M. (1996a).Effect of antibiotics contained intwo Brucella selective media on growth of *Brucella abortus*, *Brucella melitensis* and *Brucella suis.* Journal of Clinical Microbiology, 34, 426-428.
34. Marín,C.M., Jimenez de Bagüés, M.P., Barberán, M., Blasco, J.M. (1996b).Comparison of two selective media for the isolation of *Brucella melitensis* from naturally infected sheep and goats.Veterinary Record, 138, 409-411.
35. Kaden, R., Ferrari, Alm, S. E., Wahab, T. (2017). A novel real-time PCR assay for specific detection of *Brucella melitensis*. BMC Infectious Disease, 17, 230.
36. Bricker, B. J., & Halling, S. M. (1995). Enhancement of the Brucella AMOS PCR assay for differentiation of Brucella abortus vaccine strains S19 and RB51. Journal of clinical microbiology, 33(6), 1640-2.
37. Fekete,A., Bantle, J.A., Halling,S.M., Sanborn, M.R.(1990). Preliminary development of a diagnostic test for Brucella using polymerase chain reaction. Journal of Applied Bacteriology, 69, 216-227.
38. Baily, G.G., Krahn, J.B., Drasar, B.S., Stocker, N.G. (1992). Detection of *Brucella abortus* and *Brucella melitensis* by DNA amplification. Journal of Tropical Medical Hygenia, 95, 271-275.
39. Da Costa, M., Guillou, J.P., Garin-Bastuji, B., Thiébaud, M., Dubray, G. (1996). Specificity of six gene sequences for the detection of the genus *Brucella* by DNA Amplification. Journal of Applied Bacteriology, 81, 267-275.

40. Fekete, A., Bantle, J.A., Halling, S.M. (1992 a). Detection of Brucella by polymerase chain reaction in bovine fetal and maternal tissues. Journal of Veterinary Diagnostic Investment, 4, 79-83.

41. Mercier, E., Jumas-Bilak, E., Allardet-Servent, A., O'Callaghan, D., Ramuz, M. (1996). Polymorphism in *Brucella* strains detected by studying distribution of two short repetitive DNA elements. Journal of Clinical Microbiology, 34,1299-1302.

42. Ouahrani-Bettache, S., Soubrier, M.P., Liautard, J.P. (1996). IS6501-anchored PCR for the detection and identification of *Brucella* species and strains. Journal of Clinical Microbiology, 81, 154-160.

43. Fekete, A.1., Bantle, J.A., Halling, S.M., Stich, R.W. (1992b). Amplification fragment length polymorphism in Brucella strains by use of polymerase chain reaction with arbitrary primers. Journal of Bacteriology, 174, 7778-7783.

44. Romero, C., Pardo, M., Grillo, M. J., Diaz, R. J., Blasco, M., Lopez-Goñi, I. (1995). Evaluation of PCR and indirect enzyme-linked immunosorbent assay on milk samples for diagnosis of brucellosis in dairy cattle. Journal of Clinical Microbiology, 33, 3198-3200.

45. Matar, G.M., Khneisser, I.A., Abdelnoor, A.M., (1996). Rapid laboratory confirmation of human brucellosis by PCR analysis of a target sequence on the 31- kilo dalton Brucella antigen DNA. Journal of Clinical Microbiology, 34, 477-478.

46. Nielsen, K., Gall, D., Smith, P., Balsevicius, S., Garrido, F., Ferrer, M.D., Biancifiori, F., Dajer, A., Luna, E., Samartino, L., Bermudez, R., Moreno, F., Renteria, T., Corral, A. (2004). Comparison of serological tests for the detection of ovine and caprine antibody to *Brucella melitensis*. Revue Scientifique et Technique, 23(3):979-987.

47. Díaz-Aparicio, E., Marín, C., Alonso-Urmeneta, B., Aragón, V., Pérez-Ortiz, S., Pardo, M., Blasco, J.M., Díaz, R., Moriyón, I. (1994). Evaluation of serological tests for diagnosis of Brucella melitensis infection of goats'', Journal of Clinical Microbiology, 32(5), 1159-1165.

48. Delgado, S.1., Fernández, M., Cármenes, P. (1995) .Evaluation of an enzyme-linked immunosorbent assay for the detection of sheep infected and vaccinated with *Brucella melitensis.* Journal of Veterinary Diagnostic Investment, 7, 206-209.

49. Debbarh, H.S.1., Zygmunt, M.S, Dubray, G., Cloeckaert, A., Competitive enzyme-linked immunosorbent assay using monoclonal antibodies to the Brucella melitensis BP26 protein to evaluate antibody responses in infected and *Brucella melitensis* Rev.1 vaccinated sheep. Veterinary Microbiology, 53, 325-337.

50. Blasco, J.M.1., Garin-Bastuji, B., Marin, C.M., Gerbier, G., Fanlo, J., Jiménez de Bagués, M.P., Cau, C. (1994). Efficacy of different rose bengal and complement of fixation antigens for the diagnosis of Brucella melitensis in sheep and goats'', Veterinary Record, 134, 415-420.

51. Blasco, J.M.1., Marín, C., Jiménez de Bagués, M., Barberán, M., Hernández, A., Molina, L., Velasco, J., Díaz, R., Moriyón, I.(1994b).''Evaluation of allergic and serological tests for diagnosis of Brucella melitensis in sheep. Journal of Clinical Microbiology, 32, 1835-1840.

52. Blasco, J.M., Molina-Flores, B. (2011). Control and eradication of Brucella melitensis infection in sheep and goats. Veterinary Clinics of North America: Food Animal Practice, 27(1):95-104.

53. Garin-Bastuji, B., Lasco, J.M., Grayon, M., Verger, J. M. (1998). *Brucella melitensis* infection in sheep: present and future. Veterinary Research, 29, 255-274.

54. Alton,G.G., Elberg, S. (1967). Rev-1 Brucella melitensis vaccine: a review of ten yaers of study. Veterinary Bulletin, 37, 793-800.

55. Elberg, S.S. (1981). Rev-1 Brucella melitensis vaccine. Part II: 1968-1980. Veterinary Bulletin, 51: 67-73.

56. Marín, C.M.1., Moreno, E., Moriyón, I., Díaz, R., Blasco, J.M. (1999). Performance of competitive and indirect ELISAs, gel immunoprecipitation with native hapten polysaccharide and standard serological tests in diagnosis of sheep brucellosis. Clinical Diagnostic Laboratory Immunology, 6, 269–272.

57. Gasca, A., Jiménez, J.M., Díaz, L. (1995).Experiencias sobre vacunación antibrucelar de cabras adultas con la cepa Rev.1. Diputación Provincial de Cádiz.

58. Henrique, L.R., Henriques, S., Hueston, W.D., Hoblet, K.H., Shulaw, W. P.(1992). Field trials evaluating the safety and serologic reactions of reduced dose Brucella melitensis Rev.1 vaccination in adult sheep.Preventive Veterinary Medicine, 13, 205-215.

59. Delgado, S.1., Fernández, M., Cármenes, P. (1995). Evaluation of an enzyme-linked immunosorbent assay for the detection of sheep infected and vaccinated with *Brucella melitensis.* J. Vet Diagnostic Investment, 7, 206-209.

60. Jiménez de Bagués, M.P., Marin, C,M,, Barberán, M., Blasco, J.M. (1989).Responses of ewes to *Brucella melitensis* Rev.1 vaccine administered by subcutaneous or conjunctival routes at different stages of pregnancy. Annales de Recherches Veterinaires, 20, 205-213.

61. Alton, G.G. (1970). Vaccination of goats with reduced doses of Rev.-1 *Brucella melitensis* vaccine. Research in veterinary Science, 54-59.

62. Blasco, J.M., Molina-Flores, B. (2011).Control and eradication of Brucella melitensis infection in sheep and goats. Veterinary Clinics of North America: Food Animal Practice, 27(1):95-104.

63. Garin-Bastuji, B., Blasco, J.M., Grayon, M., Verger, J.M. (1998). *Brucella melitensis* infection in sheep: present and future. Veterinary Research, 29, 255-274.

64. Alton, G.G., Elberg, S. (1967). Rev-1 *Brucella melitensis* vaccine: a review of ten yaers of study. Veterinary Bulletin, 37, 793-800.

65. Elberg, S.S. (1981) Rev-1 Brucella melitensis vaccine. Part II: 1968-1980. Veterinary Bulletin, 51: 67-73.

66. Marín, C.M., Moreno, E., Moriyón, I., Díaz, R., Blasco, J.M. (1999). Performance of competitive and indirect ELISAs, gel immunoprecipitation with native hapten polysaccharide and standard serological tests in diagnosis of sheep brucellosis. Clinical Diagnostic Laboratory Immunology, 6, 269–272.

67. Gasca, A., Jiménez, J.M., Díaz, L. (1985). Experiencias sobre vacunación antibrucelar de cabras adultas con la cepa Rev.1. Diputación Provincial de Cádiz.

68. Henriques, H. S. Hueston,W.D. Hoblet,K.H. Shulaw,W.P.(1992). Field trials evaluating the safety and serologic reactions of reduced dose *Brucella melitensis* Rev.1 vaccination in adult sheep. Preventive Veterinary Medicine, 13, 205-215.

69. Delgado, S., Fernández, M., Cármenes P. (1995). Evaluation of an enzyme-linked immunosorbent assay for the detection of sheep infected and vaccinated with *Brucella melitensis*.J. Vet Diagnostic Investment, 7, 206-209.

70. Jiménez de Bagués, M.P., Marin, C.M., Barberán, M., Blasco, J.M. (1989). Responses of ewes to Brucella melitensis Rev.1 vaccine administered by subcutaneous or conjunctival routes at different stages of pregnancy. Annales de Recherches Veterinaires, 20, 205-213.

71. Alton, G.G. (1970).Vaccination of goats with reduced doses of Rev.-1 *Brucella melitensis* vaccine. Research in veterinary Science, 54-59.

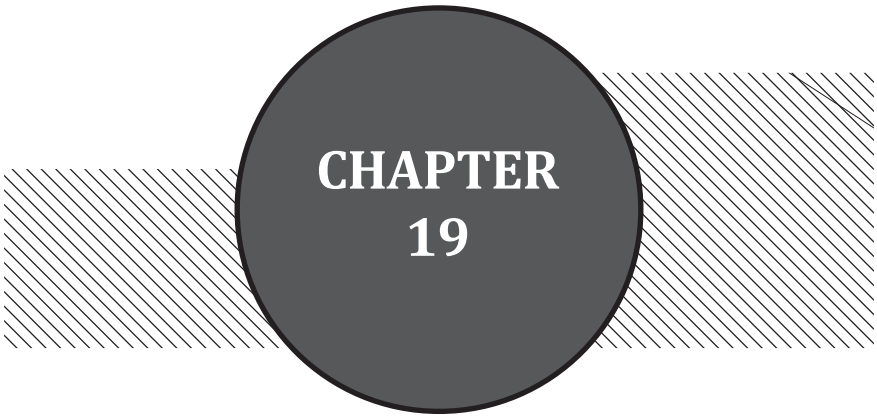
72. Fensterbank, R., Pardon, P., Marly, J. (1985).Vaccination of ewes by a single conjunctival administration of Brucella melitensis Rev.1 vaccine. Annales de Recherches Veterinaires 16, 351–358.

