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Academic Studies in Health Sciences

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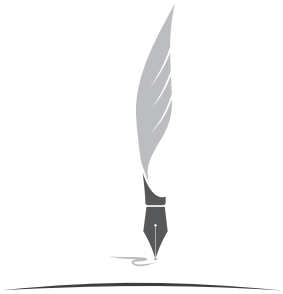

Chapter 1

LATERAL CRURAL REPOSITIONING IN RHINOPLASTY

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INTRODUCTION

Rhinoplasty is a patient-specific surgery and must be planned according to the patient's skin type, cartilage, and bony tissue characteristics (1). The shape of the nose and intranasal anatomy should be analyzed, and the anatomic variations that create pathologic conditions should be addressed carefully before every rhinoplasty. Bone and cartilage tissue constituting the nasal skeleton should be evaluated carefully.

Tip refinement is the most important part of rhinoplasty to create an aesthetically attractive nose. The size, shape, and position of the lower lateral cartilages create the appearance of the nasal tip (2). Furthermore, the positioning and the properties of the lower lateral cartilages affect the air passage of the nose by forming the nasal valve area. The tissue supporting the alar rim is the lateral crus of the greater alar cartilage. Thin or cephalically malpositioned lateral crura cause nasal obstruction by depressing nasal valves and decrease patient satisfaction as a result of nostril asymmetry and alar collapse (3).

It is not always possible to correct nasal tip deformities and positional anomalies in the cartilage structures that form the nasal tip using classic suture-and-graft techniques (4). Lateral crural repositioning (LCrep) with lateral crural strut grafting (LCSG) is one of the most advanced techniques in current aesthetic nasal tip surgery; it is an imperative method to master for surgeons who want excellent rhinoplasty outcomes.

Lateral crural repositioning with LCSG corrects tip asymmetry and malposition, especially in cases that cannot be repaired with traditional tip-plasty suturing, such as very thin, malpositioned, concave, or asymmetric tip cartilage (4,5). In addition, it corrects parentheses tip deformity and boxy nasal tip, which cannot be fixed

using other methods. In recent years, LCrep with LCSG has become a common technique used in patients with cephalic positioning of the lateral crura. It was first described by Jack P. Gunter, who claimed it an effective solution for boxy nasal tip, malposition, alar rim retraction, alar rim collapse, and pathologic conditions of the lateral crura, such as concave lateral crura (6). Lateral crural repositioning eliminates length differences between nasal projection and the dorsum, defines projection by changing the dome position, and allows the liberation of the lateral crura and fixation to the medial crura at the desired position (4).

However, every technique has advantages and disadvantages. One of the disadvantages of LCrep with LCSG is the possibility of postoperative problems, such as graft visibility and palpable hardness, especially in patients with thin skin. Furthermore, a graft of the incorrect length under the lateral crura can cause the area of the lateral crural complex insertion to protrude into the nasal cavity, occluding the external valve and the airway. Other disadvantages are that it requires extra cartilage, necessitates the elevation of the lateral crura from the underlying mucosa, and is technically challenging.

SURGICAL TECHNIQUE

A standard 1:100 000 mixture of lidocaine, 1%, and epinephrine was injected into the septum and outer nose as local anesthesia. The standard inverted V incision was made with a No. 11 blade, and bilateral marginal incisions were made with a No. 15 blade. Following skeletonization, the caudal septum was exposed gently. Hump resection was performed, after which the primary nasal dorsum height was determined. The septodorsal and bony-cartilaginous junction, or the keystone area, was shaped using a power rasp. The mucoperichondria were elevated bilaterally, and the graft was obtained. The septal L-strut

was left to support the dorsum and caudal. Before LCSG, a septal cartilage graft was obtained from each patient. Bony vault width and nasal bone spacing were evaluated in all patients, and medial or paramedian osteotomy was performed in selected patients, followed by high to low internal osteotomy for all patients. Asymmetric spreader grafts were placed to reconstruct the middle vault, followed by tip-plasty. Patients requiring repositioning were identified by measuring with a goniometer the angle between the lateral crura and the midline. Lateral crural repositioning with LCSG was performed on patients with an angle less than 30° between the lateral crura and the midline, while the surgery proceeded directly to tip-plasty in patients with an angle of 30° or more.

For patients undergoing LCrep with LCSG, the vestibular mucosa below the lower lateral cartilage was infiltrated with local anesthetic and hydrodissected and was then dissected from the caudal edge to the cephalic edge with iris scissors. The mucosal connection at the cephalic edge of the lateral cartilage was separated while leaving the cutaneous connection in the anterior caudal region intact. The lateral cartilages were freed by separating them from the accessory cartilages. Pieces of cartilage 3 to 4 mm wide and 15 to 25 mm long were removed from the septum and shaped for grafting. The graft was placed under the lateral cartilage with its tip extending 5 mm beyond the cephalic tip of the lateral crura and secured with a pair of 5-0 polyglactin 910 (Vicryl) sutures. Bilateral pockets were created anterior and caudal to the accessory cartilage dissecting the tissues in the direction of the lateral canthus, and the lateral crura supported by the lateral crural strut grafts were placed in these pockets in contact with the anterior nasal aperture. After the lateral crura and grafts were positioned in the pockets, the lateral crural strut grafts were fixed to the vestibular skin with 5-0 polyglactin 910 sutures (**Figures 1-4**).

Following middle vault modification in patients who did not undergo LCrep with LCSG, columellar supporting grafts were applied using the appropriate graft type to achieve tip definition, and the inverted V incision was sutured with 6-0 polypropylene (Prolene).

DISCUSSION

Repositioning the lower lateral cartilages and then supporting them with lateral crural struts is one of the most advanced shaping methods in the tip-plasty stage of aesthetic rhinoplasty. Changing the angle of the lateral crura and supporting them from below with cartilage strut grafts allows the correction of a host of deformities, including boxy nasal tip, lateral crural malposition, alar retraction, nasal valve insufficiency, and concave lateral crura (7). The lateral crura is the principal anatomic structure forming the aesthetic and functional character of the nasal tip, making the LCrep with LCSG technique important. With the right techniques, it is possible to create an equilateral triangular nasal tip and functionally support the alar rims to prevent nasal obstruction. In lateral crural malposition, the angle between the midline and the lateral crural insertion point is 30° or less. Studies indicate that malposition is one of the most common nasal tip deformities observed in patients undergoing primary and secondary rhinoplasty (7). A mathematical analysis of the effect of cephalic malposition on tip-plasty revealed differences in projection, rotation, and lateral crural length. Malposition has been found to affect tip-plasty and lead to the development and use of different tip-plasty techniques (8). Directing the lateral crural angle toward the medial canthus weakens the alae, resulting in parentheses tip deformity (9). New techniques have been attempted to correct this issue in noses with parentheses tip deformity and cephalic malposition (10,11).

Toriumi and others reported that alar rim grafting was not necessary in patients undergoing LCrep and LCSG because those procedures provided sufficient support to the alar rims (12,13). Various techniques have been described to support and strengthen the lateral crura, such as alar batten grafting, but malposition cannot be solved by alar batten grafts alone and requires the combination of multiple approaches. Alar batten grafting may correct nasal valve insufficiency (13). However, this technique alone is not adequate for other aesthetic deformities, such as parentheses deformity and boxy nose.

The LCrep with LCSG method, although very effective, has disadvantages and advantages. These disadvantages include the dislocation of the lateral crural complex from the created pocket and the visibility of the lateral crural structures through the skin in the long term. In addition, because LCSGs do not extend to the anterior nasal aperture, they may protrude into the nasal cavity and occlude the airway. These problems usually occur if the surgeon is still mastering the technique or has made a mistake in patient selection. Further research into the patient selection criteria for the repositioning procedure is warranted. Separate analysis of patients with normal, thick, and thin skin who underwent LCrep with LCSG revealed no differences in aesthetic satisfaction or functional improvement according to skin type (4).

CONCLUSIONS

Cephalic malposition is a common problem observed in primary and secondary rhinoplasty that affects patient satisfaction both functionally and aesthetically. Of the various techniques reported in the literature, the most effective is LCrep with LCSG. Surgeons using the technique should be aware of this possibility during their preoperative analyses and plan the operation accordingly.

LEGENDS TO FIGURES

Figure 1. *Measurement of angle between lateral crura and midline to confirm lateral crural malposition.*

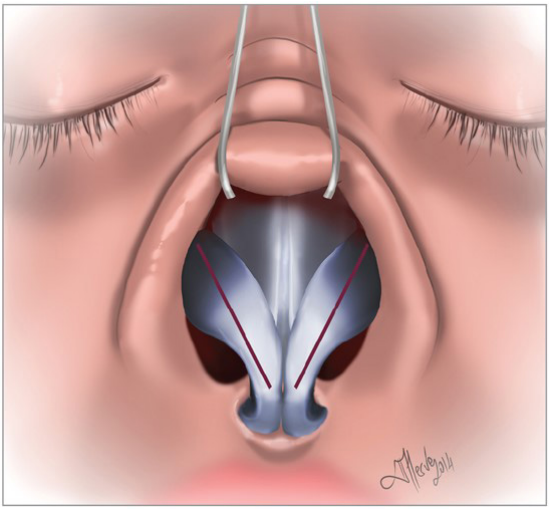


Figure 2. *Lateral cartilages exposed by separation from their point of attachment to accessory cartilages.*

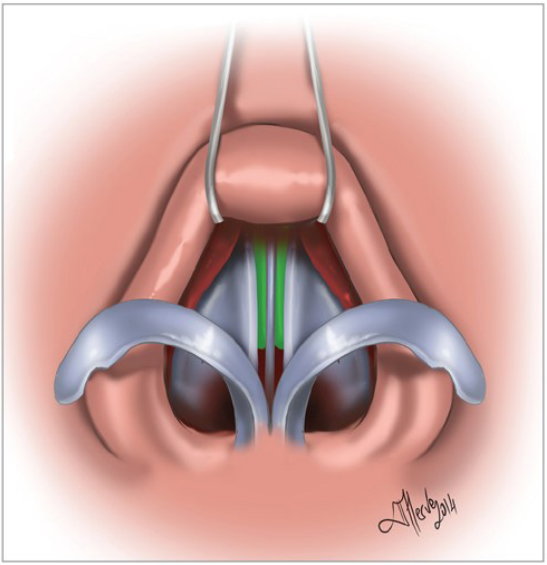


Figure 3. *Lateral Crural Strut placed under lateral crural cartilage and sutured.*

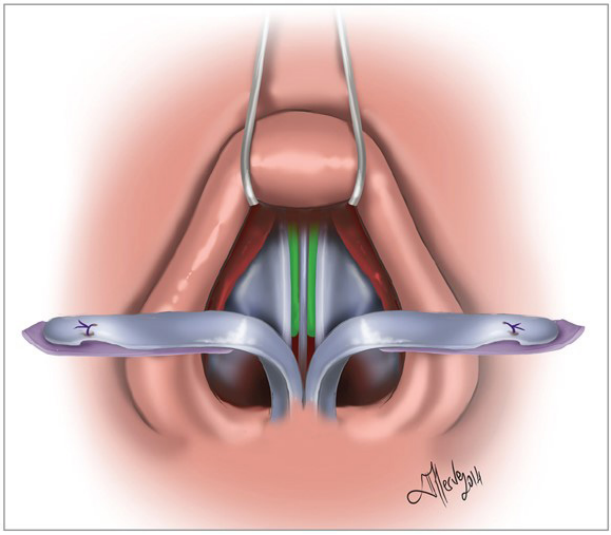
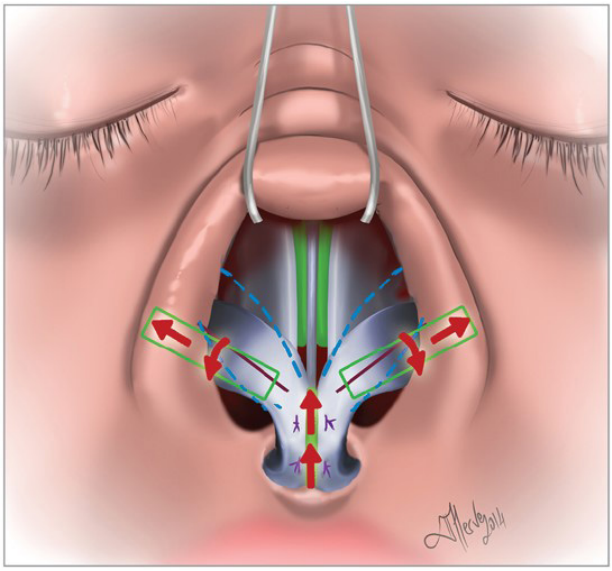


Figure 4. *Positioning of lateral cruras in pockets formed on the anterior caudal region of the accessory cartilages.*



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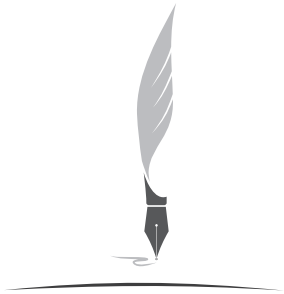

Chapter 2

DETERMINATION OF ROLES OF *INTERLEUKIN-18 (-607C/A AND -137G/C)* GENE VARIATIONS IN BREAST CANCER DEVELOPMENT, PROGRESSION AND METASTASIS

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INTRODUCTION

Many different agents, such as proinflammatory cytokines, play an important role in the pathogenesis of breast cancer, a multifactorial disease. Proinflammatory cytokines associated with the replacement of epithelial tissues are also effective in suppressing og anticancer immune response and angiogenesis increase. The inflammation process is also associated with the suppression of anticancer immune response and angiogenesis regulation. The antitumor immune response is affected by genetic variations in regulatory genes in the immune system. Cytokines are derived from tumors and their environmental components. Cytokines are known to play an important role in growth, invasion and metastasis in breast cancer.

Various studies have found that cytokine gene variations are effective in up and down regulation of cytokine production. There are studies showing that there is a significant relationship between tumor formation, invasion and angiogenesis and IL-18 gene variations. In studies conducted with human and animal models, significant relationships were found between various cancer types and increased IL-18 gene expression levels. In some studies, an increase in serum IL-18 levels was observed in the advanced stages of various tumors. In studies conducted with different populations around the world, it has been determined significant relationships that IL-18 gene variations and between various types of cancer. As a result of IL-18 gene variations in the promoter region of the IL-18 gene, production levels and IL-18 function can be affected. Thus, the tumor formation process can also be affected (1). So the purpose of this section is in addition to providing general information about breast cancer, are the determination of roles of IL-18 gene variations in breast cancer development, progression and metastasis.

BREAST CANCER

Breast cancer is a heterogeneous, complex and multifactorial disease (1). Breast cancer begins when proliferation in normal cells of the breast and division to form new cells occurs. Normal cells die when damaged and new cells replaced by these damaged cells. When these damaged cells do not die properly, the formation of new cells, although the body does not need, appears as an abnormal condition. As a result of the accumulation of additional cells, a tissue mass called as tumor occur and breast cancer develops (2). Increasing breast cancer in developed and developing countries is the most common type of cancer in women in the world. The pathogenesis of breast cancer has not been fully elucidated yet, but this disease is thought to be caused by multifactorial causes. Various factors such as diet, breastfeeding, environmental factors and genetic factors play an important role in the pathogenesis of breast cancer. Breast cancer can develop as a result of changes in some major genes such as tumor suppressor genes, growth regulatory genes and oncogenes. Single nucleotide gene variations, which are among genetic factors, play an important role in the susceptibility and progression of breast cancer. (1).

Breast cancer, one of the biggest threats to women's health worldwide, is treated with surgery. Chemotherapy, radiation therapy or both are applied after the surgery. However, there is a risk of relapse and metastasis in breast cancer. One of the important approaches in the treatment of breast cancer is known as immunotherapy. IL-18, the inducible factor of interferon gamma, can suppress antitumor immunity depend on programmed death-1 (PD-1). PD-1, a co-inhibitory receptor, is one of the main control mechanisms. IL-18 gene is known to play an important role in breast cancer susceptibility and progression (3).

It was determined that IL-18 -607C / A and IL-18 -137G / C gene variations in the promoter region of the IL-18 gene play an important role in susceptibility to various cancers such as hepatocellular carcinoma, non-small-cell lung cancer, papillary thyroid syndrome, chronic leukemia, oral squamous cell carcinoma (3). The IL-18 gene has also been found to play an important role in tumor progression in breast cancer. It was determined that in various cancers such as breast cancer, IL-18 gene expression and serum IL-18 levels were found to be significantly increased in metastatic patients compared to healthy controls and patients without metastase. IL-18 expression and serum IL-18 levels is thought to be important biomarkers in the treatment of breast cancer due to the roles of in tumor metastasis and drug resistance (2).

CLINICAL FINDINGS

Clinical presentation of breast mass varies. Some masses are found in the patient's self breast examination and others in a routine clinical breast examination. Some masses may be painful and / or discharge from the nipple (e.g. bloody, green, white, yellow) (4). Trauma to the breast (car accident with a seat belt, direct impact on the breast with a hard object) may cause breast mass upon fat necrosis or hematoma development.

Physical examination; It is similar to physical examination of patients with benign breast disease and cancer patients. Because in women, normal breast tissue is often nodular. The first aim of the physical examination is to determine whether there is a palpable mass, thickening of the skin or asymmetry. During physical examination palpable breast mass should be evaluated; soft or hard; can be fixed or movable to the chest wall or skin. The mass boundaries may have regular or discrete edges. Clinical findings such as ecchymosis, erythema,

peau d'orange or ulceration of the skin may accompany. Clinical findings such as nipple discharge and nipple withdrawal may accompany the mass. The classic characteristic of cancerous lesion is that it has irregular borders, stiff and immobile. However, these features can not be used to distinguish benign from malignant. Symptoms of advanced locaregional disease include axillary adenopathy or erythema, skin findings such as orange peel (peau d'range).

DIAGNOSIS METHODS IN BREAST CANCER

Physical examination: Examination of both breasts, neck, chest wall and axilla should be performed. Breast examinations should be performed at a time when the hormonal stimulation of the breasts is minimized, which is usually seven to nine days after the start of menstruation in premenopausal women. Evaluation of a clinically suspicious mass should not be affected by the stage of the menstrual cycle.

Inspection: The patient should be examined in both the upright and supine position. Breast examination was started with the patient in the sitting position and with arms relaxed. The patient is then asked to raise her arms above her head so that the bottom of the breasts can be controlled. Finally, the patient should place her hands on her hips and press down to contract her chest muscles so that all other retraction areas can be visualized. The examination of the breast includes:

- **Asymmetry;** general breast structure is examined.
- **Skin changes;** Darkness, withdrawal, edema, ulceration, erythema or scaly, thickened, raw skin should be checked for eczematous appearance.

- **Nipple;** Symmetry, nipple discharge or crusting should be evaluated.
- **Palpation;** regional lymph nodes and breasts need to be palpated.
- **Regional lymph node examination**; cervical, supraclavicular, infraclavicular and axillary regional lymph nodes are examined while the patient is sitting. Whether soft, mobile, hard, sensitive, fixed, it is important to note the presence and properties of nodes.
- **Breast examination:** while the patient is still in the sitting position, gently supporting the breast with one hand and examining the breasts with the other hand. The examination is continued by lifting the patient above the ipsilateral arm head, in the supine position. This allows the examiner to flatten the breast tissue onto the patient's chest. The entire breast should be examined, including breast tissue, which includes the axillary tail of Spence, extending laterally to the axis. Make sure that all breast tissue is included in the examination. The inspection technique should be systematic using concentric circles, a radial approach or vertical strips. Circular movements with light, medium and deep pressure provide palpation of the breast tissue at all levels. One hand is used to stabilize the breast while the other hand is used to perform the examination.

HISTOPATHOLOGIC CLASSIFICATION OF BREAST CANCER

Most breast malignancies arise from epithelial elements and are categorized as carcinomas. Breast carcinomas are a diverse group of lesions that differ in

microscopic appearance and biologic behavior, although these disorders are often discussed as a single disease. Breast cancers are generally divided into two main groups;

1) Non invasive or in situ carcinoms
2) Invasive carcinoms

The in situ carcinomas of the breast are either ductal (also known as intraductal carcinoma) or lobular. This distinction is primarily based upon the growth pattern and cytologic features of the lesions, rather than their anatomic location within the mammary ductal-lobular system. The invasive breast carcinomas consist of several histologic subtypes; the estimated percentages are from a contemporary population-based series of 135,157 women with breast cancer reported to the Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute between 1992 and 2001 (5) (Table 1).

Table 1: *Breast cancer histopathologic percentage*

INVASIVE DUCTAL	INVASIVE LOBULAR	DUCTAL / LOBULAR	MUCINOUS (COLLOID)	TUBULAR	MEDULLARY	PAILLARY
%76	%8	%7	%2,4	%1,5	%1,2	%1

Metaplastic breast cancer and invasive micropapillary breast cancer, all account for less than 5 percent of cases (6).

Ductal carcinoma in situ; encompasses a heterogeneous group of lesions that differ in their clinical presentation, histologic appearance, and biologic potential. DCIS is characterized by proliferation of malignant epithelial cells within the mammary ductal system, with no evidence of invasion into the surrounding

stroma on routine light microscopic examination (7). Ductal carcinoma in situ, differs from lobular carcinoma in situ with regard to radiologic features, morphology, biologic behavior, and anatomic distribution in the breast.

The traditional method used to classify DCIS lesions is primarily based on the growth pattern of the tumor and is divided into five main types (8,9).

- **The comedo type** is characterized by prominent necrosis in the center of the involved spaces. The necrotic material frequently becomes calcified; the calcifications may be detected mammographically, characteristically as linear, branching calcifications. The tumor cells are large and show nuclear pleomorphism; mitotic activity may be prominent. The comedo type is more often associated with invasion.
- **The cribriform type** is characterized by the formation of back to back glands without intervening stroma. The cells comprising this subtype are typically small to medium sized and have relatively uniform hyperchromatic nuclei. Mitoses are infrequent, and necrosis is limited to single cells or small cell clusters.

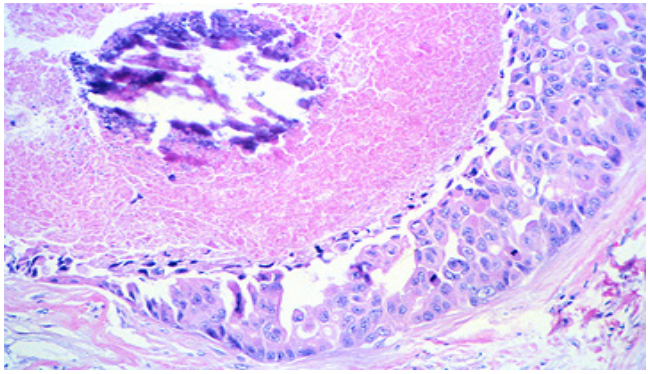

Figure 1: *Comedo duktal carcinoma in situ (10)*

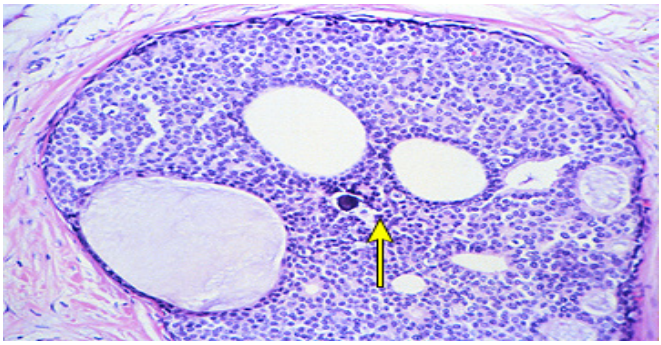

Figure 2: *Cribriform duktal carcinoma in situ (10)*

- **The micropapillary type** features small tufts of cells that are oriented perpendicular to the basement membrane of the involved spaces and project into the lumina. The apical region of these small papillations is frequently broader than the base, imparting a club-shaped appearance. The micropapillar lack fibrovascular cores. The cells comprising this type of DCIS are usually small to medium in size, and the nuclei show diffuse hyperchromasia; mitoses are infrequent.

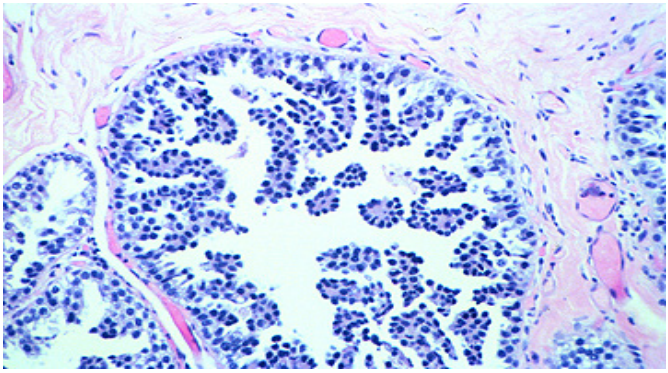

Figure 3: *Micropapillary ductal carcinoma in situ (10)*

The papillary type shows intraluminal extension of tumor cells that show fibrovascular cores and thus form true papillary, unlike the micropapillary variant. The

papillary DCIS variant is characterized by intracystic papillary carcinoma, primary or only tumor cells present in a single or cystically dilated area (11).

Some authors have proposed alternative classification systems for DCIS (12). Although they use different terminology, they all have essentially the identification of three major DCIS categories (ex, high, medium and low grade).

- Nuclear grade is the presence or absence of necrosis.

- **High grade lesions typically** show aneuploidy. It does not contain estrogen and progesterone receptors and has a high proliferative rate. It is characterized by overexpression of the human epidermal growth factor receptor 2 (HER2) oncogene, mutations of the p53 tumor suppressor gene, protein product accumulation, and angiogenesis in the stroma.

- **Low-grade lesions typically** show a diploid structure. Estrogen and progesterone receptors are positive and have a low proliferative rate. Rarely, there are or no abnormalities in HER2 / neu or p53 oncogenes.

Lesions classified histologically as intermediate grade are also intermediate between the high-grade and low-grade lesions with regard to the frequency of alterations in these biological markers.

These classification systems appear to correlate with biological prognostic markers and predict groups of patients likely to recurrence in cancer after breast-conserving surgical treatment (12,13).

In 1997, a conference was organized to reach a consensus on the classification of DCIS (14). Although the panel did not approve any classification system, they recommended routinely documenting some features in the pathology report of DCIS lesions, including nuclear class, presence of necrosis, cell polarization, and architectural pattern.

Infiltrating ductal carcinoma is the most common type of invasive breast cancer, accounting for 70 to 80 percent of invasive lesions. It is also termed infiltrating carcinoma of no special type or infiltrating carcinoma not otherwise specified (NOS). In pathological evaluation, these lesions are characterized by irregular, stiff, gray-white, sandy masses that irregularly invade the surrounding tissue. Malignant cells cause a fibrous response when they penetrate the breast parenchyma. This reaction largely constitutes the clinically coarse and palpable mass of typical invasive carcinomas. It is also responsible for breast density in radiological imaging and solid properties in sonographic imaging. Infiltrated ductal carcinomas are divided into three subgroups that include a combination of architectural and cytological features. Evaluated using a scoring system based on three parameters (15).

Well-differentiated tumors are cells that leak into the stroma. Nuclei show little or no evidence of mitotic activity.

- Moderately differentiated - Moderately differentiated tumors have cells leaking into the stroma with glandular differentiation. There are some nuclear pleomorphisms and little mitotic activity.

- Poorly differentiated - Poorly differentiated tumors consist of wells of neoplastic cells without gland structure. There is significant nuclear atypia and significant mitotic activity.

Infiltrating lobular carcinomas are the second most common type of invasive breast cancer, accounting for about 5 to 10 percent of invasive lesions. The incidence of lobular cancer in the United States is increasing faster than the incidence of ductal carcinoma. The risk of cancer associated with postmenopausal hormone therapy is more strongly associated with lobular carcinoma than with ductal cancer. Some infiltrated lobular carcinomas have a macroscopic appearance similar to that of infiltrated ductal cancers. In most cases, the mass lesion is unclear, and the excised breast tissue may be normal or have only a small rigid structure. Thus, the microscopic size of invasive lobular carcinoma may be significantly larger than that measured gross.

In addition to their different histological appearance and different mammographic characteristics, there are significant prognostic and biological differences between infiltrating lobular and infiltrated ductal cancers:

Infiltrated lobular carcinomas are more likely to be bilateral and multicenter than infiltrated ductal carcinomas (16).

Infiltrated lobular carcinomas occur in older women and are larger and well differentiated tumors (16,17). As a rule, invasive lobular carcinomas are estrogen receptor (ER)-positive, although lesions with occasional variable expression are seen.

Infiltrating lobular carcinomas metastasize to unusual sites such as the peritoneum, meninges and gastrointestinal tract (18).

Tubular carcinomas; It is relatively rare in the pre-mammography period. Tubular carcinoma is characterized by the presence of tubular or glandular structures infiltrating the stroma. These lesions have a relatively favorable prognosis compared to infiltrating ductal carcinomas and metastasis is rare (19-21).

Mucinous carcinomas; 1-2% of invasive breast cancers. It is more common in elderly patients. These lesions generally have a soft gelatinous appearance in breast examination and tend to be well confined. Prognosis is as good as tubular breast carcinomas (19-21).

Medullary carcinomas; it constitutes 1-10 percent of invasive breast cancers. Medullary carcinomas are well-limited on macroscopic examination and are usually associated with areas of bleeding or necrosis.

TUMOR, NODE, METASTASIS (TNM) STAGING CLASSIFICATION OF BREAST CANCER

Tumor staging systems provide information about the spread and severity of cancer. Today, TNM (Tumor diameter, Nod, Metastasis) system shaped by American Joint Commitee on Cancer (76) is used in the world and in our country. The criteria used to classify tumors in the TNM staging system are; tumor size (T), axillary lymph node invasion (N) and distant site invasion (M) (22).

Primary Tumor

- Tx- Primary tumor unable to be assesed.
- T0- No evidence of primary tumor.
- Tis- Carcinoma in situ
  - ✓ Tis (DCIS)- Ductal carcinoma in situ
  - ✓ Tis (Paget)- Paget disease of the nipple not associated with invasive carcinoma or /and DCIS.
- T1- Tumor ≤ 20 mm in greatest dimension
- T1mi- Tumor ≤ 1 mm in greatest dimension
  - ✓ T1a – Tumor> 1 mm, but ≤ 5mm in greatest dimension

- ✓ T1b – Tumor> 5 mm, but ≤ 10mm in greatest dimension
- ✓ T1c – Tumor> 10 mm but ≤ 20 mm in greatest dimension

- T2 – Tumor> 20 mm but ≤ 50 mm in greatest dimension
- T3 – Tumor> 50 mm in greatest dimension
- T4- Tumor of any size with direct extension to the chest wall and/or the skin (ulceration or macroscopic skin nodules).
  - ✓ T4a – Extension to chest wall, not including only pectoralis muscle adherence/invasion.
  - ✓ T4b- Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma.
  - ✓ T4c – Both (T4a and T4b)
  - ✓ T4d – Inflammatory carcinoma

Invasion of the dermis alone does not qualify as T4.