73. Xavier, M.N., Silva, T.M., Costa, E.A., Paixão, T.A., Moustacas, V.S., Jr. Carvalho, C.A., Sant'Anna, F,M,, Robles, C.A., Gouveia, A.M., Lage, A.P., Tsolis, R.M., Santos, R.L. (2010). Development and evaluation of a species-specific PCR assay for the detection of Brucella ovis infection in rams. Veterinary Microbiology, 145, 158–164.

74. Estein, S. M., Baldi, P., Bowden, R. A. (2002).Comparison of serological tests based on outer membrane or internal antigens for detecting antibodies to *Brucella ovis* in infected flocks. Journal of Veterinary Diagnostic Investment, 14, 407-411.

Apoptotic Pathways, Death Receptors And Using Phage Display To Select Apoptosis Causing Peptides And Antibody Fragments

Deniz ŞAHİN[1]



[1] Istanbul Technical University, Department of Molecular Biology and Genetics

APOPTOSIS AND APOPTOTIC PATHWAYS

Apoptosis is a process of programmed cell death (PCD) in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area [Gaoxin *et al*., 2017]. It plays a vital role in developing and maintaining the health of the body by eliminating old cells, unnecessary cells, and unhealthy cells. When apoptosis does not work properly, cells that need to be eliminated can be permanent and immortal as in cancer and leukemia.

Apoptosis can be triggered through two pathways (Figure 1). The first is the intrinsic pathway, also known as the mitochondrial pathway, which is activated by intracellular signals generated in response to cellular stress and depends on the release of pro-apoptotic factors from the mitochondria. The second is the extrinsic pathway, which is initiated by death receptors belonging to the tumor necrosis factor receptor (TNF) receptor superfamily [Gaoxin *et al*., 2017; Micheau *et al*., 2013]. Both intrinsic and extrinsic pathways converge to induce the activation of caspases which are a family of protein proteases. Ultimately, apoptotic cells are ingested by neighboring cells and phagocytes, preventing inflammation and tissue damage that might ensue upon cell lysis [Micheau *et al*., 2013].

In the regulation of apoptosis, Bcl-2 (B-cell lymphoma-2) family proteins play a central role which include the anti-apoptotic (pro-survival) and the pro-apoptotic proteins. The fluctuation in the level of these two opposing members, pro-survival and pro-apoptotic proteins, essentially determines the fate of the cell. There is a balance between these two protein groups in normal cells. However, in cancer cells, anti-apoptotic cells are overexpressed which results the survival and proliferation of cancer cells. Therefore, the anti-apoptotic members of the Bcl-2 family in cancer cells become a promising target for therapeutic purposes. The second promising target, specifically targeted by phage display and discussed below, is the death receptors on the cell surface which are a subgroup of TNF (tumor necrosis factor) receptor super family [Micheau *et al*., 2013].

All Bcl-2 family proteins share the Bcl-2 homology 3 (BH3) domain. The pro-apoptotic Bcl-2 proteins are grouped into either the BH3-only proteins (Bad, Bcl-2 associated death protein; Bik, Bcl-2 interacting killer protein; Bim, BH3-only protein BCL-2 family member; and Bid, BH3-interacting domain death agonist) or the BH1 – BH3 multi-domain proteins (Bax, Bak). The BH3 domain is crucial for homodimer and/or heterodimer formation with the anti-apoptotic Bcl-2 family proteins, which determines either repression or induction of apoptosis[Lutz *et al*., 2000]. Short peptides derived from the BH3 domain of either the BH3-only or the BH1–BH3 multidomain proapoptotic proteins have been shown to induce apoptosis in various cancer cells.

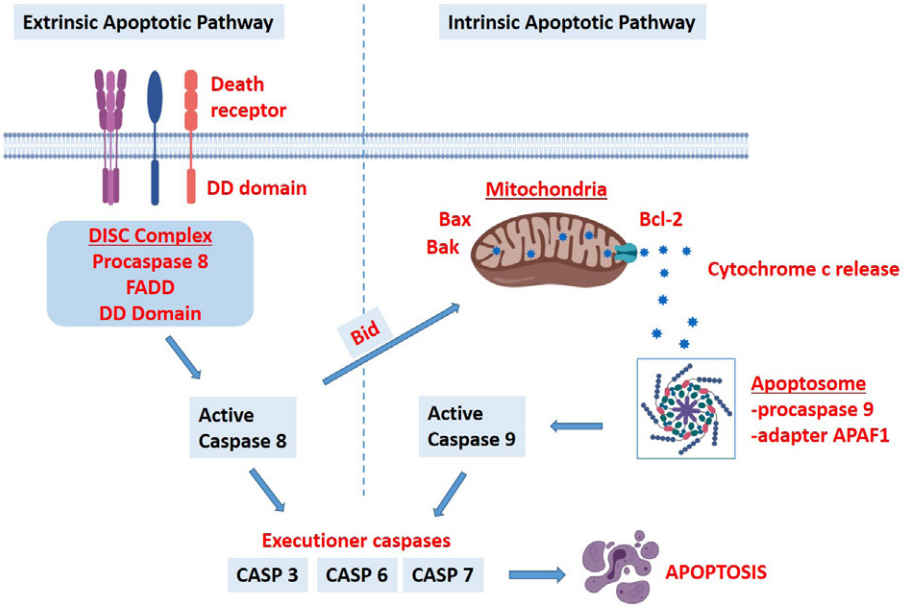


Figure 1: Two main apoptotic pathways, intrinsic and extrinsic pathways, are schematized with the main players having key roles in apoptotic signaling are indicated. (Bcl 2, B-cell lymphoma-2; Bak, Bcl-2-antagonist/killer-1; Bax, Bcl-2-associated X protein; Bid, BH3-interacting domain death agonist; apoptosis protease activating factor (APAF-1); CASP 3, caspase 3; FADD, Fas-associated death domain; DD, death receptor death domain)

INTRINSIC PATHWAY

Intrinsic apoptotic pathway is a mitochondrial mediated apoptosis pathway which requires activation of cytosolic caspases by release of essential proteins including mainly cytochrome *c* [Yang and Cortopassi, 1998] and apoptosis-inducing factor (AIF) [Susin *et al*., 1999] and endonuclease G [Li *et al*., 2001] from mitochondria into cytosol.

The control of pathway involves regulation of mitochondrial outer membrane permeabilization by Bcl-2 family proteins which are characterized by Bcl-2 homology (BH) domains [Green and Kroemer, 2004]. The Bcl-2 protein family comprises three subfamilies which contain between one and four Bcl-2 homology (BH) domains. It can be classified as anti-apoptotic (pro-survival) members and apoptotic (pro-apoptotic) members [Micheau *et al*., 2013]. [Lutz *et al*., 2000]

Bcl-2, Bcl-XL , Bcl-W, MCL1, BCL2A1 and Bcl-B are members of pro-survival proteins containing four BH domains. Most of them also contain one transmambrane domain (TM). They all block apoptosis by binding differentially to the BH-3 only proteins and preventing BH3-only protein-induced oligomerization of the pro-apoptotic Bax and/or Bak in mitochondrial outer membranes, which would otherwise lead to the release of cytochrome c and other mitochondrial intermembrane space proteins. Oligomerization of Bak and Bax at the mitochondria would change membrane permeability resulting the cytochrome c release. [Taylor *et al*., 2008]

Pro-apoptotic members that promote cell death include those containing either multiple BH domains, Bax, Bak and Bok, which lack BH4 domains or a single BH3 sequence containing Bad, Bik, Bid, Puma, Bim, Bmf, Hrk and Noxa proteins. The BH3 only proteins activate multi-domain pro-apoptotic species and disrupt the function of anti-apoptotic

Bcl-2 family members [Letai *et al*., 2002]. "BH3 only" proteins are essential regulators of apoptosis and promote cell death by inhibiting anti-apoptotic proteins and promoting insertion of Bax and Bak into the outer mitochondrial membrane. It is thought that multi-domain Bcl-2 family members form channels in the outer mitochondrial membrane through which apoptogenic proteins of the intermembranal space are released. [Taylor *et al*., 2008; Lutz *et al*., 2000]

Bcl-2 family protein activity is often regulated through phosphorylation. Phosphorylation of Bad prevents interaction between Bad and anti-apoptotic Bcl-2 and Bcl-xL proteins. Pro-apoptotic Bim phosphorylation results in ubiquitin-mediated degradation of Bim and decreased inhibition of anti-apoptotic Bcl-2 and Bcl-XL proteins. Phosphorylation of Bid in response to DNA damage prevents its activation and promotes DNA repair and cell survival pathways [Letai *et al*., 2002].

Intracellular processes following release of apoptogenic proteins: After the release of apoptogenic proteins into cytosol through PT (permeability transition) pore, cytochrome c forms an "apoptosome" in conjuction with apoptosis protease activating factor (APAF-1) and pro-caspase 9 [Zou *et al*., 1999]. Apoptosome complex promotes the activation of caspase 9, which activates effector caspases that collectively orchestrate the execution of apoptosis. AIF-apoptosis inducing factor (Susin *et al.,* 1999) and endonuclease G (van Loo *et al.,* 2001) both contribute to DNA fragmentation and subsequent chromosomal condensation [Micheau *et al*., 2013].

Caspases: Caspases are aspartate-specific cysteine proteases playing key roles in apoptosis. So far, at least 14 different caspases have been identified functioning in apoptosis and inflammation [Wolf and Green, 1999]. They are expressed as pro-enzymes containing three domains, including an NH_2 terminal, a large subunit (~20 kDa) and a small subunit (~10 kDa). Mammalian caspases are classified according to structure, molecular function, and substrate preference. Caspases-2, -8, -9, -10, and -12 are referred to as "initiator caspases" as they are closely coupled to upstream, pro-apoptotic signals. Initiator caspases cleave and activate downstream effector or "executioner" caspases -3, -6, and -7 that modify proteins ultimately responsible for programmed cell death.

Apoptotic signals trigger caspase activation involving the cleavage and recombination of the large (p20) and small (p10) caspase domains with the removal of an amino-terminal prodomain. When they are active, caspases cleave target proteins at specific amino acid residues during a programmed proteolytic cascade. Caspases cleave several proteins which at the end cause cell death.

The cleaved proteins include nuclear lamins [Lazebnik *et al*., 1995], DNA repair enzymes such as poly-ADP-ribose-polymerase (PARP) [Lazebnik *et al*., 1994], and cytoskeletal proteins such as actin [Mashima *et al*., 1995], fodrin [Cryns *et al*., 1995] and gelsolin [Kothakota *et al.,*1997].

DNA is fragmented during apoptosis which is caused in part by an enzyme known as caspase-activated DNase (CAD). Normally, CAD forms an inactive complex with the inhibitor of CAD (ICAD). During apoptosis, caspase 3 cleaves ICAD and releases CAD which triggers DNA fragmentation [Sakahira *et al.,* 1998].

Caspases can be inhibited by inhibitor of apoptosis (IAP) proteins (c-IAP1/2, XIAP, livin, survivin) that either block caspase activity through direct binding or by acting as ubiquitin ligases that target caspases for ubiquitin-mediated degradation.

EXTRINSIC PATHWAY-DEATH RECEPTOR PATHWAY

Death receptors are cell surface receptors which belong to the tumor necrosis factor (TNF) superfamily and trigger apoptosis upon ligand binding. The extrinsic apoptotic pathway can be induced through the activation of death receptors by their respective ligands which make them promising targets for phage display technique to select specific ligands activating apoptotic pathway.