Clinical Classification of Lymph Nodules

Regional lymph nodes (N) - The criteria for lymph node classification vary depending on whether the nodes are evaluated clinically or pathologically. CN or pN is used to discriminate. Pathological classification is preferred where possible.

- cNX- Regional lymph nodes cannot be assessed (eg, previously removed).
- cN0- No regional lymph node metastases (neither by imaging nor clinical exam).
- cN1 - Metastasis to movable ipsilateral level I, II axillary lymph nodes(s).
  - ✓ cN1mi - Micrometastases (approximately

200 cells, larger than 0.2 mm, but none larger than 2.0 mm).

- ✓ cN2 – Metastasis to ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in ipsilateral internal mammary nodes in the absence of clinically evident axillary node metastases.

- cN2a – Metastasis to ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures.
- cN2b Metastasis only in ipsilateral internal mammary nodes, and in the absence of clinically evident axillary node metastases.

- ✓ cN3 – Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph node involvement; or in ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement.

- cN3a - Metastasis to ipsilateral infraclavicular lymph node(s).
- cN3b - Metastasis to ipsilateral internal mammary lymph node(s) and axillary lymph nodes.
- cN3c - Metastasis in ipsilateral supraclavicular lymph node(s**).**
- The cNX category is used in cases where regional lymph nodes have previously been surgically removed or where there is no evidence of physical examination of the axilla.

cN1mi is rarely used, but in cases where sentinel lymp node biopsy is performed prior to tumor resection (eg. in some cases where neoadjuvant is treated).

Table 2: *AJCC (American Joint Comission on Cancer) cancer staging system (22).*

Stage	T	N	M
0	Tis	N0	M0
I	Tmic	N0	M0
	T1	N0	M0
IIA	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
IIB	T2	N1	M0
	T3	N0	M0
IIIA	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
IIIB	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
IIIC	T1-4	N3	M0
VI	T1-4	N0-3	M1

M; *distant metastases,* **Mx;** *Unevaluated distant metastases,* **M0;** *No evidence of distant metastases* **M1;** *distant metastases.*

PROSNOSTIC FACTORS

Parameters capable of providing information about the clinical outcome at various times during the course of the disease diagnosis or in the course of metastatic disease,

regardless of the patient's treatment, are termed prognostic factors. Conventional prognostic factors; stage, axillary lymph node involvement, tumor type, tumor diameter, histologic grade, nuclear grade, lymphovascular invasion, character component of carcinoma in situ, carcinoma in situ component ratio, skin and nipple invasion. In modern treatment, estrogen and progesterone receptor status, oncoprotein and tumor suppressor genes, tumor angiogenesis, flow cytometry studies, protease status, proliferation index and many prognostic parameters and predictive values are used.

Stage

In breast cancer, staging is based on the evaluation of primary tumor (T), lymph node involvement (N), and distant metastasis (M). Involvement of the transpectoral, internal mammarian and cervical lymph nodes is considered as distant metastasis.

Axillary Lymph Node Involvement

The most important prognostic factor on survival is axillary lymph node involvement. While recurrence occurs in 20-30% of patients with negative axillary involvement, this rate reaches 70% in patients with axillary node involvement. The number of metastatic lymph nodes involved is very important. The prognosis is poor in patients with lymph node involvement of four or more. The number of metastatic lymph nodes as well as the diameter of the metastatic lesion and its spread to the soft tissues are among the factors affecting the prognosis negatively. Sentinel lymph node biopsy has been proposed as an alternative to axillary dissection in patients with clinically negative axillary who has invasive breast cancer. Sentinel lymph node biopsy is sensitive and specific in determining axillary metastasis. Nowadays, sentinel lymph node biopsy is the most preferred because reduces the morbidity associated with axillary dissection.

However, studies have not shown that this method is equal to axillary dissection on disease-free survival.

Tumor Diameter

Tumor diameter is one of the most important prognostic factors and is used in staging with lymph node involvement. There is a relationship between increased tumor diameter and lymph node involvement. Prognosis is significantly better if the tumor diameter is 2 cm or less. Lymph node involvement rate increases with increasing tumor diameter and volume and has a negative effect on survival. Tumors have an aggressive course as the diameter increases, and they grow rapidly within them as they are aggressive (23).

Tumor Grade

Nowadays the most widely used grading system is the Modified Bloom Richardson Degree (MBRD). In this system, tumor tubule formation, nuclear characteristics and mitosis count are evaluated separately and scored. According to the result, histological differentiation of the tumor is decided (I-II-III). All invasive breast carcinomas except medullary carcinoma can be graded.

Table 3: *Modified Bloom Richardson Grade (MBRG) (23).*

FEATURE		MBRD
TUBUL FORMATION	> % 75	1
	% 10-75	2
	< % 10	3
NUCLEAR PLEOMORPHISM	Small, organized, uniform	1
	Shape and size moderataly different	2
	Shape and size clearly different	3
MITOTIC COUNT	0-9	1
	10-19	2
	>20	3

Lenfovascular ve Perinoral Invasion

Lymphovascular invasion is important in determining prognosis. This finding is strongly associated with the presence of lymph node metastasis and is considered to be a sign of poor prognosis. The presence of tumors in dermal lymphatics is a pathological indicator of inflammatory breast carcinoma.

Steroid Receptors

Determination of the presence of estrogen and progesterone in biopsy specimens diagnosed as invasive breast carcinoma has become a standard practice in the treatment protocol.

C-ERBB-2

C-erbB-2 is an epidermal protooncogene. It encodes a glycoprotein from the epidermal growth factor (EGF) family. C-erbB-2 was found to be 40-60% positive in primary breast tumors. Overexpression of this protooncogene is a poor prognostic parameter. C-erbB-2 overexpression has been shown to correlate with mitotic index and axillary metastatic lymph node involvement. However, there is no correlation between C-erbB-2 gene and other prognostic factors in breast cancer. Therefore, it is accepted as a negative independent prognostic factor. It is generally positive in hormone receptor negative tumors and indicates poor response.

p53

P53 gene is located on chromosome 17 and activates to eliminate DNA damage. Overexpression of the p53 gene in breast cancers is a poor prognostic factor. P53 inactivation is observed in 20-30% of breast cancer patients. In studies conducted in breast cancer patients, a close relationship was found between p53 expression and

high tumor grade, high proliferation index, and negative ER and PR.

MOLECULAR PROGNOSTIC FACTORS

Among the prognostic factors of breast cancer reliable in cell kinetic analyzes. These parameters are independent of axillary lymph node involvement.

PROLIFERATION RATIO

Proliferation can be measured by flow cytometry, detection of cellular proteins released during mitotic counts.

Kİ-67

It is a monoclonal antibody which develops against nuclear antigen in the whole cell cycle. Since it can enter the G0 phase, it can be considered that Ki-67 fraction assays can provide meaningful information about the proliferating cell component of a tumor.

DNA

DNA profile can be determined by flow cytometric analysis. The abnormal degree of DNA content is expressed by the DNA index. Correlate with clinical course in patients with primary breast cancer.

INTERLEUKIN-18 GENE VARIATIONS

Breast cancer, known as the most common cancer among women worldwide, is the second cause of cancer-related deaths after lung cancer in women in developed countries. In the etiology of breast cancer that can be diagnosed most frequently in women, there are various risk factors including environmental and genetic factors. Genetic factors that play an important role in breast cancer

pathogenesis include factors such as tumor necrosis factor receptor 2, ABCB1, insulin-like growth factor-1.

Chronic systemic inflammation plays an important role in the patgenesis of metabolic diseases such as diabetes mellitus and various types of cancer such as breast cancer. There are several studies showing the effect of inflammation and cytokines in breast carcinogenesis. (24). One of the important factors leading to tumor formation and development is chronic inflammation. Different chronic inflammation factors play an important role in angiogenesis, mutagenesis, invasion and stimulation of cell growth. It is thought that factors associated with angiogenesis, inflammation and thrombosis may also be associated with the development of different types of cancer.

IL-18, an important member of the IL-1 cytokine family, is localized on chromosome 11q22.2-q22.3 in humans. IL-18 it is produced by T cells, B cells and antigen presenting cells, including active monocytes, dendritic cells, macrophages that can adjust natural and inducible immune responses. IL-18 plays an important role in immune response. There are also several studies showing a significant relationship between IL-18 and tumor formation. Based on this, it is thought that there may be an important relationship between inflammation and cancer. In some studies, in comparison normal cells and cancer cells, cancer cells were found to have higher IL-18 levels than normal cells. In a study with mice, IL-18 levels were found to increase during tumor progression in breast tumor tissues and lymph nodes. In another study, it was found that serum IL-18 levels of patients with metastatic breast cancer increased and these levels may be an important biomarker in breast cancer prognosis and diagnosis. There are also studies showing significant correlations between increased IL-18 levels and angiogenesis and proliferation (1). Also in previous studies, IL-18 has been found to modulate the immune

system to attack cancer cells by inhibiting breast cancer cell progression and invasion (24).

Two common genetic variations as IL-18 -607C/A and IL-18 -137G/C have been identified in the promoter region of the IL-18 gene. It was thought that to be a significant relationship between these genetic variations in the promoter region of the IL-18 gene and different gene expression levels, protein production. IL-18 gene expression and activity are affected as a result of IL-18 gene variations in the promoter region of the IL-18 gene. There is a significant relationship between high promoter activity and increased IL-18 gene expression (1).

IL-18 -607C/A gene variation is characterized by cytosine / adenine base displacement at the -607 position in the promoter region of the IL-18 gene. IL-18 -607C/A gene variation changes the protein binding site of cyclic adenosine monophosphate. Thus, IL-18 transcription is increased. As a result of this gene variation, element binding protein region sensitive to cAMP is disrupted. IL-18 -137G/C gene variation is characterized by guanine / cytosine base displacement at the -137 position in the promoter region of the IL-18 gene (25). As a result of IL-18 -137G/C gene variation, binding sites for H4TF-1 nuclear factor are affected (IL-18-2).

Primer sequences used for IL-18 gene variations are presented in the table (Table 4) (26).

Table 4: *Primer sequences for IL-18 gene variations*

Gene Variations	Primer	Primer Sequences
IL-18 -607C/A	F1	5'-GTT GCA GAA AGTGTA AAA ATT ATT AC-3'
	F2	5'-GTT GCA GAA AGTGTA AAA ATT ATT AA-3'
	FC	5'-CTT TGC TAT CAT TCC AGG AA-3'
	R	5'-TAA CCT CAT TCA GGA CTT CC-3'

Gene Variations	Primer	Primer Sequences
IL-18 -137G/C	F1	5'-CCC CAA CTT TTA CGG AAG AAAAG-3'
	F2	5'-CCC CAA CTT TTA CGG AAG AAA AC-3'
	FC	5'-CCA ATA GGA CTG ATT ATT CCG CA-3'
	R	5'-AGG AGG GCA AAA TGC ACT GG-3'

F1: *Forward 1;* **F2:** *Forward 2;* **FC:** *Forward Control;* **R:** *Reverse*

In several studies with different populations, the relationship between IL-18 gene variations and the risk of developing breast cancer has been investigated. In some studies, significant relationships were identified between IL-18 gene variations and the risk of developing breast cancer, but some studies did not find a relationship. In a study conducted by Bao et al., it was found that there was no significant relationship between IL-18 -137G/C gene variation and IL-18 gene expression. A nonsignificant relationship was determined between the C allele of IL-18 -137G/C gene variation and increased IL-18 gene expression. In another study conducted with the Brazilian and Chinese populations by Zhao et al., IL-18 -607C/A gene variation was found to be a genetic risk factor in the development of breast cancer of the AA genotype. In another meta-analysis study conducted by Li et al., A significant relationship was determined between IL-18 -607C/A gene variation and the risk of developing breast cancer. In a study conducted with the Iranian population, there was no significant relationship between IL-18 -607C/A gene variation and breast cancer development (1). In a study conducted by Arimitsu et al, it was determined that monocyte production capacity was higher in individuals with GG genotype of IL-18 -137G/C gene variation than compared to individuals with GC

genotype. In various studies, it has been determined that PD-1 suppresses antitumor immunity dependently. In another study conducted by Khalili et al., IL-18 -137G/C gene variation was found to be a significant relationship between CC homozygous genotype and decreased breast cancer risk. In the same study, it was determined that IL-18 -607C/A gene variation was not a genetic risk factor for breast cancer development. In a study carried out with an Iranian population by Taheri et al., it was found that IL-18 -607C/A gene variation was not a genetic risk factor for breast cancer development. In the study conducted by Back et al., A significant relationship was determined between IL-18 -607C/A, IL-18 -137G/C gene variations and increased risk of breast cancer. In a study based on comparative analysis between the benign group and the malignant group in breast cancer patients, the GC genotype of the IL-18 -137G/C gene variation was found to be a genetic risk factor for the benign group. In the same study, it was found that GC genotype may be a protective factor for the healthy group. In the comparison between the healthy group and the benign group, there was no significant relationship between these gene variations and the development of breast cancer (3). In a study conducted with the Iraqi population, no significant relationship was found between IL-18 -607C/A gene variation and breast cancer development (2). In a meta-analysis study conducted with an Asian and mixed population, a significant relationship was found between IL-18 -607C/A gene variation and increased risk of breast cancer. In a study conducted with the Chinese population, AA genotype of the IL-18 -137G/C gene variation and CC genotype of IL-18 -607C/A gene variation was found to be genetic risk factors for the development of breast cancer (24). In another study conducted with the Iranian population, IL-18 -137G/C gene variation was not found to be significant relationship between CC genotype and

breast cancer susceptibility, but this genotype was found to be effective in the prognosis of breast cancer (25).

In studies conducted with different populations to investigate the effects of IL-18 gene variations in breast cancer development, progression and metastasis different results were obtained. Studies should be conducted with larger and different populations in order to determine the exact roles of IL-18 gene variations in breast cancer development and thus to develop new treatment methods.

CONCLUSION

Breast cancer is a heterogeneous and multifactorial disease whose main cause is unknown completely. Environmental factors and genetic factors play a role together in breast cancer pathogenesis. Different results have been obtained in various studies aiming to investigate IL-18 gene variations that play an important role in breast cancer development, progression and metastasis. It is thought that the different results obtained in these studies may be due to the different selection criteria of the patient and control groups in the studies and the fact that the studies were carried out with different races and populations. Identification of IL-18 gene variations occurring in IL-18 gene thought to be associated with breast cancer development, progression, metastasis will enable us to obtain general information about the mechanism of breast cancer disease and will enable us develop new drugs and treatment methods that may be effective in preventing or progressing the disease.

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Chapter 3

IMMUNOMODULATION ACTIVITIES OF PROBIOTICS

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INTRODUCTION

In the last century, the importance of intestinal flora has been better understood in maintaining a healthy life and protecting against diseases, and today the view that microflora should be supported is widely accepted all over the world. The basis of this view goes back to Metchnikoff, who said that the secret of long and healthy living of Bulgarian peasants in 1908 was due to consuming fermented dairy products. Metchnikoff had raised awareness of the concept of probiotics in those years by stating that the rod-shaped bacteria in these products positively affected the population in the colon (Fuller, 1989; Malago, Koninkx, & Marinsek-Logar, 2011).

The term "probiotic" was first used by Parker in 1974. In a meeting held in Brussels in 1994, probiotics were defined as "cultures that have a protective effect on health, have positive effects on digestive, respiratory and reproductive systems, and have been formed by one or several specific microorganisms" (Parker, 1974). Then, the World Health Organization (WHO) defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Karamese et al., 2016). Although bacteria form the majority of probiotic microorganisms, some molds and yeasts are also included in this group (Bergonzelli, Blum, Brussow, & Corthesy-Theulaz, 2005; Fuller, 1989; Malago et al., 2011).