The best characterized death receptors are Fas (CD95/Apo1), TNF receptor 1 (p55), TRAMP (Apo3/DR3), TRAIL-R1 (DR4) and TRAIL-R2 (DR5/Apo2). Fas ligand (CD95) binds Fas, TNF and lymphotoxin a bind to TNFR, TWEAK binds to TRAMP and TRAIL binds both TRAIL-R1 and TRAIL-R2 [Graves *et al*., 2014; Walczakh, H., 2013; Micheau *et al*., 2013] (Figure 2).

Death receptor ligands initiate signaling via receptor oligomerization, which results in the recruitment of specialized adaptor proteins and activation of caspase cascades. Death receptors contain an intracellular death domain (DD). With the ligand binding, death domain associates with an adaptor protein called **Fas-associated death domain (FADD)** directly or indirectly via **TNFR-associated death domain (TRADD)** [Ashkenazi and Dixit, 1998]. FADD also interacts with procaspase-8 to form a complex at the receptor called the death-inducing signaling complex (DISC) which induces the activation of caspase-8. Then, caspase-8 precipitates the activation of downstream effector caspases; caspase-3, -6 and -7.

Extrinsic and intrinsic pathways are also linked through cleaving of Bid (BH3 only protein) by pro-caspase 8, which translocates to the mitochondria to activate the intrinsic pathway [Micheau *et al*., 2013].

In addition to death receptors, the TNF superfamily comprises decoy receptors (DcR) which include DcR1, DcR2 and osteoprotegerin (OPG), which bind to TRAIL and DcR3, which binds Fas ligand. These receptors act through the sequestration of ligands and they inhibit death signaling [Ashkenazi and Dixit, 1998].

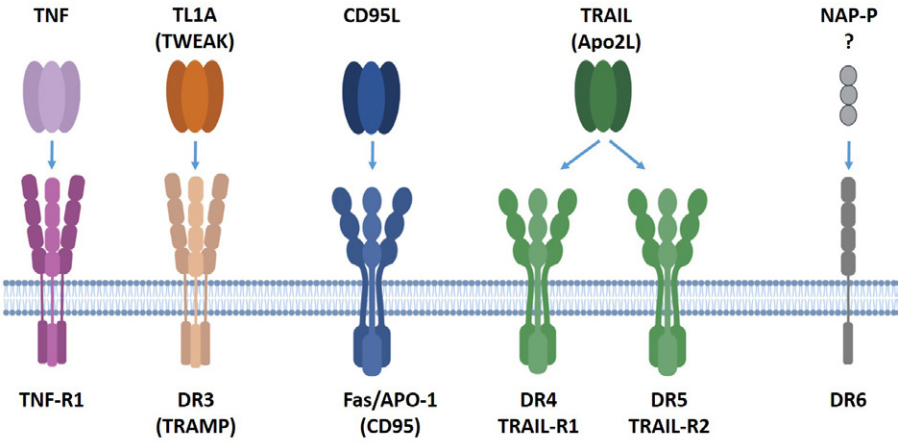


Figure 2: Death receptors and their ligands. Death receptors are transmembrane receptors that contain an intracellular death domain capable of recruiting specific adaptors defining downstream interactors and signals. Death receptors trigger two main signals. TNF-R1 and DR3 induce gene activation as primary output. Fas, DR4 and DR5, on the other hand, induce apoptosis as their primary signalig output. DR6 has been proposed to be ligated by N-APP but this is unconfirmed [Walczakh, H., 2013].

APOPTOSIS CAUSING PEPTIDES/ANTIBODY FRAGMENTS AND PHAGE DISPLAY

Several proteins are effective in both apoptotic pathways, intrinsic or extrinsic, and play critical roles in the regulation of the process. So far, different strategies were followed to develop effective molecules which may regulate or activate the apoptotic pathways. Key processes such as cytochrome c release from mitochondria can be stimulated by peptides. Several pro-apoptotic peptides have been obtained from pro-apoptotic proteins including Bax, Bad, Bim, Bid, Puma or other proteins effective in both pathways. The peptides are then delivered into the cell by conjugating with cell permeating peptides (CPP) [Barras and Widmann, 2011].

As mentioned above in "Extrinsic pathway" section, binding of ligands on death receptors may trigger apoptotic pathways that makes the mechanism a promising strategy to induce apoptosis especially in cancer cells. Ligands for death receptors as single agents or in combination with other therapeutic agents may possibly be used as anti-cancer drugs especially if they have specificity on cancer cells over normal-healthy cells.

In this context, phage display selection technique can be used for the selection of short peptide or antibody fragments as specific ligands for surface death receptors (Figure 3). The selected peptides or fragments can be tested as therapeutic molecules. Short peptides with low molecular weight generally display low toxicity, high bioavailability and solubility. They can be produced easily and at low cost. On the other hand, selected antibody fragments are larger than short peptides. Relatively higher specificity can be reached with antibody fragments which shows more specific three dimensional shape. They can also be fused with antibody fragments to form functional whole antibody molecules [Sahin, D., 2018].

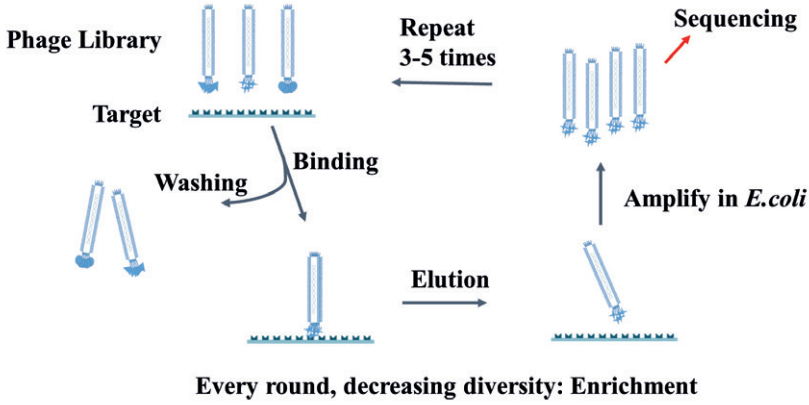


Figure 2: Biopanning procedure of a phage display library to select phages binding to desired target [Sahin, D., 2018].

Phage display is a widely used selection technique for drug discovery, studying protein-protein interactions, *in vitro* protein evolution, target specific peptide and antibody selection, detection of agonists and antagonists, and many other therapeutic and diagnostic purposes since its discovery by George P. Smith in 1985 [Smith *et al.*, 1985]. The principle of the technique is screening large peptide/antibody libraries against a target for the determination of specific peptides or antibody fragments. Target molecule can be different proteins, epitopes, cell surface receptors, whole cells, tumor tissues or several different organic or inorganic substances. Death receptors on the cell surface of

cancer cells are promising targets for phage display biopanning cycles for the selection of specific ligand peptides or antibody fragments. Negative selection against normal cells will eliminate specific colonies for normal cells, and remaining library will be used to enrich cancer specific colonies [Sahin, D., 2018].

The selected specific short peptides or antibody fragments are candidate small molecules which may trigger apoptosis in cancer cells. Listed death receptors are all possible targets for the seletion of short peptides which may activate apoptotic pathway resulting the death of cancer cell.

Both extrinsic and intrinsic apoptotic pathway members are possible targets for the selection of short peptides or antibody fragments via phage display screening. If biopanning procedure is applied against death receptors, the selected peptides are checked if they competitively inhibit the binding of death receptor and its natural ligand. The peptide may trigger apoptotic pathway also.

When the intrinsic pathway members are targeted, phage display selected peptides are better conjugated with cell penetrating peptides, to enhance the intake of the peptide into the cell. The peptide can be selected against survival proteins to inhibit their binding to pro-apoptotic proteins, or the peptide can be selected against pro apoptotic proteins and activation of apoptosis is investigated.

In a study by Vrielink *et al*. (2010), DR5 death receptor is used as target molecule to select specific short peptides via phage display technique. The selected peptides were identified that bind specifically to DR5 but not to DR4 or any decoy receptors. The best binding peptide, YCKVILTHRCY, and its both monomeric and dimeric forms, bind specifically to DR5 death receptor in such a way that TRAIL binding to DR5 is inhibited which shows competitive inhibition of ligand binding on DR5 by the selected peptide. Additionally, selected peptide and its dimeric form reduced TRAIL (DR5 ligand) induced cell death in Colo205 colon carcinoma cells. Although the selected peptide does not directly act as apoptosis causing peptide, it is usefull to dissect signalling via DR5 relative to DR4 and it can be used as a lead peptide for the development of therapeutic agents in diseases with dysregulated TRAIL-signalling.

In another study by Bing *et al*. (2006), the human proapoptotic death receptor DR5 was used again as target site for phage display selection of peptides and synthetic antibodies. Although the selected peptides and antibodies had different size and structures, all shared a tripeptide motif which was conserved within a disulfide-constrained loop of the peptides and the third complementarity determining region of the antibody heavy chains. They found that tripeptide motif is buried at the core of the interface, confirming its central role in antigen recognition. They also found that selected certain peptides and antibodies exhibited potent proapoptotic activity against DR5-expressing SK-MES-1 lung carcinoma cells which makes them candidate tools to trigger DR5 activation for extrinsic apoptotic pathway.

Similarly, in another study by Gaoxin *et al*. (2017), phage display was used to isolate a novel fully human agonistic single chain fragment variable (scFv) antibody, which targets DR5.

Although several peptides and antibody fragments were selected and developed as pro-apoptotic molecules, their activity were limited as single therapeutic agent. Although TRAIL or its derivatives have been disappointing so far despite clear evidence of clinical activity and lack of adverse events, there is still a possibility that TRAIL-based therapies in association with targeted or conventional chemotherapy [Micheau *et al*., 2013].

Because TRAIL was found to be able to induce apoptosis in cancer cell lines, multiple TRAIL-receptor agonists (TRAs) were developed for clinical application. Recombinant

TRAIL (Dulanermin) and several agonistic TRAILreceptor-specific antibodies (e.g., Mapatumumab and Conatumumab) were tested in clinical trials which confirmed broad tolerability and safety of these agents in patients. Unfortunately, none of the TRAs achieved a therapeutic effect in clinical trials. Two main reasons for that; first, in many cancer cells, TRAs need to be combined with sensitizing agents to break resistance of cancer cells. Second, so far TRAs with comparably low agonistic activity have entered clinical trials and TRAs with superior agonistic activity need to be searched and developed for clinical trials.

One example for developing different strategies for better apoptotic activity was about a novel TRA molecule, named APG350, which was an agonistic fusion protein and designated to imitate the TRAIL-R1/TRAIL-R2 interaction sides of TRAIL. It has two single-chain TRAIL-receptor binding domains covalently linked to the Fc-portion of a human IgG1 molecule and forms dimers in which each one binds to three TRAIL-receptors. As a result of hexavalent binding mode with six-TRAIL-receptors, superior apoptosis-inducing activity was achieved in a variety of human tumor cell lines and primary tumor cells such as colon, liver, breast and lung cancer. APG350 also reduced the size of subcutaneously implanted human colon carcinoma cell line Colo205 in mice. It was also showed that TRAIL-R agonists based on the hexavalent structural concept represent a very promising novel, next-generation TRA for the treatment of PDAC (pancreatic ductal adenocarcinoma cells) [Legler *et al*., 2018].