Many of the probiotic microorganisms are intestinal flora elements and their number may decrease in cases such as aging, disease, and using medication. It has been determined that even if the probiotics exist in the flora, it should be taken with foods between 10^6-10^8 CFU/g in order to observe the positive effects of them (Champagne, Gardner, & Roy, 2005). Although probiotics show

their activation by clinging to the digestive tract, they reproduce slowly and cannot colonize for a long time. They are metabolically active in this process; however, it may take time to see their positive effects (Marco, Pavan, & Kleerebezem, 2006).

Microorganisms Used as Probiotics and Their Properties

Probiotic bacteria are more resistant to stomach acid, bile salts and lysozyme enzyme than other bacteria. *Lactobacillus* species are generally found in large amounts in the small intestine, while *Bifidobacterium* are larger in the large intestine (Walter, 2008). Probiotic bacteria control the growth of unwanted microorganisms in the intestines and balance the intestinal flora with the antimicrobial substances they produce as a result of the destruction of glucose, such as acetic acid, lactic acid, bacteriocin. The number of probiotic bacteria in the intestinal flora of healthy individuals stabilizes over time; however, conditions such as excessive use of antibiotics, stress, fatigue, improper nutrition, excessive alcohol intake, various diseases and intestinal surgeries cause reduction of the number of these bacteria (Parvez, Malik, Ah Kang, & Kim, 2006). One of the important features of probiotic bacteria is their ability to attach to the intestinal wall. This attachment is necessary for them to have a biological effect. Probiotic bacteria prevents the attachment of pathogenic microorganisms by clinging to the intestinal wall. They also reproduce rapidly without being affected by bowel movements during digestion. Lactic acid bacteria such as *Lactobacillus* sp., *Pediococcus* sp., *Streptococcus* sp., *Leuconostoc* sp., *Lactococcus* sp., *Propionibacterium* sp. constitute the most important and most common group of probiotics that regulate the intestinal flora of the host, strengthen mucosal and systemic immunity and provide beneficial

effects on health. All lactic acid bacteria, which are used for making yogurt, except *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*, are the members of intestinal flora. Additionally, *Bifidobacterium* species (especially *Bifidobacterium bifidus*, *Bifidobacterium longum*, and *Bifidobacterium breve*) are the most commonly used probiotic bacteria as well as lactic acid bacteria (Aydoğdu, Karameşe, Altoparlak, & Karameşe Aksak, 2019; Karamese et al., 2016; Sanchez et al., 2017). In addition to these two groups (*Lactobacillus* and *Bifidobacterium*), it has been shown in studies that some bacterial strains, mold and yeast species may also have probiotic characteristics. The list of most used probiotic microorganisms is shown in Table 1.

Table 1: *Most used probiotic microorganisms*

Lactobacillus sp.	*L. bulgaricus*	*L. rhamnosus*
	L. salivarius	*L. delbrueckii*
	L. curvatus	*L. plantarum*
	L. lactis	*L. cellebiosus*
	L. acidophilus	*L. brevis*
	L. helveticus	*L. reuteri*
	L. johsonli	*L. casei*
	L. fermentum	*L. gasseri*
Bifidobacterium sp.	*B. thermophilum*	*B. breve*
	B. bifidum	*B. adolescentis*
	B. animalis	*B. lactis*
	B. infantis	*B. longum*
Bacteriodes sp.	*B. capillus*	*B. ruminicola*
	B. suis	*B. amylophilus*
Bacillus sp.	*B. subtilis*	*B. coagulans*
	B. lentus	*B. cereus*
	B. pumilus	*B. licheniformis*
Propionibacterium sp.	*P. shermanii*	*P. freudenreichii*
Pediococcus sp.	*P. cerevisiae*	*P. pentosaceus*
	P. acidilactici	

Streptococcus sp.	*S. intermedius* *S. thermophilus* *S. lactis*	*S. cremoris* *S. diacetilactis*
Leuconostoc sp.	*L. mesenteroides*	
Molds	*Aspergillus niger*	*Aspergillus oryzae*
Yeasts	*S. cerevisiae* *S. boulardii*	*Candida torulopsis*

Microorganisms to be used as probiotics are expected to meet certain criteria. The main criteria are being of human origin, being non-pathogenic and non-toxigenic, adhesion to the intestinal mucosa, adaptation and well colonization, antagonistic effect against pathogenic microorganisms, producing antimicrobial agent, being resistant to antibiotics, stimulating the immune system, regulating immune response, and making a positive impact on host health (Ouwehand, Salminen, & Isolauri, 2002; Shi, Balakrishnan, Thiagarajah, Mohd Ismail, & Yin, 2016; Yousefi et al., 2018).

Mechanisms of Action of Probiotics

Probiotics show their beneficial effects on the host through three mechanisms, which are determined to reduce the number of pathogenic microorganisms, to change microbial metabolism, and to improvement or regulation of the immune system (Bermudez-Brito, Plaza-Diaz, Munoz-Quezada, Gomez-Llorente, & Gil, 2012; Ouwehand et al., 2002; Singh, Sharma, Babu, Rizwanulla, & Singla, 2013). In recent years, studies investigating the beneficial effects of probiotic bacteria on humans have raised. The beneficial effects and probable action mechanisms of probiotics are summarized in Table 2.

Table 2: *The beneficial effects and probable action mechanisms of probiotics*

Beneficial effects	**Probable action mechanisms**
Prevention of urogenital infections (Hanson, VandeVusse, Jerme, Abad, & Safdar, 2016)	• Preventing the development of pathogenic bacteria by producing organic acids (lactic acid, acetic acid, etc.) and antimicrobial compounds (H_2O_2, bacteriocin, etc.) • Binding to urinary and vaginal area cells • Having co-aggregation ability
Resistance to pathogenic bacteria (Imperial & Ibana, 2016)	• Competing with pathogenic bacteria concerning nutrients and receptors • Providing non-suitable conditions for pathogens in the intestinal system (pH, short-chain fatty acids and bacteriocins, etc.) • Removing pathogens from the mucosal surface with various surface proteins • Increasing mucus production • Producing antitoxins • Providing beneficial effect on intestinal flora
Regulation of the intestinal system (van Baarlen, Wells, & Kleerebezem, 2013)	• Changing the composition of the intestinal flora • Increasing mucosal and systemic immune activity • Increasing the lifespan of epithelial cells • Ensuring barrier integrity • Preventing overgrowth of intestinal bacteria
Anti-carcinogenic effect (Gorska, Przystupski, Niemczura, & Kulbacka, 2019)	• Changing the physicochemical conditions in the colon • Binding mutagens and reducing absorption of mutagenic compounds • Producing anti-carcinogenic or anti-mutagenic compounds • Stimulating apoptosis mechanism • Providing inhibition of fecal microbial enzymes with mutagenic and carcinogenic effects
Controlling of some allergic diseases (Michail, 2009)	• Reducing sensitivity to peptides • Preventing substances with antigenic effects from passing into the circulatory system

Controlling of respiratory diseases (Y. Wang et al., 2016)	• Increasing the number and activity of phagocytic cells in the lungs • Increasing the number and activity of phagocytic cells in the lungs • Reducing allergic airway inflammation by inducing non-antigen-sensitive T regulatory cells
Prevention and treatment of infectious diarrhea (Guarino, Guandalini, & Lo Vecchio, 2015)	• Stabilizing the natural flora in the treatment of rotavirus and other viral diarrhea • Inhibiting the attachment of the virus to the mucosa • Increasing the efficacy of mucin-encoding genes in the intestinal epithelium of the host to increase the barrier effectiveness of mucus • Increasing viral excretion by increasing bacterial adhesion • Shortening viral excretion time • Repairing epithelial cells by stimulating Toll-like receptors • Strengthening tight junction areas and increasing cytokine release
Assimilation of cholesterol (Tomaro-Duchesneau et al., 2014)	• Being able to assimilate cholesterol in the presence of bile salts • Binding cholesterol to the cell wall • Converting cholesterol to coprostanol
Reduction of lactose tolerance (Oak & Jha, 2019)	• Digesting lactose with bacterial β-galactosidase enzyme
Regulation of immune system (Karamese et al., 2016; Kim et al., 2016; Maldonado Galdeano, Novotny Nunez, Carmuega, de Moreno de LeBlanc, & Perdigon, 2015; Yan & Polk, 2011)	• Regulating the host immune response • Increasing secretory IgA release • Increasing B lymphocyte production • Increasing phagocytic activity • Increasing the number of apoptosis • Modulating dendritic cell function • Increasing expression and release of IL-10, TGF-β and PGE2 • Reducing TNF-α, IL-1 and INF-γ expression • Activating regulator T cells • Increasing natural killer cell activity

Probiotics and Immune System

The gut microbiota helps in the development of the host immune system. The interaction between immune system and microbiota should be in balance, and any disturbances of this interaction would result in inflammatory disorders. The increased rate on the emerging disorders such as rheumatoid arthritis, cardiovascular disease, inflammatory bowel disease, and metabolic syndrome has direct the scientists to investigate the possible interaction between immune system and microbiota (Sommer & Backhed, 2013).

The mechanisms of regulation of innate and adaptive immune responses by probiotics are not completely known; however, many researches have been reported that probiotic bacteria help to regulate and increase the innate and adaptive immune response (Figure 1). The consumption of probiotics may effects the innate immune response via inhibiting of pathogenic microorganisms, elevating of mucin production, reducing the intestinal permeability, and increasing the Natural Killer (NK) cell activity, phagocytic capacity and macrophage activation (Gill & Rutherfurd, 2001). On the other hand, it may also effects the adaptive immune response via elevating the production of immunoglobulins, and coordinating the production of cytokines and other regulatory elements (Paineau et al., 2008). This modulation of immune system can be performed via innate cell surface pattern recognition receptors (PRRs) carried out by dendritic cells (DCs), macrophages and monocytes, or lymphoid cell activation (Cross, 2002; Isolauri, Sutas, Kankaanpaa, Arvilommi, & Salminen, 2001; Ruiz, Hoffmann, Szcesny, Blaut, & Haller, 2005).

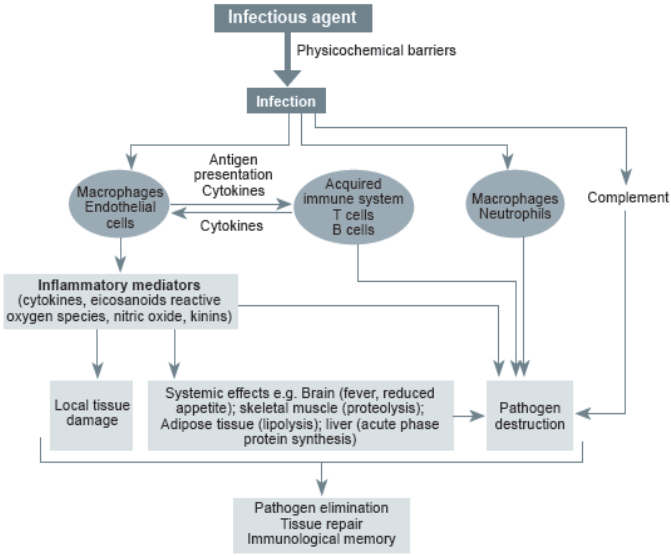

Figure 1: *A general overview of the immune system (Bamola, Sharma, & Chaudhry, 2013).*

Furthermore, probiotics can affect the release of some immune modulators including cytokines and chemokines by unique T cell subsets. These mediators play a crucial role of the regulation of immune response in mucosal site and are responsible for the maintenance of intestinal homeostasis (Marques & Boneca, 2011). Most studies have reported a significant decrease in the production of pro-inflammatory cytokines such as Interleukin-12 (IL-12) with a simultaneous increase in the production of anti-inflammatory cytokines such as Interluekin-10 (IL-10) (Mohamadzadeh et al., 2011; S. Wang et al., 2012). In addition to these two effector pathways, a new hypothesis is proposed that certain probiotic microorganisms can activate T cell subsets such as T helper 9 (Th-9) and T helper 17 (Th-17) (Lopez et al., 2012). Thus, the studies about the immunomodulatory activity of probiotics

on some diseases including inflammatory and allergic diseases have been getting attention and increasing day by day.

Dendritic Cell Maturation

The related studies indicated that the maturation of DCs is one of the most important factor that affecting the modulation activity of probiotics. DCs plays key role on the differentiation of naive CD4+T cells into Th-1, Th-2 or even Th-3, determination of the Th-1/Th-2 balance, and development of tolerance (Braat et al., 2004; Steinman & Nussenzweig, 2002). The cytokines are another factor for the differentiation of naive T cells. Some probiotics triggers the production of IL-10 to restrict the mucosal inflammation by influencing the monocyte-derived DCs. The DCs enhance the T regulatory cells activity and shows anti-inflammatory effects. It has been shown that specific probiotic strains can stimulate the secretion of specific cytokines. The lactobacilli-induced cytokine response by *L. casei, L. rhamnosus, L. reuteri*, *L. johnsonii,* and *L. gasseri* in DCs triggers Th-1 and Th-17 response for the production of IL-10 to restrict the mucosal inflammation by influencing the monocyte-derived DCs (Chuang et al., 2007; Evrard et al., 2011). Furthermore, majority of *Bifidobacterium* species elevate the IL-10 production, while IL-12 and Tumor Necrosis Factor-alpha (TNF-α) levels remain reduced (Round & Mazmanian, 2009). Taken together, DCs have potential to drive regulatory T-cell subtypes, and are fundamental for bacterial interception.

T Cell Responses and Cytokine Production

The release of mediators such as cytokines, including ILs, TNFs, transforming growth factor (TGF), interferons (IFNs), and chemokines from immune cells including DCs, lymphocytes, macrophages, and mast cells (Azad, Sarker,

& Wan, 2018; Savan & Sakai, 2006). Environmental cytokines have a potential to reveal pro-inflammatory (IL-1-β, IL-6, TNF-α) and anti-inflammatory (IL-10, TGF-β) responses. Additionally, cytokines have key roles on Th-1 differentiation and cellular immune response by the production of IL-12 and IFN-γ; Th-2 differentiation and humoral immune response by the production of IL-4, IL-5, IL-10, and IL-13; Th-17 differentiation and anti-microbial response by the production of IL-1-β, IL-6, IL-17A, IL-22, IL-23, and TGF-β; T regulatory differentiation and immune-suppression by the production of IL-10, IL-35 and TGF-β (Hardy, Harris, Lyon, Beal, & Foey, 2013) (Figure 2).