CONCLUSION

Apoptosis is a process of programmed cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area. It is triggered through two main pathways; intrinsic and extrinsic apoptotic pathways. Several enzymes and receptors have key roles in those pathways. Triggering apoptosis by using apoptosis causing molecules (peptides, proteins and antibody fragments) is a promising strategy to cause cell death especially in cancer cells. So far, several peptides and antibody fragments have been developed to activate the apoptotic pathways. One of the widely used technique to select peptides and antibody fragments as apoptosis causing ligands which specifically bind on death receptors (surface receptor proteins active in extrinsic pathway) is phage display. Surface death receptors can be used as target molecules in phage display selection steps to screen specific peptides and antibody fragments which bind on target protein, interfere with natural interaction between receptor and its natural ligand, and cause apoptosis. So far several selected molecules have been tried in clinical trials, but using selected ligands as single agent to trigger apoptosis showed limited efficiency. Using them in association with targeted or conventional chemotherapy, development of death receptor agonists with superior activity, using the selected ligands in different configurations may overcome the limited efficiency problem of the ligands in clinical trials.

REFERENCES

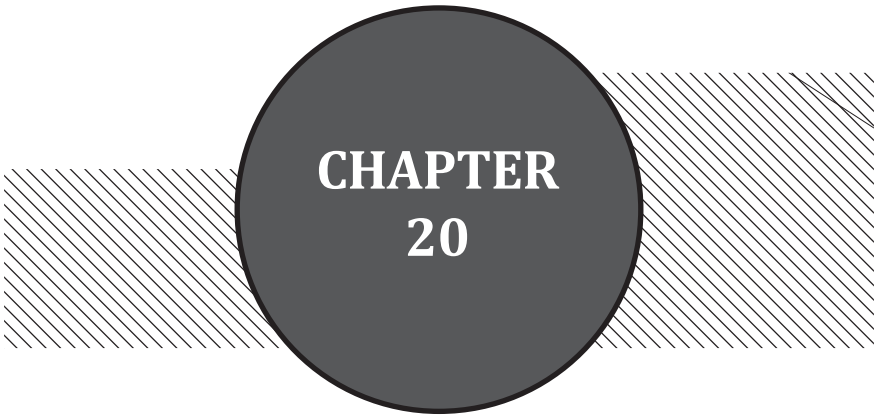
1. Ashkenazi A., Dixit V. M., (1998), "Death receptors: signalling and modulation", Science. 5381, 1305-1308
2. Barras, D. and Widmann, C., (2011), "Promises of Apoptosis-Inducing Peptides in Cancer Therapeutics", Current Pharmaceutical Biotechnology, 12, 1153-1165
3. Bing, L., et al., (2006), "Activation of the Proapoptotic Death Receptor DR5 by Oligomeric Peptide and Antibody Agonists", J. Mol. Biol., 361; 522–536.
4. Cryns, V.L., et al., (1996), "Specific cleavage of alpha-fodrin during Fas and tumor necrosis factor-induced apoptosis is mediated by an interleukin-1-beta converting enzyme/Ced-3 protease distinct from poly(ADP-ribose) polymerase protease", J. Biol. Chem. 271, 31277-31282.

5. Gaoxin, L., et al., (2017), "A Novel Fully Human Agonistic Single Chain Fragment Variable Antibody Targeting Death Receptor 5 with Potent Antitumor Activity In Vitro and In Vivo". Int. J. Mol. Sci., 18, 2064
6. Graves, D. J., et al. (2014), "Apo2L/TRAIL and the Death Receptor 5 Agonist Antibody AMG 655 Cooperate to Promote Receptor Clustering and Antitumor Activity". Cancer Cell, 26:177-189.
7. Green, D.R. and Kroemer, G., (2004), "The pathophysiology of mitochondrial cell death", Science. 5684, 626-629
8. Irmler, M., et al., (1997), "Inhibition of death receptor signals by cellular FLIP", Nature. 6638, 190-195
9. Kothakota, S., et al., (1997), "Caspase-3 generated fragment of gelsolin: effector of morphological change in apoptosis", Science. 5336, 294-298.
10. Lazebnik, Y.A. et al., (1994), "Cleavage of poly(ADP-ribose) polymerase by a proteinase with properties like ICE", Nature, 6495, 346-347.
11. Lazebnik, Y.A. et al., (1995), "Studies of the lamin proteinase reveal multiple parallel biochemical pathways during apoptotic execution", Proc. Natl. Acad. Sci. U. S. A. 92, 9042-9046.
12. Legler, K., et al., (2018), "The novel TRAIL-receptor agonist APG350 exerts superior therapeutic activity in pancreatic cancer cells", Cell Death and Disease, 9:445
13. Letai, A. et al, (2002), "Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics", Cancer Cell., 2, 183-192.
14. Li, L.Y., Luo, X. and Wang X. (2001) Endonuclease G is an apoptotic DNase when released from mitochondria. Nature. 6842, 95-99.
15. Lutz, R.J., (2000), "Role of the BH3 (Bcl-2 homology 3) domain in the regulation of apoptosis and Bcl-2-related proteins", Biochem Soc Trans, 28, (2):51-6
16. Mashima, T., et al., (1995), "Identification of actin as a substrate of ICE and an ICE-like protease and involvement of an ICE-like protease but not ICE in VP-16-induced U937 apoptosis", Biochem Biophys. Res. Commun. 217, 1185-1192.
17. Micheau, O., Shirley, S., Dufour, F., (2013), "Death receptors as targets in cancer". Br. J. Pharmacol. 169, 1723–1744.
18. Raucher, D., et al., (2009), "Therapeutic peptides for cancer therapy. Part II – cell cycle inhibitory peptides and apoptosis-inducing peptides", Expert Opin. Drug Deliv., 6(10):1049-1064
19. Sahin D., (2018), "Phage Display And Methods To Present The Targets To The Phage Display Library: Whole Cell Biopanning", In Kilic, B., Atik, D., and Dogan, S. (Eds), "Innovative Approaches in Health Sciences", (pp.89-98), Ankara, TR, Gece Publishing
20. Sakahira, H., Enari, M., Nagata, S., (1998), "Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis", Nature. 6662, 96-99.
21. Smith, G.P., (1985), "Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface". Science, 228(4705), 1315-1317.
22. Susin, S. A., et al., (1999) "Molecular characterisation of mitochondrial apoptosis-inducing factor", Nature. 6718, 387-389.
23. Taylor, R.C., Cullen S.P., and Martin, S.J., (2008), "Apoptosis: controlled demolition at the cellular level". Nature Reviews, Molecular Cell Biology, 9; 231-241.
24. Vrielink, J., et al. (2010), "Synthetic constrained peptide selectively binds and antagonizes death receptor 5". FEBS Journal, 277; 1653–1665

25. Walczak, H., (2013), "Death Receptor–Ligand Systems in Cancer, Cell Death, and Inflammation". Cold Spring Harb Perspect Biol; 5:a008698.
26. Wolf, B .B. and Green, D.R. (1999), "Cell death by caspase family proteinases", J. Biol. Chem. 274, 20049-20052.
27. Yang, J.C., Cortopassi, G.A., (1998), "Induction of the mitochondrial permeability transition causes release of the apoptogenic factor cytochrome c", Free Radical Biol. Med. 24, 624-631.
28. Zou, H., et al., (1999), "An APAF-1-cytochrome c multimeric complex is a functional apoptosome that activates procaspase 9", J. Biol. Chem. 274, 11549-11556

Power in Nursing

Hava GÖKDERE ÇİNAR[1]



[1] Department of Nursing, Faculty of Health Sciences , Uludag University, Bursa, Turkey PhD & Instructor

INTRODUCTION

Although it is hard to attain professional power in nursing, it is not impossible. Even though the profession of nursing has many limitations that have subsisted from past to present, such as elements questioning its acceptability as a profession; nurses today have the capacity of turning these limitations into a professional opportunity (Sepasi et al. 2017; Dikmen at al. 2016). Nurses' position of communicating with individuals receiving healthcare service the most, approaching individuals as a whole and affecting the decisions with their large number increases the value of the profession of nursing (Karaöz 2004; Karataş 2018). In other words, the fact that they are in constant contact with people by the benevolent nature of nursing, are a part of a big healthcare team and have a numerical superiority within the healthcare sector could be considered sufficient reasons for professional improvement and change opportunities. (Sepasi et al. 2017). Thus, in order for nursing services to be qualified, effective and efficient as required, it is necessary to increase the autonomy of nurses and allow them to make their own decisions concerning professional issues; it other words to strengthen them (Er and Altuntaş 2014).

Strengthening of nurses may have positive consequences concerning personal, institutional and patient care within the healthcare system. Strengthened nurses can start and sustain independent behaviors to achieve specific goals and by this way increase the effectiveness of the study (Burkhard and Nathaniel, 2013). Studies describe three factors that have a positive effect on strengthening. They are; reaching knowledge structures, support and resource. Considering the close relationship of strengthening with organizational effectiveness and patient safety especially in the dimension of support; it is important for managers in healthcare organizations to place a greater emphasis on strengthening nurses (Baykal and Türkmen 2014).

CONCLUSION

What is power?

Power is an abstract concept that is hard to analyze and define (Sepasi et al. 2017). The term power is derived from the Latin word poter. The word connotates strength, control, capability, as well as fulfilling and achieving something (Burkhard, Nathaniel 2013). In addition, sociologists and political scientists have also attempted to define power for a long time. In the broadest sense; **power** is the ability of affecting and changing the behaviors of others. **Power** is the competence of reaching the results despite resistance. According to another definition; **power** is the ability of persons or organizations to achieve desired goals using resources (Karaöz 2004). Power is also the capacity of having an influence on a person or a group. It makes persons or groups accept different opinions even if these opinions contradict with their demands. Power reveals the capacity of many people to attain what they desire with a collective action. This is because power is among the issues emphasized by institutions at the present time where rapid changes occur (Cruz et al. 2009). In the literature, it is suggested that nurses should know and use power resources in order to affect health policies of institutions and countries, achieve goals of institutions and thus improve the health level of society by providing a quality and cost-effective healthcare service and maintain their personal and professional development (Başaran and Duygulu 2014; Marquis and Huston 2009; Huber 2010). Rather than an ideal, "being powerful" is a feature expected by an organization from its managers and employees to achieve goals (Korkmaz and Abaan 2005).

What is power in nursing?

As a basis of nursing practices, nurses always try to influence individuals in order to improve their health. Therefore, having power for nurses is perceived as being a part of a profession. It is required for nurses to have power in order to influence their colleagues, as well as patients, physicians and other members of profession (Baykal and Türkmen 2014; Marquis and Huston 2009).

Today nurses should be a strategic planner who can understand the complexity of clinical practice, a human resources specialist, a risk manager and a high-quality specialist. On the other hand, the work that are accepted as profession should be independent, self-directed and nurses should control the content of their work (Sepasi 2017). Because a powerful nurse can also increase the power of their patients and affect their outcomes positively (Spence et al. 2016).

An extensive study implemented in different countries has indicated that the sense of weakness among nurses and lack of control over their work affect the quality of patient care negatively and reduce job satisfaction. This condition should be overemphasized from the viewpoint of care quality and patient safety. It depends on the amount of professional power for nurses to provide their patients a high-quality care and become independent (Sepasi 2017).

The importance of being powerful for nurses is also emphasized by international organizations in the area of health. World Health Organization (WHO) published the Munich Declaration titled "Nurses and Midwives: a Force for Health" at the end of the Second Ministerial Conference on Nursing and Midwifery in Europe in 2000. In the declaration, it is stated that nurses and midwives are a force for health and it is required to make an effort for strengthening nurses and midwives in all member countries. International Council of Nurses (ICN) also considers nurses a force in planning healthcare services and developing convenient and effective regional, national and international health policies and stresses the importance for nurses to serve actively in this respect (Başaran and Duygulu 2014; www.icn.ch; http://www.t-hasak.org).