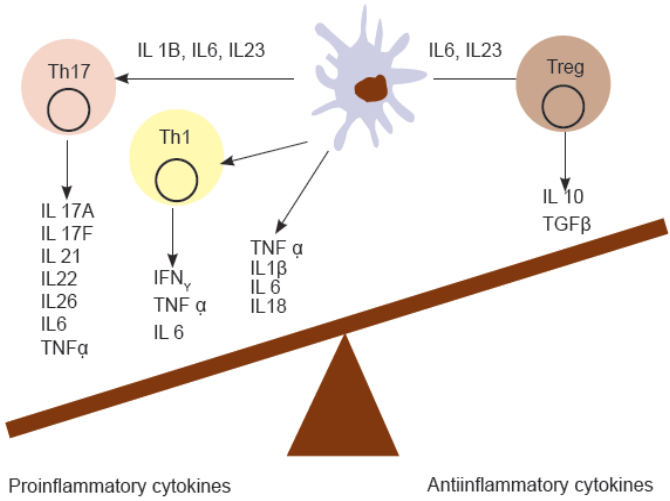

Figure 2: *Pro-inflammatory and anti-inflammatory cytokine balance (Bamola et al., 2013)*

In current literature, there are evidences suggesting that an important mechanism by which probiotics provide health benefits is through the modulation of cytokine production; however, the benefits are strain-dependent concerning pro- and anti-inflammatory effects. The probiotic strains of the *Lactobacillus* and *Bifidobacterium*

genera as well as *Leuconostoc* and *Lactococcus* genera have shown capacity to modulate cytokine expression including TNF-α, TGF-β, IL-1-β, IL-6, and IL-10 (Aydoğdu et al., 2019; Karamese et al., 2016). Additionally, other studies had also reported that most of the *Lactobacillus* and *Bifidobacterium* strains induce the cytokine production by intestinal epithelial cells, monocyte-derived DC and peripheral blood mononuclear cell (PBMCs) in *in vitro* experiments (Candela et al., 2008; Latvala et al., 2008; Pozo-Rubio et al., 2011). It was reported that *L. rhamnosus* GG, *L. gasseri*, *B. bifidum*, and *B. longum* led to an increase in Th-1-associated mediators secreted by PBMCs (IFN-γ and IL-12) (Martin et al., 2009). Oral administration of *Lactobacillus paracasei* also elevated IL-12 levels (Ichikawa, Miyake, Fujii, & Konishi, 2009). *L. rhamnosus* Lcr35 led to an increase in the levels of IL-23 and IL-17, while *B. breve* and *L. plantarum* reduced those levels (Evrard et al., 2011; Ghadimi, Helwig, Schrezenmeir, Heller, & de Vrese, 2012; Paolillo, Romano Carratelli, Sorrentino, Mazzola, & Rizzo, 2009).

Antibody production

The studies proved that daily consumption of probiotics has an important influence on the gut barrier and are able to stimulate the production of IgA by B cells (Azad et al., 2018; Link-Amster, Rochat, Saudan, Mignot, & Aeschlimann, 1994). A study stated that *L. rhamnosus* GG increased the levels of IgM, IgG or IgA antibodies in children after rotavirus vaccination (Kaila, Isolauri, Saxelin, Arvilommi, & Vesikari, 1995). Furthermore, another study reported that *Bifidobacteria* could also promote IgA production in children who consumed a probiotic mixture containing *Bifidobacterium animalis ssp. lactis* Bb12 (Fukushima, Kawata, Hara, Terada, & Mitsuoka, 1998). These results show that oral consumption of probiotics induced serum IgA production;

however, there is a difference between the local IgA and serum IgA measurements. On the other hand, the use of *Bifidobacterium lactis, L. fermentum*, and *Lactobacillus paracasei* ssp. *paracasei* caused significantly increase on the levels of specific IgG1, IgG3, and IgM as well as IgA (Kaila et al., 1995; Olivares et al., 2007).

IgA is known as the most important antibody of the mucosal immune response to microbial antigens; however, IgG has recently been raised as another key factor for mucosal innate immune response (Roopenian & Akilesh, 2007; Sabirov & Metzger, 2008). For this reason, the recent studies for developing vaccines have been focused on the production of mucosal IgG along with serum IgG and mucosal IgA (Bamola et al., 2013). It is well known that probiotics increase the total and pathogen specific secretory IgA (sIgA) levels. Thus, sIgA can protect against intracellular pathogens by neutralizing microbial components (Ohland & Macnaughton, 2010).

Conclusion

The probiotic consumption and treatment is seen as a promising research area. Probiotics can modulate the activity of both innate and adaptive immune system cells including DCs, macrophages, T cells (Th-1, Th-2, Th-17, T regulatory, T cytotoxic), B cells, NKs, epithelial cells and granulocytes. Furthermore, they increase the functions of gut barrier by influencing cytokine production and stimulating B cells, and modulate the gut microbiota and mucosal immune response in the host. However, as known, probiotics do not have the same effects in each individuals. Thus, selection of probiotic strains or mixture should be formulated for considering the mechanisms of disease and its interactions with immune system. Understanding of the mechanisms are crucial for the development of new strategies on therapeutic approaches that influence the gastrointestinal flora to maintain health and prevent diseases.

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Chapter 4

PEDIATRIC ONCOLOGY PATIENTS AND THEIR FAMILIES

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1. INTRODUCTION

Today, mortality rates have decreased with the treatment and follow-up of this disease, and childhood cancers, which are considered chronic diseases, have a significant affect on both the child and the family. The response of each child to this disease, which is a source of stress all by itself, also varies with different variables. The disease causes cognitive, social and emotional problems, as well as physical problems in children. The absence of school life and children's friends who have to fight school-age cancer have a number of negative effects on the child. That is why these children should be rehabilitated by specialists with a multidisciplinary perception. The child should be supported in accordance with the characteristics of the child's development and the condition of the disease. The psychological and psychosocial needs of the family should be provided without being ignored in this process.

2. EPIDEMIOLOGY OF CHILDHOOD CANCERS

Cancer is a disease that begins in any part of the body with uncontrolled cell growth and can spread to other parts of the body (Denny, Wechsler, & Sakamoto, 2016). Childhood cancers are referred to as chronic diseases that are largely unknown and difficult to prevent in literature before the age of 18 (Muslu & Kolutek 2018).

Childhood cancers are rare compared to other types of cancer. In developed countries, the rate of this disease among all cancers is less than 1%. In Sub Sahran-African countries, childhood cancers among all cancers are between 4% and 5% due to the ratio of the young population in the general population. Around 215,000 new childhood cancer cases are emerging globally annually

(WHO, International Childhood Cancer Day: Much remains to be done to fight childhood cancer, 2016).

According to a study, the incidence of childhood cancer in a wide geographical variation ranges from 160 to 170 per million. (WHO, World Cancer Report, 2014) According to WHO;

- More than 300,000 children and young people are diagnosed with cancer every year.

- 80% of children diagnosed with cancer survive in high-income countries.

- while only 20% of children diagnosed with cancer can survive in low and middle-income countries.

- Childhood cancers can often be cured with inexpensive generic drugs and effective treatments.

Childhood cancers are classified internationally as follows (ICCC,1996):

- Leukemia
- Lymphoma
- Brain and spinal canal tumors
- Sympathetic system tumors
- Retinoblastome
- Kidney tumors
- Liver tumors
- Bone tumors
- Soft tissue sarcomas
- Gonad and germ cell tumors
- Epithelial tumors
- Other malignant neoplasms

Leukemia is the most common type of cancer in childhood cancer. Lymphoma and central nervous system tumors are associated with leukemia in both sexes. As we

look at young people aged 15-24, notably testicular cancer in men and thyroid cancer in women, brain, meninges and central nervous system are at the forefront. (Turkey Cancer Statistics, 2015)

The cause of the highest cancer deaths in children and adolescents are brain, other nervous system tumors and leukemia. Leukemia has a higher mortality rate in adolescents than in children. According to SEER, the survival rate of all types is 83% at the age of 0-14; 85% at the age of 15-19. In leukemia, this rate is 87% at 0-14 years, and 72% at 15-19 years old. (American Cancer Society, 2019).

3. REACTIONS OF PEDIATRIC ONCOLOGY PATIENTS TO DISEASE ACCORDING TO DEVELOPMENTAL CHARACTERISTICS

The disease is a source of stress that has negative effects for each child and not only physically but also psychologically affects the child. (Sezgin, Ekinci, & Okanli, Psychosocial Problems and Nursing Approaches in Childs with Cancer, 2007) (Peykerli, 2003)

That is why each child can react differently to the disease depending on different variables. These variables are;

- Age of the child
- The period of psychosexual and psychosocial development in which the child is in
- Personality of the child
- Diagnosis of the disease
- Effect of the disease on the child
- Hospitalization status and duration

- Change of the disease in the child's life

- Sociocultural and socioeconomic living conditions in which the child and the family are in. (Basbakkal, Sonmez, Celasin, & Esenay, 2010) (Peykerli, 2003).

Kids in infancy which is a vulnerable age to adverse situations, facing cancer and experiencing hospital life, can have bad effects on the child The child may experience divisive anxiety and depression in the attachment period with the idea that he/she will break apart with his/her mother. At the same time, he/she may experience regression because of the constraints caused by the disease and the stages of treatment. Regression is represented in the form of a regression of the abilities that the person acquires. Regression may appear as eating disorder in a child who is self-feeding, speech regression, repeated urinary incontinence in a child who has completed toilet training (Sezgin, Ekinci, & Okanli, Psychosocial Problems and Nursing Approaches in Childs with Cancer, 2007) (Cavusoglu, 1997).

Children of pre-school age have fears towards their body (castration complex, etc.). That is why children with cancer during this period may be afraid of the processes performed on their body for the purpose of examinations and treatment. Depending on the peculiarities of this period, children may feel guilty about the fact that the disease has happened as a result of his/her mistake. The child with guilt can return to his/her inner world and experience an introvert (Yildiz, 2019) (Peykerli, 2003).

School-age children can now establish a logical relationship of cause and effect between events. Hence their reactions are different from those of previous periods. Even if he / she understands why he / she should be staying in the hospital or taking medication, he / she may oppose therapy. Because peers and school life are of great importance for the child in this time period.

The child is seperated from his / her peers and school environment because of his / her illness, and is disturbed by this situation. He / she has concerns about having his / her friends left behind. He / she feels guilty in that respect. At the same time the child may also have the idea that he / she will be rejected by his / her friends, experiencing differences in appearance and body functions. Feeling isolated as if a wall were being constructed between him / her and them, the child starts showing distressed behaviour. As a consequence of age, the child also starts to understand the concept of death and he / she is having a difficult time dealing with it. And he / she may overreact to it one day, and he / she may turn in on him / herself the next (Oguzhan & Erden, 2012; Beytut, Bolisik, Solak, & Seyfioglu, 2009).

Throughout this period, adolescents who seek to gain independence have cancer and experience the hospital process causes adolescents to worry about restricting their independence. During this time one of the biggest concerns they have is the future anxiety. Their appearance is very important for adolescents. That's why they can worry about even the slightest negative changes in their bodies (Yildiz, 2019).

Becoming distanced from schools and peers is also a factor that causes adolescents to respond to cancer and treatment process (Baykoc, 2006).

Cancer disease causes different reactions in each individual. In general, the following reactions can be observed;

- Depression
- Anxiety
- Denial
- Anger

- Hostile manners
- Mourning reaction
- Pathological dependence
- Regression
- Aggressive attitude and resistance
- Guilt
- Fear
- Detach himself/herself from society and turn in upon himself/herself (Peykerli,2003).

4. EMOTIONAL, SOCIAL AND COGNITIVE PROBLEMS IN CHILDREN WITH CANCER

Cancer, which is one of the most likely diseases to cause psychological problems, brings psychosocial and mental problems as well as physical problems. This is due to the fact that treatment is difficult and has noticeable effects on the child (Sezgin, Ekinci, & Okanli, Psychosocial Problems and Nursing Approaches in Childs with Cancer, 2007) (Hersh & Wiener, 1993).

Many psychosocial problems have been encountered in studies relating to children with cancer and their psychosocial problems afecting their perspective on life and their life quality, in particular body perception, uncertainty of the future, decrease in self-esteem and fear of relapses. (Elcigil & Tuna, Nurse's role in the care and problems of children who ended cancer treatment, 2011)

According to the study results, there are differences between healthy children and children with cancer in dealing with events. This result has also shown that children with cancer experience a higher level of depression and anxiety than healthy children. It implies children with

cancer have lower quality of life than healthy children. (Nazari et al., 2017)

According to the results of Sezgin and Ekinci's research to compare the depression levels of cancerous and healthy children between the ages of 7-14, the average of depression scores of children with cancer depression of healthy children was found higher than their average points (Sezgin & Ekinci, Comparison of Depression Levels of Cancer and Healthy Children, 2006).

A study on Swedish children and adolescents between the ages of 8 and 18 compared the levels of depression, anxiety and self-esteem of healthy and cancer-treated children. It has been indicated that adolescents and children with cancer treatment have a higher level of depression and anxiety, lower psychological well-being and physical self-conception than healthy children (Essen, Enskar, Kreuger, Larsson, & Sjöden, 2000).

The greatest risk of treatment methods in children with acute lymphoblastic leukemia and central nervous system tumors are neurocognitive effects. It is noted that children under 6-8 years are more at risk, especially in girls (Butler, Rizzi, & Bandilla, The Effects of Childhood Cancer and Its Treatment on Two Objective Measures of Psychological Functioning, 1999). It is believed that the cause of neurocognitive deficiencies is intrathecal or systemic chemotherapy (Butler & Copeland, Neuropsychological Effects of Central Nervous System Prophylactic Treatment in Childhood Leukemia: Methodological Considerations, 1993).

These deficiencies causes neurological disorders such as motor function disorders, attention and memory problems, sens organs-related disorders such as hearing, learning disability, internal and external adaptation problems. The child experiences these problems also causes problems in his/her academic and social life

(Elcigil & Tuna, Nurse's role in the care and problems of children who ended cancer treatment, 2011).

5. REHABILITATION IN PEDIATRIC ONCOLOGY

Loss and abnormalities in the physical, anatomical or psychological structure and function are referred to as disorder. Children suffering from such cancer disorders may experience psychological and cognitive difficulties in addition to problems such as social dysfunction disorders, self-care problems, communication problems, movement restrictions. These problems and difficulties lead to a decline in the quality of their lives. Cancer rehabilitation includes all interventions aimed at achieving social, psychological, physical and professional functions in the most useful way possible for cancer patients (Michaud, Ried, & Mcmahon, 2001).

Rehabilitation of children with cancer requires a comprehensive interdisciplinary approach. By looking at various types of cancer, recovery in surviving children is a consequence of rehabilitation. This success accompanied by a number of problems faced by children. The main objective of rehabilitation is to minimize the negative effects of disease and treatment. Disability as a result of disorders may impose restrictions on the child's performance of roles in accordance with age and society.

Another goal of rehabilitation is to ensure that individuals are able to adapt easily to roles suitable to society and age by eliminating these restrictions Limitations caused by the same disorder may be different for each child. The goal of rehabilitation is to promote the functioning of the child individually in each affected area and to reduce the burden of disability In spite of the disorder efforts should always be directed at achieving

full freedom. 6 basic intervention strategies for this. These are;

a) Prevention or correction of additional secondary disability
b) Increasing functions in the affected system
c) Increasing the effectiveness of the unaffected system
d) Use of adaptive equipment to improve functions
e) Changing the social and professional environment
f) Using psychological techniques to improve the patient, patient's performance and family (Michaud, Ried, & Mcmahon, 2001).

Pediatric rehabilitation unit should consist of professional groups, such as physician, physiotherapist, rehabilitation nurse, psychologist, child development specialist, social researcher, oncologist, language and speech therapist, special educator, therapeutic recreation expert, prosthesist-orthosis specialist. In the process of treatment, the child, the family and the rehabilitation team should be in close and comprehensive communication (Wells & MacBride, 2006).

6. PSYCHOSOCIAL SITUATIONS AND REACTIONS OF PEDIATRIC ONCOLOGY PATIENTS

The process of diagnosing and treating cancer is traumatic not only for the child, but also for his / her family. Having a child with cancer as a parent is described as one of the most traumatic experiences that a family can experience. (Gibbins, Steinhardt, Beinart, 2012).

Studies have revealed that parents experience some problems in the process after the diagnosis. These are sleep problems, change in social functions, somatic problems, disturbances in body functions and negative emotions such as depression and anxiety (Sawyer, Antonıou, Toogood, Rıce, & Baghurst, 1993).