What are power resources and classifications?

In the literature, it is seen that power resources are classified in various ways. According to Mc Clelland, power originates from two resources as "positive (social) power and negative (personalized) power". While personalized power includes behaviors for personal goals, social power includes behaviors for group goals. Mechanic (1973) classifies power resources as; "collective, legitimate, identity power, wealth and speciality power". Etzioni (1975) suggests that power has three resources as; "punishing, rewarding and normative (canonical) power". Kanter (1979) classifies power resources as; "formal and informal power" (Başaran and Duygulu 2014; Karadaş 2018). French and Raven, on the other hand, classify power as; identity, speciality, rewarding, compelling and legitimate power, which is the most frequently used classification.

Identity power originates from a person's characteristics (charisma). If an individual has characteristics that are liked and adored by another person and the person is attached to him/her or tries to identify with him/her because of that, the power of that individual is called identity power. Speciality power develops when a person has special knowledge and skills needed by other people. Rewarding power develops as a result of providing rewards valued by another person and group. Compelling power is mainly based on fear. Compelling power develops by exposing other people to unpleasant experiences, giving punishments or withdrawing rewards. Legitimate power, on the other hand, is a power type originating from a person's official position/status. In general, speciality and identity power types, personal power types, legitimate, compelling and rewarding power types

are called positional power types (Korkmaz and Abaan 2005, Baykal and Türkmen 2014).

What are the power resources used by nurses?

In the nursing literature; power is used in the meaning of authority, status, decision making, independence, responsibility and autonomy (Korkmaz and Abaan 2005). Every nurse is responsible for their work, service, personal and professional development. A successful strengthening will provide more power and knowledge, ability to control in practices and autonomy to the profession of nursing (Ulupınar 2011). Especially for nurses working in the clinical area, power requires being intellectually, physically and emotionally talented and getting prepared for interpreting, planning, applying and efficiently assessing. Nurses' awareness of certain care actions to carry out, freedom to fulfil them and ability of deciding to fulfil them represent their clinical power (Cruz et al. 2009).

Power in nursing is an ability of attaining and using necessary resources to achieve nursing goals. Benner, a nursing leader identified six power resources in nursing care; Transformative Power: ability of helping the individual who is given care change the image of self-respect, Holistic Power: ability of helping the individual/individuals who is/are given care return to normal life, Defensiveness Power: ability of removing the obstacles, Therapeutic Power: ability of establishing patient-nurse relationship to accelerate recovery, Participative Power: power originating from the care relationship with the patient, Problem Solving Power: ability of being sensitive to clues and researching in order to solve problems throughout the care process (Benner 1984; Karaöz 2004).

SUGGESTIONS

Powerful nurses have a more successful professional power and will undeniably improve their professional development further. Strengthening of nurses will make them more efficient and satisfied in their work. In addition, it will be easier to provide a high-quality and safe patient care and start and accept changes after the strengthening process. In order for nurses to have an active role in developing health policies that may support both patient care and professional development, they are required to understand power and encourage strengthening. While there is no consensus among nurses concerning how to attain professional power; every professional department interprets how to attain power. The power of knowledge is used in developing care for individuals who work in patient care process and it is required for those who work in management and academic departments to exist and update in the socio-political area. Additionally, revealing the power type used by nurse managers and expectations of managers will light the way for resources to be used by nurses who make and will make nursing services efficient in these institutions to achieve their organizational goals in the future. Thus, both nurses and managers have important responsibilities for nurses to be powerful.

REFERENCES

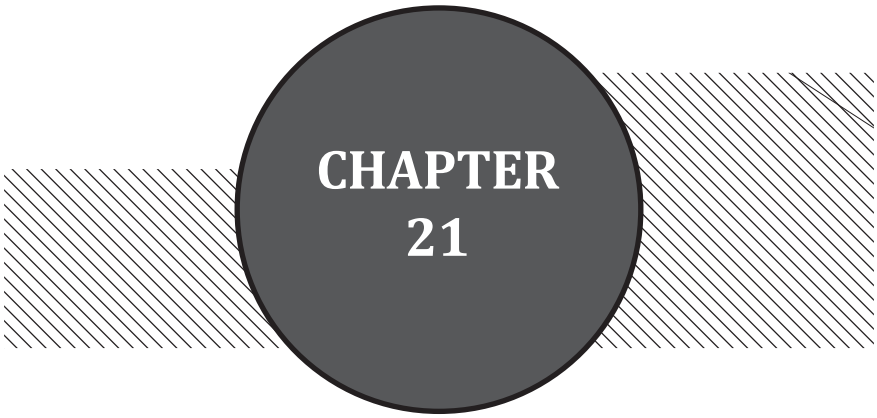
1. Başaran, S. ve Duygulu, S. (2014). Hemşirelikte Güç Kavramının Analizi. Hacettepe Üniversitesi Hemşirelik Fakültesi Dergisi, 62-73.
2. Baykal Ü.t., Türkmen E.E. (Ed.) (2014) Hemşirelik Hizmetleri Yönetimi. Akademi Basın ve Yayıncılık, İstanbul, 133-141.
3. Benner P (1984) From Novice To Expert: Exellence And Power İn Clinical Nursing Practice. Menlo Park, C A: Addison- Wesly.
4. Burkhard, M.A, Nathaniel K.A. (2013). Çağdaş Hemşirelikte Etik. Alpar, Ş., Bahçecik, N., Karabacak, Ü. (Çeviri Editörleri). 3. Baskı, İstanbul Medikal Yayıncılık, İstanbul, 469-479.

5. Cruze, D.A.L.M., Pimenta, C.A.M., Pedrosa, M.F.V., Lima, A.F.C. ve Gaidzinski, R.R. (2009). Nurses' Perception of Power Regarding Their Clinical Role. Rev Latino-am Enfermagem, 17:2, 234-239.
6. Er F., Altuntaş S.(2014) Hemşirelikte Personel Güçlendirme. Sağlık ve Hemşirelik Yönetimi Dergisi. 3; 1, 155-160.
7. Dikmen Y., Yılmaz D.K., Usta Y.Y. (2016) Hemşirelerin Otonomi Düzeylerinin Bazı Değişkenler Açısından İncelenmesi. Uluslararası Hakemli Hemşirelik Araştırmaları Dergisi. 8;72-87.
8. Huber DL. (2010) Leadership and Nursing Care Management (4th. Ed.) By W. B. Sounders Company, London,166-179.
9. Karaöz, S. (2004). Hemşirelerin Politik Gücü. C.Ü Hemşirelik Yüksek Okulu Dergisi, 8:1, 30-36. Karataş 2018
10. Korkmaz, G. ve Abaan, S. (2005). Servis Sorumlu Hemşireleri Yöneticilerindeki Algıladıkları Güç Tipleri ve Tercihleri. Hacettepe Üniversitesi Hemşirelik Yüksekokulu Dergisi, 26-42.
11. Marquis BL, Huston CJ. (2009) Leadership Roles and Management Functions in Nursing Theory & Applications (6th. Ed.)
12. Münih Deklerasyonu (2000). Hemşireler ve Ebeler Sağlık için bir Güç. http://www.t-hasak.org Erişim: 2017.
13. Sepasi RR, Abbaszadeh A, Borhani F, Rafiei H. (2016) Nurses' Perceptions of the Concept of Power in Nursing: A Qualitative Research. J Clin Diagn Res. 10(12): LC10-LC15. PMID:
14. Sepasi RR., Borhani F., Abbaszadeh A. (2017) Nurses' perception of the strategies to gaining professional power: A qualitative study. Electronic Physician. 9; 7, 4853-4861
15. Spence Laschinger HK, Gilbert S, Smith LM, Leslie K. (2010) Towards a comprehensive theory of nurse/patient empowerment: applying Kanter's empowerment theory to patient care. J Nurs Manag. 18(1): 4-13
16. Participation of Nurses in Health Services Decision Making and Policy Development. Reviewed and revised in (2008). ttp://www.icn.ch/PS_D04_ ParticipationDecisionMaking.pdf. Erişim: 2017
17. Ulupınar, S. (2011). Hemşirelikte güçlendirme. Yoğun Bakım Hemşireliği Dergisi, 15:2, 77-84.

Stigmatization in Genetic Diseases

İlkay KESER[1]

İbrahim KESER[2]



[1] Assist.Prof. Dr., Akdeniz University, Nursing Faculty, Department of Psychiatric Nursing, Antalya, Turkey

[2] Prof. Dr., Akdeniz University, Medical Faculty, Department of Medical Biology and Genetics, Antalya, Turkey

INTRODUCTION

Stigma has been known since the early days of history, but it has become more important in recent years. Stigmatization is difficult to understand without psychological, sociological, anthropological, political dimensions and interdisciplinary evaluation. Stigmatization can be defined as a situation in which people discredit their reputation in society because of a attribute, and place them in a low position. Different types of stigma may have harmful effects. Poor psychological well being, poor quality of life and poor self esteem are related stigmatization. Stigmatization is a modifiable environmental risk factor. Also, stigma can be defined as a sign, and a definition that causes the individual to be rejected by the society, rejected, looked down and excluded (Schulze & Angermeyer, 2003). The person who was seized by Goffman for the first time means that his or her acceptance of life has been differentiated and treated differently (Yıldırım, 2016). Stigma has an effect on the individual. Stigma perception may be indistinguishable from discrimination in the minds of those who experience it (Williams, 2010). Goffman has grouped the types of traits that cause stigmatization under the titles "Visibility", "Controllability", "Inheritance" (Goffman, 1963). Explain the visibility of the stigma by referring to the visible staigma and the concealable stigma. Visible stigma states that people have certain characteristics that they can not escape from judgments and discrimination, and that there are certain features that can be stored in the society in the case of hidden stigmas. Some signs such as ideology, denomination, homosexuality, substance abuse, schizophrenia are examples of the signs that cause the stigma, visible deformation, physical deformation, obesity, sex, skin color etc. According to controllability, stigmatization can be classified as situations in which people are out of their choice and out of control. Controllable stigmas, party sympathy, smoking, overweight, child abuse are issues. Uncontrollable stigmas are the stigmas of life, such as race and religion, which are caused by things that are not in your hands. People are less criticized because of uncontrollable features; stigmatization can be experienced more intensely because people are thought to have created the controllable stigma with their own choices (Hogg &Vaughan, 2014). Stigmatization according to heredity can be classified as inherited and later acquired. These are characteristics such as birth marks, gender, ethnicity. Stigmas earned later include topics such as education and occupation (Goffman, 2014).

When the stigmatization is considered in the historical process, it is seen that different belief systems, cultures, subcultures affect the attitudes towards diseases, besides the social capacity, the functions of the individual, the identity of the individual and the family structure affected the stigma or stigmatization of the individual. In the historical process of medieval aging, the stigmatization of the bodies with angry iron has left its place in the modern world to be embodied as hatred, execution in the brains and negative attitudes and behaviors.

Societies have become more prone to stigmatization, especially in epidemics and mental illnesses, the widespread influences. It is stated that the diseases are labeled, but some diseases also bring about the conditions that cause the stigma (Yıldız et al., 2012). Those who suffer from the stigma of the illness can be isolated from social care, discriminated against health services, insurance or employment, and are subject to other injustices (Savulescu & Kerin, 1999).