According to a study conducted by Cimete and Kuğuoğlu, while in a family with cancer child, the father experienced excessive weight loss and mother had hypertension; in other family, mother had sleep problems and repeated anxiety of loss, and the father had diabetes. In another family, it has been learned that there is anger towards all other children. In all families, it has been learned that an economic collapse caused by the disease affects them negatively (Cimete & Kuğuoğlu, 2002).

Families of pediatric oncology patients show some common reactions to the disease in this process. With the Kubler Ross's Stage Approach, it is possible to explain these reactions as follows;

The first stage is denial. Like a patient at this point, his family is shocked and denies the disease. The second phase is anger. "Why me?" question arose, and anger was directed towards to all, especially to God. The third stage is the negotiation. At this point, anger is set aside and dealt with circumstances such as being a good person once the disease passes. The next stage is depression, and at this stage the patient begins to realize that he/she is defeated by the disease and mourn deeply. Then there is a phase of acceptance, which is devoid of emotion. Although the parent at this stage now carries the desire to prolong the patient's life, he has given up the struggle and is secretly ready for anything. It is the stage where help and support are needed most. It is not possible to distinguish these stages with precise lines from each other. Parents can also experience the same stage repeatedly, according to

the psychosocial processes in which they are in and the condition of the child's illness (Zengin et al., 2012).

Families with cancer children have a high degree of emotional stress. The most important factor in experiencing feelings such as unhappiness, loneliness, fear, anxiety, sadness is that they feel helpless. The result is the inability to understand the severity of the disease, completely abstraction from social activities, not interesting in anything except illness (Barg, Pasacreta, & Nuamah, 1998) (Goldberg-Arnold, Fristad, & Gavazzi, 1999).

7. PSYCHOLOGICAL AND PSYCHIATRIC SUPPORT IN CHILDREN AND ADOLESCENTS WITH CANCER

Young children are very quickly worried about hospitalization in case of illness, leaving the family and fear of medical procedures. Because these kids are very sensitive about leaving the family and break out of their routine, and they can feel like they're being punished. Therefore, children need to be constantly visited and relieved by their parents in order to prevent them from feeling punished and abandoned. Communicating with other children who have had surgery is an important way to reduce their anxiety and help them understand that they are not alone (Lansky, List, & Ritter-Sterr, 1989, Ulutaş & Pekdoğan 2019).

Children in early childhood also have sensitivity to body integrity. That's why medical procedures on their bodies can cause them concern. For this reason, it is necessary to inform children without going into too much medical details that are appropriate for children's developmental level before the procedure is carried out (Yildiz, 2019).

Disease and hospitalization can cause regression in children. This may be the form of a regression in the behavior that children have previously acquired or starting back to the behavior they have left. It is necessary to ensure that parents understand that regression is part of children's efforts to cope with stress. Parents should not be angry with them and treat them overly intolerant for this behavior of children. Because in general, these behaviors spontaneously decrease after the acute stage of the disease. In cases where it does not decrease, professional help should be applied (Lansky, List, & Ritter-Sterr, 1989, Cosanay &Ulutas 2019).

When surgery is planned, the child needs to prepare for it. Introducing the child's room, the staff to perform the procedure and the tools to be used will help to reduce their fears. The questions asked by children must be answered in accordance with their age. Similarly, it is important to inform the child of circumstances that may occur after the procedure. The best approach for children to receive this information is 3-5 days before for 3-6 years; 1-2 weeks for 6-12 years; 12-18 years immediately after surgery decided (Cihangir Altay, 2008).

Some behavioral interventions are effective in reducing children's discomfort and anxiety rates in medical procedures. Giving positive reinforcers to the child before and during medical functions will help to reduce the fear of the child. In the same way, prizes and presents make it easier for the infant to participate for medical procedures (Cihangir Altay, 2008).

Children need to feel physically comfortable in order to have a minimum of stress levels and anxiety. Therefore, pain control must be carried out continuously and necessary interventions should be made.

School-age children with strong investigative motivation want to be aware of everything related to

diseases and methods of treatment. Often they ask difficult questions about the causes and consequences of the disease. As they learn about their own diseases, they experience the pride of it and use it effectively after they start school. Because of these characteristics, the questions asked by these children should be answered as much as they ask. Parents often refrain from informing the child about the disease. When this happens, the child can learn this information from elsewhere in a more disturbing way. This may increase the child's personal distress. Therefore, it is important for the family and doctors to answer the questions together. According to a study conducted, a few training sessions conducted by doctors with patients without parents, the connection between children with the doctor is strengthened and the process to be performed on their body and it is concluded that in the treatment the child shows more consent (Lansky, List, & Ritter-Sterr, 1989).

School-aged children are more concerned about privacy than pre-school children. The privacy of the child must therefore should be respected in all interventions.

It creates specific problems for adolescents to get sick during the transition from childhood to adulthood. The major concerns of teenagers during this time are independence, appearance, sexuality, acceptance and future plans. Their efforts against autonomy and self-determination are inevitably threatened by illness and hospitalization. Forced addiction, loss of control and compliance problems are associated with disease and treatment. Because of these reasons, every teenager may experience anxiety and stress in different ways at different times (Ettinger & Heiney, 1991).

In the process of treatment, pediatric centers should be used as much as possible rather than adult centers. Because these centers are typically equipped

to pay attention to the psychosocial and developmental characteristics of adolescents. The most important thing that adolescents desire during the course of treatment is understandable explanations and a reassuring approach to possible treatments. At this point it is important to allow and encourage the adolescent to participate in medical decisions as much as possible. Adolescents within the limits can be given control in the programming of their treatments. Especially in adolescents of older age, this condition will help to control anxiety and stress. When the adolescent is ready, individual and group therapies should also be started (Lansky, List, & Ritter-Sterr, 1989).

8. PSYCHOLOGICAL AND PSYCHOSOCIAL SUPPORT FOR FAMILIES IN PEDIATRIC ONCOLOGY

The diagnosis of cancer has rapid changes and a lasting effect not only in the child, but also in the family. Families face many different stressors throughout the disease. In this process, they must both control their stress and endure the change. The need to quit control and the need to keep up with the medical environment requires great support for the family (Kerr, Harrison, Medves, & Tranmer, 2004).

How the diagnosis of cancer is reported to parents and children affects the harmony, confidence and cooperation of the child and family in the process of treatment. This form of reporting should be made according to the needs of the patient, the family and the condition (Hersh & Wiener, 2001).

It is argued that parents communicate with the child about cancer also has a positive effect on the child's adaptation to the disease and treatment (Last, Veldhuizen, & A, 1996). In families where cancer communication is more likely, children have less defense and psychological distress; more social competence and self-sufficiency have

been found. (Varni, KAtz, Colegrove, & Dolgin, 1996). But some families think they have to protect themselves from the fear and suffering of their children, and see the diagnosis of cancer as a threat to their protective personality traits. In this process, families who also experience feelings such as guilt, despair, denial, anger, fear may have difficulty accepting and rejecting the diagnosis. (Goldberg-Arnold, Fristad, & Gavazzi, 1999). For this reason, especially parents should be supported to accept the diagnosis when they first learn it and share it with the child. Telling the child about the diagnosis should be in accordance with the developmental characteristics of the child and honestly.

Children feel the fear, anxiety and stresses of their family and are negatively affected by it. Therefore, psychological and psychosocial support should be applied in the process of illness, not only the sick child, but all of the family members.

In recent years, health professionals have become aware of the complexity of caring for children and family, who face cancer. No health professional alone is able to fully meet the needs of the family. In a multidysplinary team work, it is important to implement appropriate interventions, trying to predict the psychological state of families throughout the course of the disease. The basis of these interventions lies in identifying the strengths and weaknesses of families. After these are determined, sessions are organized at certain intervals and the information is synthesized. Sessions should preferably be conducted by a mental health specialist from competent members of the rehabilitation team. The sessions should be conducted in a way that will comfort the family as much as possible and in a form of communication that is not threatening. At the same time during the sessions, the family should be told how to support the child, according to the characteristics of the child's development (Hersh & Wiener, 2001).

9. SCHOOL LIFE OF PEDIATRIC ONCOLOGY PATIENTS AND PROBLEMS RELATED TO EDUCATION

The school is an environment that meets the needs of the child in terms of achievement, socialization and self-identification as an important member of society (Davis, 1989). A successful school life is an important factor for the cancer child to feel normal. According to studies, children with cancer experience some difficulties in school life depending on the disease and treatment. These children who are at risk in terms of school phobia and adaptation problems with the effect of increased absenteeism and treatment, difficulties in cognitive function, weak academic achievement, learning disabilities. problems begin to appear (Bessell, 2001).

The fact that children miss a significant amount of school during the treatment process has significant consequences in academic and social relations. In the process of treatment, children with cancer are subjected to treatments that threaten the central nervous system, such as chemotherapy and radiotherapy. It is known that these treatments negatively affect the central nervous system of children. Children 5 years and under are more susceptible to this disease and treatment. A general decrease in cognitive functions of some children is observed, while others have deficiencies in attention, concentration and mathematical functions (Wallace, et al., 2001).

Some studies have shown that the use of radiotrapia and interectal methods limits the cognitive development of the child. It has been determined that close to 50% of children who are treated with chronic irradiation needed special education (Hill et al., 1998).

The physical symptoms caused by cancer and its treatment in children can be effective in the child's

attendance to school. Examples of these symptoms include pain, fatigue, weakness, nausea, vomiting, insomnia, sores in the mouth and lack of appetite influenced by swallowing disorders. At the same time, due to limitation of the movement of the child caused by problems with extiryremia, depending on the location of the tumor, and the risk of getting infection due to suppression of the immune system also causes the child to absent to school. The thought of retracting from friends due to physical insufficiency that develops due to illness is a reason why the child does not want to go to school by causing low self-perception (Collins, and others, 2002) (Patenaude & Kupst, 2005).

The literature shows that many long-term effects occur as a result of treatment. As a result of the effects such as neurocognitive disorders, organ damage, delay in growth, infertility, as well as the visible effects such as weight loss, amputation and hair loss, the child may think that he or she will be excluded by his or her friends and at the same time will not be able to keep up with his / her friends due to fatigue caused by the disease. (Chekryn, Deegan, & Reid, 1986).

Healthy children may have a lack of information related to the disease. So they may think that cancer is contagious, and because of physical insufficiency, they might want to stay away from their cancerous friends. In this case, the cancer child feels more stressed and can experience social isolation (Prevatt, Heffer, & Lowe, 2000).

Researchers have found that children with cancer have increased risk-taking behaviors, anxiety levels and feelings of anger, aggression, depression, helplessness. (Lawson, 1977). School life helps children overcome emotional difficulties by moving away from the role of patient. Therefore, long-term hospitalization and

treatment due to the concerns of children to return to school after as hard as it is, and to provide hope for the future will help you gain a sense of control in their own lives (Lansky, Cairns, & Zwartjes, 1983).

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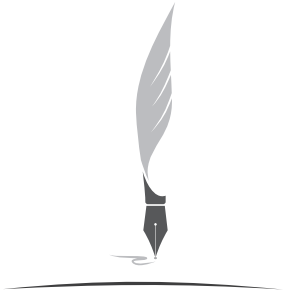

Chapter 5

TREATMENT OF VITILIGO DISEASE

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INTRODUCTION

Vitiligo is a white skin disease of different shapes and number of skins [1] (Figure 1). This disease is also called white spot disease, leukoderma and white leprosy [2]. It develops due to melanin pigment and melanocyte loss in the skin and manifests itself with depigmented macules [3]. Vitiligo may also be congenital. Although the disease has no effect on mortality and physical morbidity, it is an asymptomatic disease. However, depigmentation does not look nice on the skin and may cause psychological problems, especially in people with dark skin color [4]. The most prominent sites of vitiligo are the face, dorsal parts of the hands, nipples, axilla, navel, sacrum, inguinal and anogenital regions [5] [6].

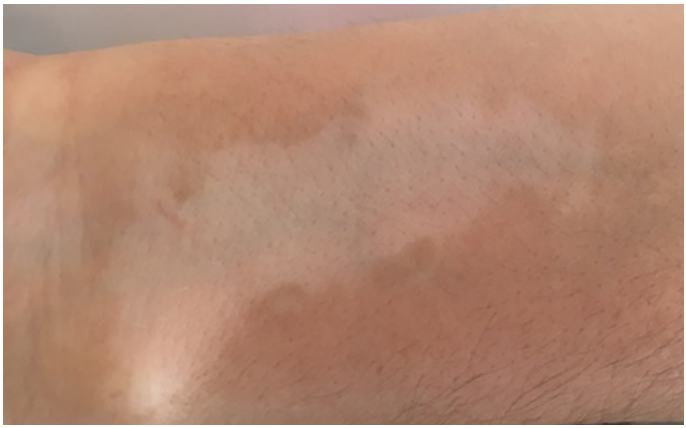


Figure 1. *Depigmented macules in vitiligo.*

1. Classification of Vitiligo

Vitiligo has different classifications It was classified in two different ways according to the distribution of images and lesions and the current classification method.

1.1. Classification according to image and distribution of lesions

a.Acrophacial vitiligofasiyal

Acrophacial vitiligo distributions are symmetrical lesions. Mucocutaneous junction areas such as face, hands, feet and lips are seen at the end of the body.

b.Vitiligo vulgaris

Vitiligo vulgaris can be randomly distributed to different parts of the body.

c.Dermatomal vitiligo

The lesions in dermatomal vitiligo are mostly concentrated in a single area of the body.

d.Common vitiligo

Generally, the lesions are prominent on the bone protrusions and can be found symmetrically or asymmetrically in the body (Figure 2).

e.Halo nevus / Sutton nevus

Unlike others, this type is characterized by a hypo or depigmented ring, oval or circular, markedly delimited around a pigmented stain [7].

1.2. Current classification

a.Localized vitiligo

It is divided into two as focal and segmental vitiligo.

1. Focal vitiligo

In focal vitiligo, there are one or more vitiligo macules locally.

2. Segmental vitiligo

There are single or multiple vitiligo macules as dermatomal.

b. Common vitiligo

It is divided into two as acrophacial and vitiligo vulgaris.

1. Acrophacial vitiligo

Too many lesions are seen on the face and distal parts of the extremities.

2. Vitiligo vulgaris

Symmetrical or asymmetrically distributed lesions.

c. Universal vitiligo

The whole body was involved with vitiligo macules.

d.Mix Form

Segmental and vulgaris or segmental and acrophacial forms coexist [8].

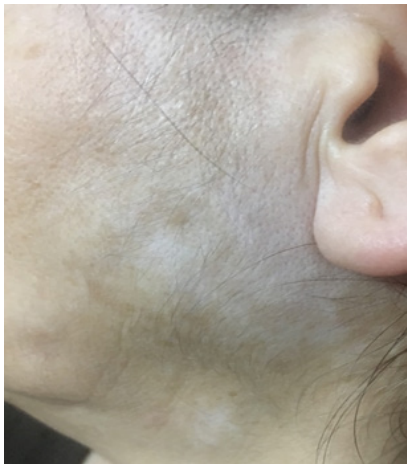


Figure 2. *Symmetrically located vitiliginous macules on the extensor surfaces of the extremities*

2. Genetics of Vitiligo

The familial predisposition of vitiligo was revealed in 1933 and there have been many studies on the genetic factors that cause the disease. In a study, the incidence of vitiligo among first degree relatives was found to be 11.5%. The average age at onset of vitiligo patients of the Caucasian race is 24, which is another proof that generalized vitiligo is genetic. In the largest study of vitiligo with twins performed to date, the association for generalized vitiligo in monozygotic twins was found to be 23%. This is much greater than the risk of vitiligo in siblings of vitiligo patients in the general population. These results support the effect of genetic factors in the disease. This means that genetic factors and environmental factors also play an important role. Perhaps environmental factors may even play a more important role than genetic factors. Some genes in the human leukocyte antigen region are known to be associated with vitiligo. T-cell receptor, T-cell receptor signaling, T-cell activation, innate immune response, and genome-wide association of chemokine / cytokine receptors have been found to be associated with vitiligo.In addition, melanocyte cells were associated with vitiligo. Melanocytes play a role in regulation of cell death and apoptosis during oxidative stress [9] [10].