In the historical process, it is seen that the ending individuals are subjected to stigmatization. Sudden outbreaks in ancient Greece, leprosy and veins in the 1300s; In the 1980s, it was believed that those who were arrested by AIDS were ill or they were guilty or sinful (Özdemir, 2010; Gary, 2005). The illnesses were seen as a sign of disgrace, and these stigmatized persons were healthy and normal separated; they are condemned and discredited. In the 18th and 19th centuries; tuberculosis, poverty and mental retardation

(Barış, 2002); In the 1900s, cancer was associated with death, and stigmatization and discriminatory diseases. Despite the history of cancer, tuberculosis, leprosy, syphilis and epilepsy stigmatized diseases; AIDS is at the top of stigmatized diseases (Read&Horre, 2001; Oran&Şenuzun, 2008) found that there are more stigmatisms against persons with a mental illness for biological and genetic reasons; reported that mental illnesses based on genetic factors(Phelan, 2002).

Stigmatization plays a role not only in the outbreak but also in the emergence of the disease. Van Zelst (2009) has drawn attention to the gene-environment interaction. Sometimes genetic predisposition leads to disease. According to this model, individuals exhibit adverse structural interpersonal interactions in areas of social livelihood that behaviorally represent genetic load. For example, an individual can be stigmated with a single paranoid behavior or speech. Although this phenomenon does not develop in the context of psychiatric diagnosis, different and unusually perceived behavior such as schizophrenia is stigmatized. As a form, this is a structural stigma. This process continues as stigmatization after the disease. The main theme of stigmatization and structural discrimination is the perception of the social difficulty component. This social defeat shapes specific negative beliefs about themselves and others. Negative interactions are complicated by the difficulties and illnesses of the societal area and affect the onset and onset of the illness.

As the genetic basis of diseases began to emerge, stigmatization has also begun on hereditary diseases. The real danger is in genetic-based diseases that have emerged as inherited stigmas. Stigma affects all of family members as single by single, especially in familial diseases. In genetically based diseases, a new concept has emerged for stigmatizing the person or family of clinical findings: Genetic stigmatization.Genetic stigmatization may be due to chromosome-based abnormalities in the human genome or diseases at gene levels.

The Human Genome and Its Phenotypes

We, as Homo sapiens, have 46 chromosomes from which we take half of our mother and half of our father. Two of the 46 chromosomes are sex chromosomes, XX in women and XY in men. X and Y chromosomes determine the sex of a person. Most women are 46, XX and most men are 46, XY. However, research shows that a single sex chromosome, such as 45, X, or 45, Y, is defined as sex monosomes of some individuals at several births. Unlike monosomes, XXY, which is three or more sex chromosomes, some of which are 47, XXX, 47, XYY, or 47, are defined as sex chromosome polyomies. Additionally, in the karyotype and the sex phenotype incompatibility, some males are born 46,XX due to the translocation of a tiny section of the sex determining region of the Y chromosome. Similarly, some females are also born 46,XY due to mutations such as deletions in the Y chromosome. Clearly, there are not only females who are XX and males who are XY, but rather, there is a range of chromosome complements, hormone balances, and phenotypic variations that determine sex. Sex determination is among the most interesting areas of study in modern genetics, with research spanning a large range of topics, including developmental mechanisms, behavior, sex chromosome biology and evolution. The biological differences between men and women result from two processes: sex determination and differentiation (Goodfellow, 1993; Willard, 2003).

A phenotype that is the opposite of genetic sex causes stigmatization. For example, the karyotype is 46,XX and the name of the person named Fatma in the male phenotype is called “Male Fatma” in Turkish meaning “Erkek Fatma”. There are a number of cultures, for example, in which greater gender diversity exists and sex and gender are not always neatly divided along binary lines such as male and female or homosexual and heterosexual. It is apparent, then, that different cultures have taken different approaches

to creating gender distinctions, with more or less recognition of fluidity and complexity of gender(Wood,1997).

Genetic Tests and Stigmatization

The numerical and structural abnormalities in the genome make up a clinical manifestation in a person. If this clinical finding leaves a visible trail and if this person is known to be genetically identical, both that person and his or her family may become a clear target for genetic stigmatization. When genetics, which develops with technology, starts routine tests on the diagnosis of diseases, it has come to our attention that genetic tests are not known by others (Botkin, et al. 2015). In 1995, the American Society of Human Genetics (ASHG) and American College of Medical Genetics and Genomics (ACMG) published a joint statement titled ''Points to Consider: Ethical, Legal, and Psychosocial Implications of Genetic Testing in Children and Adolescents (American Society of Human Genetics Board of Directors,1995). This publication was influential in guiding clinicians and families during an era in which a number of new genetic tests, particularly predictive or predispositional testing, were being introduced into clinical medicine.

Since 1995, clinicians have gained substantial experience with genetic testing in a number of clinical contexts, and research has improved the evidence on which professional recommendations can be developed. The ethical, legal, and social issues in genetic and genomic testing have been subject to special scrutiny for several reasons.

First, for some heritable conditions, genetic testing can provide powerfully predictive information about the individual's future health status. Professionals, and society more broadly, have been concerned about the impacts of such predictive power on the psychological well-being of those found to be at increased risk, as well as concerns about stigma and discrimination.

Second, genetic information about one individual provides presumptive information about other ''blood'' relatives. The family or kindred nature of genetic information poses ethical, legal, and social challenges for the appropriate management of that information in clinical and research contexts.

Third, genetic and genomic information is complex, and health risks associated with this information are often probabilistic. This means that special care and expertise are important in ordering and interpreting many genetic tests. Finally, genetics has a troubled history, evident during the first half of the twentieth century, when genetic concepts were misunderstood and misused to the detriment of vulnerable groups in society.

Genetic and genomic tests are not uniquely challenging with respect to ethical, legal, or psychosocial considerations, but these features justify careful thought and an element of caution as we assess the benefits and risks of these evolving technologies.

Cytogenetics and molecular diagnostics have both undergone several revolutions since the fields began in 1959 and in 1976, respectively(Kan et al., 1976). Cytogenetics started with chromosome analysis and matured with increasingly detailed banding and then fluorescence in situ hybridization. Most recently, the field has seen the introduction of chromosomal microarray analysis for deletions and duplications. Molecular diagnostics has transitioned from hybridization-based techniques to Sanger sequencing with the increasingly common utilization of next-generation-sequencing (NGS) based techniques.

In both fields, the increased coverage and increased resolution of the current technologies confer high analytic validity, but both platforms create problems with interpretation.

First, a significant challenge is the difficulty in distinguishing between pathogenic variants and rare polymorphisms, resulting in the identification of ''variants of uncertain significance.''

Second, there are difficulties in interpreting variants and copy-number alterations whose significance is incompletely understood because of reduced penetrance or a lack of sufficient data on clinical associations.

Third, these technologies result in the identification of variants unrelated to the indication for testing (secondary or incidental findings). These challenges arise from our evolving understanding of the fine structure and variation in the human genome. At the present time, the contrast between our ability to identify genetic variants and our ability to fully interpret the information gives rise to many of the ethical issues in this domain.

Predictive Tests and Children

The first American Society of Human Genetics- American College of Medical Genetics and Genomics (ASHG-ACMG) pediatric testing statement, there has been a modest volume of clinical research about the impact of predictive testing in highrisk families during the 20 years. To date, this limited research has not found evidence of significant psychosocial harms in children(Wade et al., 2010). Perhaps the most significant finding is that, even without testing, children and many families create narratives about a child's genetic status. That is, some families simply assume that their children are destined to have, or not have, the familial condition. Further, the baseline uncertainty about risk status can cause psychosocial distress in the absence of genetic testing. Over the last two decades, there has been a general shift toward greater parental discretion in the face of clinical uncertainty about the best interests of the child (Wilfond et al., 2009). This broad shift is not exclusive to genetics but has implications for genetic testing. As parents consider the best course of action regarding genetic testing of their children, it remains important for parents to be aware that informed adults make a range of choices about predictive and reproductive testing, and thus many adults decline such testing.

Delaying of Genetic Test and Stigmatization

Postponing genetic testing to adulthood allows children the opportunity to make their own decisions. This is especially important for the small subset of conditions where a minority of at-risk adults opt for genetic testing, such as for Huntington disease. Approaching parents (and children, when appropriate) with respectful but directive recommendations, along with acknowledging flexibility, might be an effective approach to forging a therapeutic alliance with families. Encouraging families to consider such decisions over a period of time might convince some families that testing will be helpful in their particular context, or it might become clear that it will be most appropriate to defer testing until adulthood. The ASHG offers the following recommendations: Unless there is a clinical intervention appropriate in childhood, parents should be encouraged to defer predictive or pre-dispositional testing for adult-onset conditions until adulthood or at least until the child is an older adolescent who can participate in decision making in a relatively mature manner. Adolescents should be encouraged to defer predictive or pre-dispositional testing for adult-onset conditions until adulthood because of the complexity of the potential impact of the information at formative life stages. Providers should offer to explore the reasons why parents or adolescents are interested in predictive or pre-dispositional testing for adult-onset conditions. Providers can acknowledge that, in some cases, testing might be a reasonable decision, but decisions should follow thorough deliberation.

New Technologies in the Genetic Testing and An Individual Genome

Updated technology to enable whole-exome sequencing and whole-genome sequencing has become more accurate, more efficient, and less expensive. For the purposes of this statement, we use the term ''genome-scale sequencing'' to mean either whole-genome or

whole-exome sequencing. Given these technical improvements, genome-scale sequencing can be considered in a variety of clinical and research contexts. These include diagnostic testing, predictive testing for childhood-onset conditions, pharmacogenetic testing, and testing in children with cancer to inform diagnosis or therapy. Genome-scale sequencing creates a tension between the need to generate a comprehensive analysis of an individual's genome to address a clinical challenge and the need to limit problems created by a wealth of data, including secondary findings and findings of uncertain clinical significance.

Genetic diagnostics have been transformed from karyotype to chromosomal microarray analysis(Beaudet et al., 2008). Chromosomal microarray analysis is now a standard diagnostic test for a wide variety of conditions, including developmental delay with and without dysmorphic features, autism spectrum disorders, and multiple congenital anomalies, in the pediatric population(Miller, et al., 2010). The tecniques based on arrays has increased the utility of cytogenetic testing by increasing the rate of positive diagnoses (allowing the identification of much smaller deletions and duplications than cytogenetics alone), and with increasingly precise definition of breakpoints and gene content for deletions and duplications, it has allowed the identification of many new syndromes (Bejjani & Shaffer 2008). However, these tests also allow the identification of copy-number alteration of disease-associated genes unrelated to the initial reason for study, allow the identification of excessive homozygosity indicating potential consanguinity or incest, and have a significant likelihood of identifying a variant of uncertain significance. Chromosomal microarray analysis also has the potential to identify secondary findings. Therefore, chromosomal microarray analysis, like sequencing, raises ethical considerations that warrant obtaining informed consent from the child's parents, a practice that has not been routine for traditional chromosome analysis.

Genetic Stigmatization from the Member to the Family

As genetically, carrier testing of adolescents has historically been controversial, and professional statements generally do not support routine carrier testing of adolescents outside of pregnancy or reproductive planning (Borry et al., 2005a; Committee on Bioethics 2013). Hypothetical concerns include stigma, discrimination, and potential confusion over affected versus carrier status(Wade et al., 2010). It is notable that a significant body of literature addresses carrier screening in adults. Outside of some specific populations, there is little documentation of discrimination around carrier status in recent years, and most adult carriers without a family history do not appear to have significant short- or long-term differences in anxiety. In contrast, adult siblings of individuals affected by recessive or X-linked conditions often have strong views on whether or not they wish to know their carrier status and how it might affect their reproductive decision making. Some studies have reported that siblings show transient anxiety and depression after carrier testing(Fanos& Johnson 1995; Kenen& Schmidt 1978; Sorenson et al., 2003; Van Riper 2005).