3. Clinical Outlook and Course of Disease

Asymptomatic lesions in vitiligo are most common in the face during childhood [11]. Lesion sizes are variable and spread over time to the environment [12]. In vitiligo, depigmented regions are well delimited and are usually surrounded by a hyperpigmented ring. Limits can sometimes become swollen as a result of weak inflammation. This condition is called jinal marginal inflammatory vitiligo. This condition is called jinal marginal inflammatory vitiligo. Flat lesions in the affected area of vitiligo are seen as dark without pigmentation

[13]. The boundaries are fully visible and irregular. Lesions may disappear spontaneously in a short time or may disappear with total depigmentation within a few weeks. In the course of the disease, vitiligo macules in general and the formation of new macules can be seen. Single, sharp-bound macules are more likely to disappear spontaneously. The presence of lesions located on the face, neck, chest, arms and legs are prognostic factors in vitiligo. Poor prognostic factors include widespread lesions, mucosal involvement of vitiligo, and macules on the fingers and toes [14-15].

Sometimes itching may occur on the skin with vitiligo lesions. Lesions may cause erythematous and painful conditions when exposed to sunburn. On the lesions with vitiligo, the hairs become whitened and this is called leukotrichy (Figure 3). Hypopigmented and depigmented lesions predominate in areas exposed to the sun and symmetrically above the bone protrusions. Lesions are often seen in body openings such as the nose and mouth [16].

Vitiligo is usually insidious onset and can be noticed after tanning in summer. The natural course of the disease is often unpredictable. Especially considering that the face is exposed to the sun, the protection of this area and the continuous use of sunscreen creams may make the treatment more effective [17-18]. Both sun protection factor (SPF) 30 and above, as well as products containing zinc oxide and titanium oxide can be used for this purpose [19].

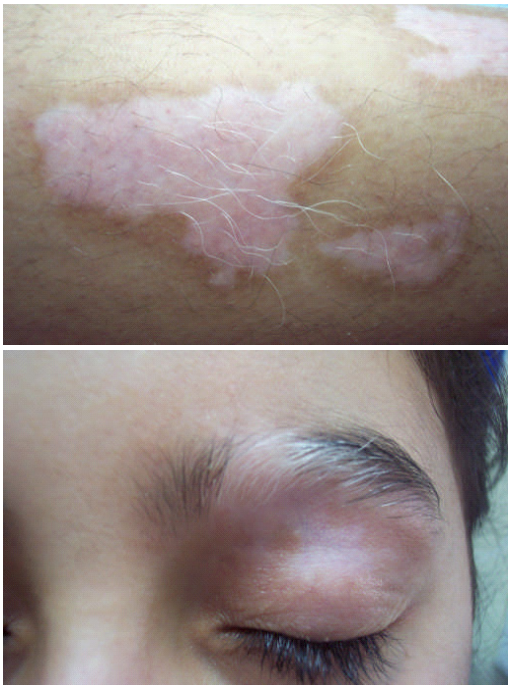


Figure 3. *White hairs on vitiligo [20]*

4. Epidemiology of Vitiligo

The incidence of vitiligo epidemiology was found to be 0.14-8.8%. It is generally accepted that the incidence is around 1-2% in the world [21]. Vitiligo is more common in young adults and women. It is most commonly seen in the 10-30 age range. 1% of the population of the United States of vitiligo, the 0.36% in Denmark, 0.33% of Libya, Turkey was determined to affect 0.15% [22-23].

5. Pathogenesis in Vitiligo

The histopathology of Vitiligo was first described by Brocq and Kaposi in the nineteenth century. Kaposi reported the disappearance of pigment granules in the basal layer cells of the epidermis with vitiligo. They also

reported acromia and hyperpigmentation at the margins of vitiligo lesions [24].

The etiology of vitiligo has not been fully elucidated. However, genetic factors, autoimmunity, viral infections, oxidative stress and toxic metabolites are thought to be responsible for the pathogenesis of the disease [25]. Factors such as sunburn, fungal, bacterial infections, recurrent trauma, and emotional stress trigger the disease [26].

6. Normal function of melanocytes

Melanin is a pigment that absorbs ultraviolet (UV) directly. It plays an important role in protection from the harmful effects of sun rays. In healthy body skin, melanin synthesis is carried out by melanocytes located in the basal layer. Melanin is synthesized primarily from hydroxy phenylalanine or tyrosine as the amino acid. The main enzyme of this synthesis is tyrosinase. Melanin synthesis occurs in specific organelles called melanosomes. These chain reactions in melanosomes are called "Raper Mason Road ". Melanozomlarda gerçekleşen bu zincir reaksiyonlarına "Raper Mason Yolu" denir. Melanocytes transfer the melanin produced by their dendritic extensions to keratinocytes. In a healthy adult, approximately 3-5% of epidermal cells (more dense in sun-exposed areas) are composed of melanocytes. The number of melanosomes and melanin content in the melanosomes are associated with the difference in skin color [27].

Slorninski et al. emphasized the possible role of melanocyte receptors among the causes of Vitiligo. According to this hypothesis, the destruction of melanocytes in vitiligo begins with the disruption of melanogenesis order. These reactions result in highly uncontrolled free radicals and toxic melanogenesis products. They destroy melanocytes and keratinocytes. These devastated keratinocytes and melanocytes cause an autoimmune response in the body because they act as

antigens [28]. Pigment cells are normally found outside the skin, inner ear, intraocular, brain, and other organs. Therefore, mechanisms that cause melanocyte loss may also affect other organs containing melanocytes [29-34].

7. Etiology of Vitiligo

There is no clear precise information on the occurrence of vitiiligo disease. However, three theories have been proposed, such as autoimmune theory, neural theory, and melanocyte self-destruction theory [35].

a. autoimmune theory: The detection of vitiligo disease in patients with autoimmune disorder [36].

b. neural theory: According to neural theory, vitiligo disease leads to a neurochemical mediator that selectively destroys melanocytes at the nerve endings. Segmental dermatomal forms of vitiligo support this theory.

c. melanocyte self-destruction theory: Melanocytes have been suggested to self-destruct against cytotoxic melanin precursors that cause vitiligo [37].

The most valid of these theories is melanocyte self-destruction theory [38]. The reason for the acceptance of this theory is the presence of reactive T lymphocytes and antibodies against melanocytes in vitiligo patients. At the same time, vitiligo may coexist with other autoimmune diseases and respond to immunomodulatory therapies [39]. Other hypotheses related to the formation of vitiligo are based on langerhans cells, free radicals, skin lymphoma, melanocyte growth factors, melatonin receptor, viral disease and apoptosis [40-52].

8. Treatment in Vitiligo

Although there are many treatment options in vitiligo, all treatment options have advantages and disadvantages. Treatment of vitiligo should be individualized [53].

Because vitiligo treatment varies according to the prevalence of the lesions, localization, patient age, skin type and response to previous treatments. In the treatment of vitiligo, it is important to inform the patients, talk to the patients about the prognosis of vitiligo and explain the treatment options with their advantages and disadvantages.

Vitiligo treatment takes a long time. Response to treatments before 3 months is not evaluated and this affects the compliance of patients to treatment. The aim of the treatment of the disease is: (1) to stop depigmentation and (2) to stimulate repigmentation. Phototherapy, topical treatments and surgery are used in the treatment. Repigmentation can occur spontaneously with treatment. Repigmentation is achieved by re-collecting melanocytes from hair follicles or surrounding normal skin to depigmented skin [54]. Short-term systemic steroids in vitiligo are used to suppress the disease activity and concealer camouflage methods are used to increase the cosmetic acceptability of the disease.

Although it is easy to diagnose vitiligo, treatment is a disease that requires patience. It is always difficult to treat a patient with vitiligo. Recently, the introduction of calcineurin inhibitors, targeted NB-UVB and surgical methods in the treatment of vitiligo and successful results have started a new era. However, further elaboration of the mechanisms in the pathophysiology of the disease will shed more light on other studies.

Surgical interventions in the treatment of vitiligo may be considered as the last option in vitiligo with poor response to treatment and non-progressive vitiligo. Depigmenting agents (Monobenzylether hydroquinone, Ruby laser) are optionally applied to the remaining normal skin in patients with very common, stubborn and progressive lesions who cannot produce repigmentation [55].Vitiligo is a disease that progresses

without being stable. Sometimes it can be seen in latent periods and rarely self-healing may occur in some of the patients. Unfortunately, patients with vitiligo may also require psychotropic drug therapy and psychotherapy. Dermatologist and psychiatrist cooperation is needed in this disease [56].

The role of nutrition as a support for vitiligo treatment is also very important. This event has been noticed with the development of vitiligo in many developing countries in malnourished children. Nutrition is very important during the initial propagation period of vitiligo. Therefore, conservative treatment with the necessary vitamins, trace elements and protein sources can improve the course of the disease, especially in children. Copper, vitamin B12, folic acid, zinc, manganese, nickel, cobalt, calcium, iron, ascorbic acid, and alfatocopherol may affect the pigmentation process as nutritional therapy [57]. Oral and topical corticosteroids, ACTH, levamisole, cyclophosphamide, cyclosporine, topical fluorouracil, human placental extract, polypodium leukotomes can be used from immunomodulatory-immunosuppressive agents.

The treatment approach in vitiligo can be summarized as follows;

1.Topical treatment

- Corticosteroids

- Calcipotriol

- Tacrolimus

- Pimecrolimus

- Phototherapy / Photochemotherapy (narrow band UVB, PUVA)

- Other: prostaglandin E2 analogue, placental extract (melagenina), nitrogen

mustard, 5-fluorouracil

2. Systemic treatment

- Corticosteroids

- Phototherapy / Photochemotherapy

- Immunomodulators: Levamisole, vitamins, trace elements

- Immunosuppressives: Cyclophosphamide, azathioprine, cyclosporine

3.Surgical treatment

- Grafting techniques: Autologous skin grafts, cellular grafts (cultured or not)

- Lasers: Excimer laser and Helium-Neon laser

- Micropigmentation

8.1. Topical treatments

8.1.1. *Corticosteroids*

Depending on the duration of the disease, the localization and extent of the lesions, weak, moderate-acting or potent preparations may be selected [58]. Corticosteroid drugs are used in the treatment of vitiligo in both adults and children. The success rate in this regard is between 45-60%. However, long-term use of corticosteroids has side effects. There are risks such as epidermal atrophy, telangiectasia, striae, glaucoma and systemic absorption. To eliminate these side effects, it is more reasonable to start with strong steroids and continue with weaker ones. Topical corticosteroids may be the first-line therapeutic in patients with vitiligo spreading to less than 10% of the body surface. Strong corticosteroids are usually administered once a day for 3-4 months [59-60].

8.1.2. *Calcineurin inhibitors*

Calcineurin is an enzyme that activates calcium. Separates phosphate groups from target proteins. Cytokines are a good target for immunosuppression. They also play a major role in transcriptional activation. Tacrolimus and pimecrolimus are calcineurin inhibitors and are thought to act by suppressing cytotoxic T lymphocyte reactions to melanocytes in the treatment of vitiligo. The effects of topical steroids and calcineurin inhibitors are known to be equivalent. They do not show side effects as well as topical steroids. Calcineurin inhibitors have side effects such as transient pruritus, burning sensation, and erythema [61].

8.1.2.1. *Tacrolimus*

Tacrolimus is an important drug used as an alternative to topical steroids for facial and neck lesions of pediatric vitiligo patients [62] (Figure 4).

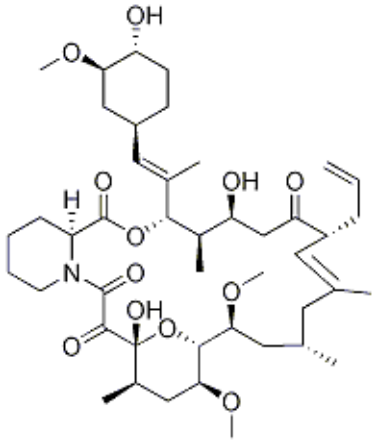


Figure 4. *Structure of tacrolimus [62].*

Pharmacological properties of tacrolimus

Tacrolimus is an immunosuppressive drug belonging to the macrolide lactone group such as cyclosporine,

rapamycin and ascomycin. It was first obtained from a fungus called Streptomyces Tsukubaensis in 1984. It is used to prevent tissue rejection in organ transplant patients. In recent studies, topical and systemic tacrolimus has been shown to be effective in the treatment of inflammatory skin diseases.

Mechanism

Tacrolimus shows its therapeutic effect by inducing repigmentation and inhibiting T cell activation by proinflammatory cytokine production and release [63]. It is not clear how tacrolimus exerts its immunosuppressive effect. However, it is believed that by inhibiting calcineurin, it inhibits antigen-specific T cell activation and the release of inflammatory cytokines such as interleukin-2 (IL-2), IL-4, IL-5.

Tacrolimus binds to tacrolimus binding protein after crossing the cell membrane. Tacrolimus binding protein is a cytolosic protein and is commonly found in T lymphocytes. Tacrolimus and tacrolimus binding protein form a complex. This complex inhibits calcineurin away from phosphate active sites. Calcinein physically inhibits transcription factor interaction to prevent dephosphorylation. This inhibits the release and synthesis of cytokines and IL-2.

The ability of tacrolimus to reduce IL-2 synthesis is almost 100 times greater than cyclosporine. When administered topically, the drug acts on basophils, mast cells and T cells and dendritic cells. Skin atrophy with topical steroids does not occur with tacrolimus because calcineurin does not play a role in collagen synthesis.

Pharmacokinetics and metabolism

Tacrolimus can be administered orally, topically and intravenously. The absorption rate of the drug after

oral administration is variable and is between 13% and 23%. Therefore, the oral dose should be administered 3-4 times a day to achieve blood concentration similar to intravenous administration. Intake with food also affects absorption. Absorption is reduced when taken with fatty foods. No dose reduction is required in mild hepatic and renal insufficiency. The physical state of the skin changes the absorption when applied topically. Topical application of the drug to healthy skin does not cause absorption and is not detected in the blood. It is absorbed very little when applied topically to intact skin. Absorption from damaged tissue is 7 times higher than intact tissue.

8.1.2.2. *Pimecrolimus*

Pimecrolimus has been reported to achieve repigmentation between 26-60% in mixed study groups of adult and pediatric patients. When pimecrolimus and mometasone were compared in children with localized vitiligo, both drugs were found to be equally effective.

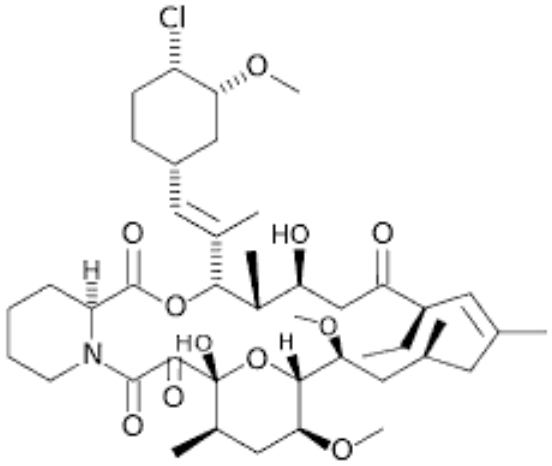


Figure 5. S*tructure of pimecrolimus [63].*

Pharmacological properties of pimecrolimus

It is a selective inhibitor of pimecrolimus, an antiinflammatory ascomycin macrolactam derivative. Pimecrolimus allows the production of pro-inflammatory cytokines and mediators in T cells and mast cells. With a high affinity, Pimecrolimus binds to macrophilin-12 and inhibits calcium-dependent phosphatase calcineurin.