Many research assessing adolescent or childhood carrier testing are small and address individuals with a family history of X-linked conditions (e.g., Duchenne muscular dystrophy, hemophilia, and fragile X syndrome) and autosomal- recessive conditions; provide a summary of some of the early literature in this area(Borry et al. 2005a; Borry et al. 2006b). These small studies documented high short-term recall and a number of potentially beneficial psychosocial outcomes, including relief in those who are non-carriers, relief from uncertainty in both carriers and non-carriers, and positive reappraisal of self-esteem and self-image. Additionally, these studies also suggested that adolescents found to be carriers felt able to plan for future parenthood and that most were open about the condition and their carrier status, sharing with family, and planning to tell partners(Ja¨rvinen et al., 2000a; Ja¨rvinen et al., 2000b; Barlow-Stewart et al., 2003; McConkie-Rosell et al., 2008; McConkie-Rosell et al., 2012b).

Does Newborn Screening occur the Stigmatization?

Also, newborn screening (NBS) is one of the most effective public-health programs of the last century. The ASHG strongly supports NBS programs and encourages genetic professionals into support NBS in their communication with patients, colleagues, and policy makers. NBS is conducted by state-based public-health programs in the US. For the first four decades of the programs, there was substantial variability between states on the conditions targeted (American Academy of Pediatrics,2000). NBS is conducted under state mandates in all but two US states or territories (Wyoming and the District of Columbia). However, 43 states permit parents to refuse NBS for either religious or philosophical reasons. The number of parents who opt out of NBS is exceedingly small(Faden et al., 1982; Liebl et al.,2002)

Adoption and Genetic Stigmatization

Approximately 2% of children are adopted, and many children are living in foster care in the US. Prospective adoptive parents might want genetic information about a child to inform their decision on whether or not to adopt. But previous consensus statements of the ASHG and ACMG have advocated that indications for pre-adoption testing closely parallel the indications applied to children living with their biological parents(American Society of Human Genetics Social Issues Committee, 2000) The rationale for these recommendations rests on concerns that harms might come to the child without sufficient benefit to balance the scales. If such concerns are valid for children living with their biological parents, then the standards for genetic testing should be the same for all children. The ''principle of equity'' articulates the idea that prospective adoptive parents are entitled to no more information at the time of taking custody of a child than the child's birth parents could obtain (Freundlich 1998). It is possible that a child with an untreatable genetic disorder would be better off with parents specifically chosen because of their ability to deal with this difficult circumstance.

An obvious objection is that knowledge of the disorder might so restrict the pool of willing parents that the child is made ''unadoptable.'' Another concern is that adults responsible for the placement of adoptive children most likely do not have the specialized genetics knowledge that would be required for assigning children to ''matched'' families. Another argument for matching is that prospective, adoptive parents' interests would be harmed by failure of the adoption agency to make the best possible choice of home on the basis of the full range of relevant information about the child. However, there is no assertion of a paralel responsibility of the prospective parents to undergo genetic testing themselves. The argument of matching creates the possibility that some parents might find themselves to be genetically unsuitable to adopt. Also, this is a genetic stigmatization.

Common Genetic Diseases and Stigmatization

For example, related with genetic testing and its results in the families.Using grounded theory methodology, this study examined the experiences of six BRCA1/2 gene mutation carriers. Three types of stigmatization were identified: stigmatization by anticipation, stigmatization through rejection, and stigmatization by affiliation. Participants described potential impacts on their womanhood, felt threatened by others, and revealed fears that their children would inherit their stigmatization. These findings indicate the importance of psychological support in the follow-up of such patients (DiMillo et al.,2015).

Women carrying BRCA1 or BRCA2 genetic mutations have an overall heightened risk of developing breast or ovarian cancer (up to 80%) (American Cancer Society, 2007). Furthermore, carriers' children have a 50 percent chance of inheriting the BRCA mutation (Di Prospero et al., 2001). Despite the positive aspects of genetic testing for breast cancer,

such as knowledge of risk, research demonstrates potential negative psychological implications when undergoing this type of genetic testing (Di Millo et al., 2013). Specifically, many negative psychological effects such as anger, distress, and vulnerability (Esplen et al., 2009), and heightened anxiety and depression, including worry about cancer (Van Oostrom et al., 2003), affected self-concept, quality of life, and stigma (Vodermaier et al., 2010), have been reported by women who have been found to possess mutations of the BRCA1 or BRCA2 genes.

One consequence of living with mutations of the BRCA1 or BRCA2 genes that needs further research is the stigma associated with receiving this test result (Vodermaier et al., 2010). In general, stigmatized individuals are those that "by virtue of their social membership in a social category are vulnerable to being labeled as deviant, are targets of prejudice or victims of discrimination, or have negative economic or interpersonal outcomes" (Crocker and Major, 1989). Goffman(1963) further distinguished between discredited and discreditable stigmas.An individual with a discredited stigma is one who believes that his or her stigma is evident to others immediately. On the contrary, one with a discreditable stigma believes that his or her stigma is not instantly known by others, nor is it instantaneously perceivable by those present. In addition, as a consequence of the anticipatory loss due to potential prophylactic surgeries, participants felt stigmatized when comparing themselves with other "normal" women. In the same vein, previous research has demonstrated that BRCA1/2 gene mutation carriers sometimes describe feeling lonely, feeling different, feeling isolated, and devastated, following the receipt of their test result (Kenen et al., 2006). Participants often described fearing external reactions; in other words, they feared that they could experience prejudice due to possessing the genetic mutation.

For instance, certain participants described that they were hesitant to share their test result with others because they feared the manner in which others would react. Specifically, participants feared whether others would pity them or judge that they were not competent in their job due to an emotional weakness caused by the test result. It appears that participants were afraid that if their children were found to possess the same genetic mutation, they might also experience the stigma and associated consequences that their mother had already felt as a carrier of the BRCA1 or BRCA2 gene mutation. As a result of this fear, participants described a desire to protect their children from stigma. Finally, this situation engendered feelings of guilt among participants, who felt that they may have passed on this genetic difference to their descendants (Di Millo, 2015).

Genetic stigmatization and/or discrimination that can be identified as genetic is defined as differential treatment, denial of rights, privileges or opportunities or other adverse treatment, based solely on genetic information, including a family history (Gostin, 1991;Goh, et al, 2013). Stigmatization /discrimination can also happen to individuals who have a genetic diagnosis, but who are asymptomatic or who will never become significantly impaired (Billings et al., 1992). Many investigations into the concerns about genetic discrimination have been published (Harmon, 2008). The Australian Genetic Discrimination Project (Taylor et al., 2007) reports cases of genetic discrimination against healthy people, with most related to insurance and employment domains, informal and social contexts, and within health services. Those having neurodegenerative conditions were more likely to report discrimination or social stigma across multiple domains (Taylor et al., 2008). Half the participants in an Australian bowel cancer study withdrew participation when told that the required genetic testing could impact life insurance Access (Keogh et al., 2009). Huntington's disease (HD) is a neurodegenerative disorder with progressive psychiatric, cognitive, and motor symptoms. Currently, no treatments are available to delay the onset of disease. It is inherited via an autosomal dominant fashion with almost 100% penetrance (Myers et al., 1998). Direct gene mutation detection

methods (via blood test) have been available since 1993, leading to highly accurate predictive testing with a sensitivity and specificity of almost 100% (Meiser & Dunn, 2000), and confirmation of a disease mutation may occur years before any symptoms appear. HD gene testing measures the number of expanded CAG trinucleotide repeats in exon 1 in the HD gene. If there are too many repeats, the test result and individual are gene-positive and will eventually develop HD symptoms. However, a positive test result provides little information regarding age of onset, rate of progression, or symptom severity. If the repeat size is found to be in the normal range (usually less than 35 repeats), then the individual is considered gene-negative, and is not at risk for developing HD.

And Genetic Stigmatization Everywhere

The quantification and verification of the relationships between genetic stigmatization and/or discrimination and the domains of insurance, employment, education, and social relationships is much needed (Treloar et al., 2004).

Concern about genetic testing and the risk of stigmatization and/or discrimination can result in individuals refusing testing and limiting their options to take advantage of available interventions that might lower the morbidity and mortality associated with genetic disorders. Discrimination fears can also prevent individuals from research participation, thus delaying treatments and potential cures. As more illnesses with a substantial (and testable) genetic component are revealed, there is an increasing need for education, knowledge, and tolerance for having genetic risk, as well as resources to ameliorate genetic discrimination, including more awareness of obligations and rights relating to genetic discrimination. The results of many studies suggest that there is a pressing need for increased education, intervention strategies, and support programs, particularly in the arena of insurance. Continuing education of insurance workers, and clear application forms and information about the insurance issues relating to genetic tests will be helpful in making the process transparent and understandable.

CONCLUSION

Although prevention is key to public health, the potential drawbacks associated with this form of genetic testing should be taken into account. Particularly, psychological support seems as important as medical followup, as the stress and anxiety produced through stigmatization are significant and can present in many ways. It follows that healthcare professionals must collaborate to ensure that followup for persons with genetic mutations includes psychological screening and adequate support in order to accompany them through this seemingly tumultuous journey. Deficiencies in genetics education extend from the preservice training of most health-care professionals to postgraduate internships, residency and fellowship training, and continuing medical and professional education for actively practicing health-care professionals. Many of those efforts are driven by the development of competencies that focus on content knowledge and related clinical skills. Up-to-date programs should be developed in schools and colleagues for the use of individual, familial and social genetic data and prevention of stigmatization.

REFERENCES

1. American Academy of Pediatrics (2000). Serving the family from birth to the medical home. Newborn screening: a blueprint for the future - a call for a national agenda on state newborn screening programs. Pediatrics 106, 389–422.
2. American Cancer Society, Inc. (2007) Detailed guide: Breast cancer: What is breast cancer? Available at: http://www.cancer.org/docroot/CRI/content/CRI_2_4_1X_What_is_breast_cancer_5.asp?sitearea

3. American Society of Human Genetics Board of Directors; American College of Medical Genetics; Board of Directors (1995). Points to consider: ethical, legal, and psychosocial implications of genetic testing in children and adolescents. Am. J. Hum. Genet. 57, 1233–1241.
4. American Society of Human Genetics Social Issues Committee; American College of Medical Genetics; Social, Ethical, and Legal Issues Committee (2000). Genetic testing in adoption. Am. J. Hum. Genet. 66, 761–767.
5. Barış, Y. İ, (2002), "Dünyada Tüberkülozun Tarihçesi", Toraks Dergisi, 3(3): 338-340.
6. Barlow-Stewart, K., Burnett, L., Proos, A., Howell, V., Huq, F., Lazarus, R., and Aizenberg, H. (2003). A genetic screening programmefor Tay-Sachs disease and cystic fibrosis for Australian Jewish high school students. J. Med. Genet. 40, e45.
7. Beaudet, A.L., and Belmont, J.W. (2008). Array-based DNA diagnostics: let the revolution begin. Annu. Rev. Med. 59, 113–129.
8. Bejjani, B.A., and Shaffer, L.G. (2008). Clinical utility of contemporary molecular cytogenetics. Annu. Rev. Genomics Hum. Genet. 9, 71–86.
9. Billings PR, Kohn MA, de Cuevas M, et al. (1992) Discrimination as a consequence of genetic testing. Am J Hum Genet 50:476–482.
10. Borry, P., Fryns, J.P., Schotsmans, P., and Dierickx, K. (2005a). Attitudes towards carrier testing in minors: a systematic review. Genet. Couns. 16, 341–352.
11. Borry, P., Fryns, J.P., Schotsmans, P., and Dierickx, K. (2006b). Carrier testing in minors: a systematic review of guidelines and position papers. Eur. J. Hum. Genet. 14, 133–138.
12. Botkin JR , Belmont JW, Berg JS. Berkman BE., Bombard Y, Holm IA., Levy HP., Ormond KE., Saal HM., Spinner NB., Wilfond BS., and McInerney JD(2015).Points to Consider: Ethical, Legal,and Psychosocial Implications of Genetic Testing in Children and Adolescents. American Journal of Human Genetics 97, 6–21, July 2, 2015.
13. Committee on Bioethics; Committee on Genetics; and American College of Medical Genetics and Genomics Social, Ethical, and Legal Issues Committee (2013). Ethical and policy issues in genetic testing and screening of children. Pediatrics 131, 620–622.
14. Crocker J and Major B (1989) Social stigma and selfesteem: The self-protective properties of stigma. *Psychological Review* 96(4): 608–630.
15. Di Prospero LS, Seminsky M, Honeyford J, et al. (2001) Psychosocial issues following a positive result of genetic testing for *BRCA1* and *BRCA2* mutations: Findings from a focus group and a needs assessment survey. *Canadian Medical Association Journal* 164(7): 1005–1009.
16. Di Millo J, Samson A, Theriault A, et al. (2013) Living with the BRCA genetic mutation: An uncertain conclusion to an unending process. *Psychology, Health, & Medicine* 18(2): 125–134.
17. Di Millo J, Samson A, Thériault A, Lowry S, Corsini L, Verma S, and Tomiak E. (2015) Genetic testing: When prediction generates stigmatization. Journal of Health Psychology, 2015, Vol. 20(4) 393–400
18. Esplen MJ, Stuckless N, Hunter J, et al. (2009) The BRCA Self-Concept Scale: A new instrument to measure self-concept in *BRCA1/2* mutation carriers. *Psycho-Oncology* 18: 1216–1229.

19. Faden, R., Chwalow, A.J., Holtzman, N.A., and Horn, S.D. (1982). A survey to evaluate parental consent as public policyfor neonatal screening. Am. J. Public Health 72, 1347–1352.
20. Fanos, J.H., and Johnson, J.P. (1995). Perception of carrier status by cystic fibrosis siblings. Am. J. Hum. Genet. 57, 431–438.
21. Freundlich, M.D. (1998). The case against preadoption genetic testing. Child Welfare 77, 663–679.
22. Gary, F. A., (2005), "Stigma: Barrier To Mental Health Care Among Ethnic Minorities", Issues In Mental Health Nursing, 26(10): 979-999.
23. Goffman, E., (1963), Stigma: Notes On The Management of Spoiled Identity, ABD: Prentice-Hall, Inc.
24. Goffman, E., (2014), Damga: Örselenmiş Kimliğin İdare Edilişi Üzerine Notlar, (Çev. L. S. Ş.Geniş), Ankara: Heretik Yayıncılık.
25. Goh AMY., Chiu E, Yastrubetskaya O, Erwin C, Williams JK.,. Juhl AR, Paulsen JS., and the I-RESPOND-HD Investigators of the Huntington Study Group, Perception, Experience, and Response to Genetic Discrimination in Huntington's Disease: The Australian Results of the International RESPOND-HD Study. GENETIC TESTING AND MOLECULAR BIOMARKERS Volume 17, Number 2, 2013 Pp. 115–121,2013.
26. Goodfellow PN. SRY and sex determination in mammals. Annual Review of Genetics, 1993, 27:71–92.
27. Gostin L (1991) Genetic Discrimination: the use of genetically based diagnostic and prognostic tests by employers and insurers.Am J Law Med 17:109–144.
28. Ja¨rvinen, O., Hietala, M., Aalto, A.M., Arvio, M., Uutela, A., Aula, P., and Ka¨a¨ria¨inen, H. (2000). A retrospective study oflong-term psychosocial consequences and satisfaction after carrier testing in childhood in an autosomal recessive disease: aspartylglucosaminuria. Clin. Genet. 58, 447–454.
29. Ja¨rvinen, O., Lehesjoki, A.E., Lindlo¨f, M., Uutela, A., and Ka¨a¨ria¨inen, H. (2000). Carrier testing of children for two X-linked diseases: A retrospective study of comprehension of the test results and social and psychological significance of the testing. Pediatrics 106, 1460–1465.
30. Kan, Y.W., Golbus, M.S., and Trecartin, R. (1976). Prenatal diagnosis of sickle-cell anemia. N. Engl. J. Med. 294, 1039–1040.
31. Kenen R, Arden-Jones A and Eeles R (2006) "Social separation" among women under 40 years of age, diagnosed with breast cancer and carrying a *BRCA1* or *BRCA2* mutation. *Journal of Genetic Counselling* 15(3): 149–162.
32. Kenen, R.H., and Schmidt, R.M. (1978). Stigmatization of carrier status: social implications of heterozygote genetic screening programs. Am. J. Public Health 68, 1116–1120.
33. Keogh L, Van Vliet CM, Studdert D, et al. (2009) Is uptake of genetic testing for colorectal cancer influenced by knowledge of insurance implications? Med J Aust 191:255–258.
34. Liebl, B., Nennstiel-Ratzel, U., von Kries, R., Fingerhut, R., Olgemo ller, B., Zapf, A., and Roscher, A.A. (2002). Very highcompliance in an expanded MS-MS-based newborn screening program despite written parental consent. Prev. Med. 34, 127–131.
35. McConkie-Rosell, A., Heise, E.M., and Spiridigliozzi, G.A. (2012). Influence of genetic risk information on parental role identity in adolescent girls and young women from families with fragile X syndrome. J. Genet. Couns. 21, 59–71.

36. McConkie-Rosell, A., Spiridigliozzi, G.A., Melvin, E., Dawson, D.V., and Lachiewicz, A.M. (2008). Living with genetic risk: effect on adolescent self-concept. Am. J. Med. Genet. C. Semin. Med. Genet. 148C, 56–69.
37. Meiser B, Dunn S (2000) Psychological impact of genetic testing for Huntington's disease: an update of the literature. J Neurol Neurosurg Psychiatry 69:574–578.
38. Miller, D.T., Adam, M.P., Aradhya, S., Biesecker, L.G., Brothman,A.R., Carter, N.P., Church, D.M., Crolla, J.A., Eichler, E.E., Epstein, C.J., et al. (2010). Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am. J. Hum. Genet. 86, 749–764.
39. Myers R, Marans K, MacDonald ME, eds. (1998) Huntington's disease. Academic Press, San Diego.
40. Oran, N.T. ve Şenuzun, F., (2008), "Toplumda Kırılması Gereken Bir Zincir: HIV/AIDS Stigması ve Baş Etme Stratejileri", Uluslararası İnsan Bilimleri Dergisi, 5(1): 1-16.
41. Özdemir, H., (2010), Salgın Hastalıklardan Ölümler 1914-1918, 2. Baskı, Ankara: Türk Tarih Kurumu.
42. Phelan, J. C., (2002), "Genetic Bases of Mental Illness-A Cure For Stigma?", Trends in NeuroSciences, 25(8): 430-431.
43. Read, J. and Ha(o)rre, N., (2001), "The Role of Biological and Genetic Causal Beliefs in the Stigmatisation of Mental Patients", Journal of Mental Health, 10(2): 223-235.
44. Savulescu J, Kerin J (1999) The "geneticisation" of disease stigma. The Lancet, December 1999. Pp:16
45. Schulze B, Angermeyer MC. Subjective experiences of stigma. A focus group study of schizophrenic patients, their relatives and mental health professionals. Soc Sci Med 2003; 56(2): 299-312. Sorenson, J.R., Jennings-Grant, T., and Newman, J. (2003). Communication about carrier testing within hemophilia A families. Am. J. Med. Genet. C. Semin. Med. Genet. 119C, 3–10.
46. Taylor S, Treloar S, Barlow-Stewart K, et al. (2007) Investigation genetic discrimination in Australia: perceptions and experiencesof clinical genetics service clients regarding coercion to test, insurance, and employment. Aust J Emerg Techn Soc 5:63.
47. Taylor S, Treloar S, Barlow-Stewart K, et al. (2008) Investigating genetic discrimination in Australia: a large-scale survey ofclinical genetics clients. Clin Genet 74:20–30.
48. Treloar S, Taylor S, Otlowski M, et al. (2004). Methodological considerations in the study of genetic discrimination. Community Genet 7:161–168.
49. Van Oostrom I, Meijers-Heijboer H, Lodder LN, et al. A (2003) Long-term psychological impact of carrying a *BRAC1/2* mutation and prophylactic surgery: A 5-year follow-up study. *Journal of Clinical Oncology* 21(20): 3867–3874.
50. Van Riper, M. (2005). Genetic testing and the family. J. Midwifery Womens Health 50, 227–233.
51. Van Zelst C.(2009) Stigmatization as an enviromental risk in schizoprenia: a user perspective. SchizophrBull. 2009;35:293-6.
52. Vodermaier A, Esplen MJ and Maheu C (2010) Can self-esteem, mastery and perceived stigma predict long-term adjustment in women carrying a *BRCA1/2*- mutation? Evidence from a multi-center study. *Familial Cancer* 9: 305–311.
53. Wade, C.H., Wilfond, B.S., and McBride, C.M. (2010). Effects of genetic risk information on children's psychosocial wellbeing: a systematic review of the literature. Genet. Med. 12, 317–326.

54. Wilfond, B., and Ross, L.F. (2009). From genetics to genomics: ethics, policy, and parental decision-making. J. Pediatr. Psychol. 34, 639–647.

55. Willard HF. Tales of the Y chromosome. Nature, 2003, 423:810–813.

56. Williams JK, Erwin C, Juhl AR, Mengeling M, Yvonne B,, Hayden MR, Quaid K, Shoulson I, Taylor S, Paulsen JS(2010) In their own words: Reports of stigma and genetic discrimination by people at risk for Huntington disease in the International RESPOND-HD study Am J Med Genet B Neuropsychiatr Genet. 2010 September ; 153B(6): 1150–1159.

57. Wood JT. Gendered lives: communication, gender, and culture, 2nd ed. Belmont, California, Wadsworth Publishing Company, 1997.

58. Yıldırım Z. Türk toplumunda epilepsi ve stigmanın değerlendirilmesi, epilepsi hastalarında stigma ve depresyonun ilişkisinin araştırılması. Bakırköy Ruh Sağlığı ve Sinir HastalıklarıEğitim ve Araştırma Hastanesi. Uzmanlık Tezi. 2016.

59. Yıldız, M., Özten, E., Işık, S., Özyıldırım, İ., Karayün, D., Cerit, C. vd., (2012), "Şizofreni Hastaları, Hasta Yakınları ve Majör Depresif Bozukluk Hastalarında Kendini Damgalama", Anadolu Psikiyatri Dergisi, 13: 1-7