8.2. Analogs of vitamin D

Vitamin D receptors are thought to be effective in the treatment of vitiligo by immunomodulation and melanogenesis stimulation. It has been observed that 10 of 18 children with vitiligo alone had moderate level and 10 of 12 children with topical steroid combined with repigmentation. No significant side effects other than mild irritation were observed [64].

8.3. Pseudocatalase

Combined treatment with topical activation of pseudocatalase with low dose narrowband-UVB has been successful. Because oxidative stress plays an important role in the etiology of vitiligo, pseudocatalase eliminates epidermal H_2O_2 in treatment. It has been reported that more than 75% repigmentation of face and neck lesions was achieved in 66 of 71 patients, in 48 of 61 body trunk lesions and in 40 of 55 patients with limb lesions by applying this combined treatment in pediatric vitiligo cases for 8-12 months [65].

9. Treatment approaches other than pharmacotherapy

A. Biofeedback Therapy: Biofeedback is used, such as training to control muscle tension, blood flow and temperature. In addition to decreased muscle tension and increased blood flow, relaxation feedback is achieved

through biofeedback training. This treatment method allows the patient to recognize and soothe his or her autonomic symptoms, increase the feeling of relaxation, feel better, reduce symptoms and increase the patient's sense of physical control.

B. Relaxation training: The aim of this training is to increase parasympathetic activity and minimize sympathetic activity. This treatment approach includes progressive muscle relaxation, self-education and imagination. Other approaches to relaxation and meditation (breathing exercises, self-talk, and others) are also used [66-67].

C. Hypnosis: Provides psychological relief to the patient with hypnosis [68].

D. Psychotherapy: Patients generally do not want to go to a psychiatrist. Even psychiatric intervention alone can initiate recovery. Such patients may need longer-term and supportive psychotherapy. In some psychotherapeutic approaches, it was seen that the patient's recollection of the events and emotions that he had experienced and suppressed previously caused the loss of skin symptoms [69].

E. At the same time, selective serotonin reuptake inhibitors (SSGI) have been found to be beneficial for psychiatric syndromes frequently encountered in skin disorders [70].

10. Photochemotherapy treatment approach

It is the mainstay in the treatment of vitiligo by exposure to sunlight alone or by oral or topical applications of photo sensitizers. PUVA treatment refers to the use of psoralen combined with UVA light (figure 6). The most commonly used psoralens are 8-methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP, bergapten) and

trioxalene (4,5,8-trimethoxipsoralen, TMP). Trioxsalen is no longer commercially available. Psoralens can be administered either topically (topical PUVA) or orally (oral PUVA) [71]. Repigmentation with 5-MOP yields similar response rates to 8-MOP. 5-MOP seems to be more suitable for the treatment of vitiligo. The response to psoralen UVA treatment in children is better than in adults.

Figure 6. *Puva unit shape [71].*

10.1. *Topical puva treatment*

Treatment with topical administration of psoralen is an appropriate treatment option for vitiligo cases with limited lesions involving less than 20% of the body.

10.1.1. *Application of topical puva treatment*

Apply 0.05-0.1% 8-MOP to the vitiligo-affected area, after 30 minutes the patient is exposed to the source of artifical UVA at an appropriate distance. The duration of the first exposure is approximately 30 seconds, which is

gradually increased by 15-30 seconds to reach a period of 10 minutes. This application is done 2-3 times a week. In this treatment, normal skin and eyes should be protected. The affected part is then washed with soap and water and a sunscreen cream is applied to protect it from sunlight. Side effects include local bullae formation, erythema, pruritus, perilesional hyperpigmentation and pain. The advantage of this treatment is the absence of systemic or ocular toxicity due to a decrease in total UVA dose [72].

10.2. *Systemic puva treatment*

Systemic PUVA treatment is applied in patients who are resistant to topical PUVA treatment and the distribution rate of lesions is above 20%.

10.2.1. *Application of systemic puva treatment*

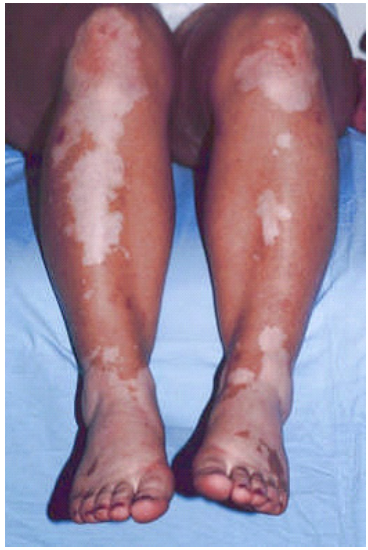
In this treatment, the most commonly used 8-MOP (0.4-0.6 mg / kg) is taken orally 1.5-2 hours after UVA application. The initial dose for patients with vitiligo is 0.5-1.0 j / cm^2. The dose is gradually increased until asymptomatic minimal erythema of the affected skin occurs. This application is done in 3-4 sessions per week.

The molecular effects of psoralens in vitiligo are not known. PUVA stimulates the proliferation of melanocytes and provides proliferation of the remaining reserve follicular melanocytes in vitiligo lesions. In addition, similar changes occur in the border of the pigment around the lesion. Repigmentation occurs as a result of proliferation of stimulated melanocytes and the passage of melanocytes into the depigmented epidermis [73]. UV radiation, as well as factors with anti-inflammatory and immunosuppressive effects; it also regulates the production and function of cell-surface molecules, including adhesion molecules ICAM-1, cytokine

receptors (IL-R 1 and 2) and growth factor receptors. UV also induces apoptosis of skin-infiltrating T cells [74].

The main factors affecting the repigmentation of PUVA are patient age, motivation, skin type, severity of the disease, localization, number of sessions and maintenance of the dose at the appropriate dose. Perifollicular repigmentation pattern is observed predominantly in treatment with psoralens, while oral and topical corticosteroids form a diffuse repigmentation pattern. In addition, the rate of repigmentation is higher in the treatment with oral topical corticosteroids. Although diffusion repigmentation with steroids occurs rapidly, its effect is short-lived since it is not over the melanocyte reservoir. It is suggested that the lesion is converted to depigment again with the removal of the stimulus.

In conclusion, since the rate and stability of repigmentation in vitiligo is related to the treatment method, the most optimal result can be achieved by combining different methods (Figure 7).



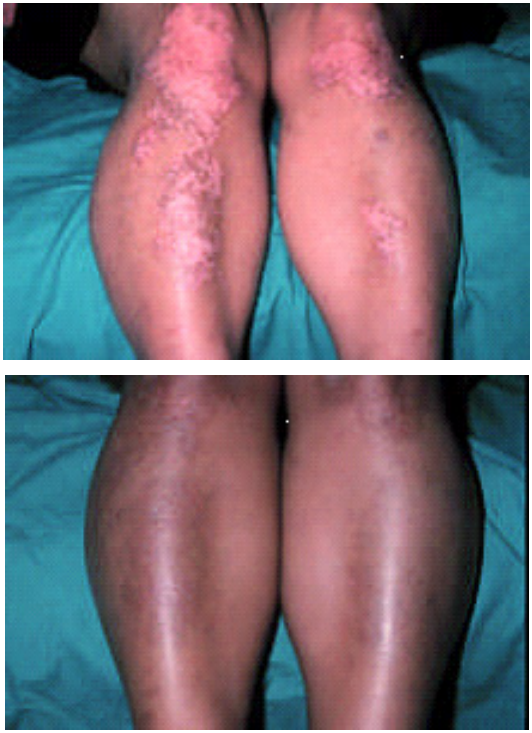


Figure 7.*A case of vitiligo with significant repigmentation of systemic PUVA before, during and after treatment [75].*

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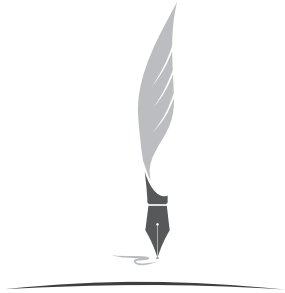

Chapter 6

MYOCARDIAL PERFORMANCE INDEX IN LATENT RHEUMATIC HEART DISEASE OF CHILDREN

Şeyma KAYALI

INTRODUCTION

Rheumatic heart disease (RHD) is the most common cause of acquired heart disease and still a serious public health problem in developing countries (1,2). RHD development is leaded by an attack of acute rheumatic fever (ARF) with cardiac involvement. However,almost 50% of RHD cases do not have an ARF history because of the silent progression of carditis and termed as latent RHD (3). Latent RHD diagnosis is a great challenge for developing countries and echocardiography is reported as the most sensitive tool in detecting subclinical RHD by several publications (4-6). In addition, World Heart Federation (WHF) published and updated an echo based guide for identification of these cases in 2012 and determined the terms of 'borderline' and 'definite RHD'(7).

Although, conventional echocardigraphy including M Mode and Doppler examinations provides the RHD diagnosis, determination of valvuler damage severity and systolic functions, it is not sufficient to reveal myocardial damage due to rheumatic cardiac involvement. Tissue Doppler imaging (TDI) is a feasible and reproducible tecnique which helps to measure cardiac deformation by assessing diastolic functions (8,9). Myocardial performance index derived from TDI is an index reflects left ventricle systolic and diastolic functions together and has a prognostic value in various heart diseases (10-12).

In the literature, several studies reported myocardial dysfunction by using TDI in acute rheumatic carditis, however, the data about myocardial deformation in latent RHD is still scarce especially at pediatric age (13,14).

We aimed to evaluate myocardial injury and ventriculer functions in subclinical RHD by TDI derived indexes and calculation of MPI.

Material- Methods:

Participants and diagnosis

A total of 40 patients with the diagnosis of latent RHD under the age of 18 were enrolled the study group in one year period and divided in to two groups as borderline and definite RHD. The control group was consisted from 22 gender and age matched healthy children.

Echocardiographic evidence and absence of ARF history were required for the establishment of latent RHD diagnosis. Pathological valve insufficiency was defined according to the criteria of WHF in 2012. Rheumatic mitral valve insufficiency definition should have the criteria of insufficiency jet which were seen in at least two sections with at least 2 cm jet length and at least 3 m/s systolic peak flow velocity. Rheumatic aortic valve insuffiency was defined as insufficiency jet which were seen at least two different sections, at least 1 cm jet length and >3 m/s diastolic flow rate (7).

The determination of subgroups including borderline and definite RHD was also made according to the criteria of WHF which describes valvular morphogical changes. Pathological valve regurgitation as well as at least two morphological criteria such as prolapse, thickening of anterior leaflet..etc for mitral valve and coaptation defect, reduced leaflet mobility… etc for aortic valve were assessed as definite RHD (7).

Pathological valve insuffiency in the absence of morphological changes or at least two rheumatic morphological changes without pathologic valve insuffiency was assessed as borderline RHD.

Demographic data, weight, height, body surface area, heart rate, systolic and diastolic blood pressure were recorded. Children who had other comorbid diseases

and /or taking regular medications were excluded from the study.

Study group participants were also asked for penicilin prophylaxis and duration of clinical follow up.

The present study was approved by scientific committee of Keciören Training and Research Hospital and performed in accordance with the Declaration of Helsinki.

Echocardiography:

All study participants underwent a standard echocardiography including two dimensional, M Mode and colour Doppler examinations and also tissue Doppler imaging at the same time while they were in supine or left lateral decubitis, rest position. Echocardiographic measurements were perfomed with Vivid 3 (General electric Medical Systems, Milwauke, USA) by the aid of 3 mHz transducer according to recommedations of American Society of Echocardiography (15). All images were recorded in the echocardiography device memory for later review and all measurements were made over three consecutive heart beats and averaged results were presented.

Conventional echocardiography:

In the parasternal long axis position, interventriculer septum, left ventricular posterior wall thickness, left ventricular (LV) dimensions at the end of systole and diastole were measured by M-mode method. Ejection fraction(EF) and fractional shortening (FS) showing left ventricular systolic functions were calculated according to these data by using following formulas;(15)

$$\text{EF: } \frac{\text{LV end diastolic volüme} - \text{LV end systolic volüme}}{\text{LV end diastolic volüme}} * 100$$

$$FS: \frac{\text{LV end diastolic dimension} - \text{LV end systolic dimension.}}{\text{LV end diastolic dimension}} * 100$$

Valve insuffiency were evaluated in apical four chamber, apical five chamber and parasternal long axis views by color Doppler echocardiography.

The diastolic flow velocity of LV were obtained in the apical four chamber view by placing the sample volümeon the tip of mitral valve leaflets by pulse Doppler echocardiography. Diastolic early filling peak velocity (E), diastolic late filling peak velocity (A) and their ratio to each other (E/A) were also measured(16).

Tissue Doppler echocardiography:

Records were obtained by color Doppler and pulsed tissue Doppler tecnique in apical four chamber view in tissue Doppler echocardiography. The sample volume were adjusted as 2-5 mm length and placed at the junction of the LV posterior wall and mitral annulus. Doppler beams were aligned as closely parallel as possible to the myocardial segment to optimize signal quality and the angle between Doppler beam and longitidunal movement of LV was kept as small as possible.

We measured the early myocardial peak velocity (E'), atrial systolic peak velocity (A'), systolic myocardial wave velocity (S) and time intervals including isovolumetric contraction time (IVCT) from the end of A'wave to the onset of S wave, isovolumetric relaxation time (IVRT) from the end of S wave to the beginning of S wave, ejection time (ET) from the beginning to end of S wave. Decelaration time (DT) was also measured from the early myocardial peak velocity(16,17).

Myocardial performance (MPI) index were calculated according to Formula (18):

$$\frac{IVRT + IVCT}{ET}$$

Statistical analysis:

Statistical analysis were made by SPSS version 21 software(SPSS Inc., Chicago, IL, USA).). Results were expressed as mean± standart deviation (SD). The differences between two groups were evaluated by Pearson chi square for categorical variables and Student's t test for continous variables. One way ANOVA test was used in comparison of groups more than two. Post Hoc test (Tukey-HSD) was used as secondary test in order to determine the different group when a significant difference found according to test of One way ANOVA. To estimate correlations between parameters, Pearson's correlation analysis was used. A p value <0.05 was accepted as statistically significant.

Results:

Of the 40 patients, 19 (47.5%) were determined as definite RHD and 21(52.5%) as borderline RHD. There was no statistical difference between study and control group in terms of demographic features, anthropoemetric measurements and clinical examinations including blood pressure and heart rate (Table 1).

There were only two patients with valvuler regurgitation of grade two or more in definite RHD group, the others in study group had valvar regurgitation of less than grade 2. The characteristic features of valvuler involvement at the time of evaluation were summarized in Table 2.

There were no differences in terms of M Mode measurements between groups. Marginally significant decreasing in E/A ratio were present in study group by pulsed wave Doppler echocardiography (Table 3).

There were statistically significant differences in terms of TDI results including IVRT and MPI (Table 4). Other parameters of LV were not significantly different.

In the study group, no correlation was found between TDI results and follow up interval. However, statistically significant positive correlation was determined between the degree of mitral insuffiency and mitral E, E/A and E/E' ($p<0.05$).

Discussion:

It is a known fact that RHD is a disease of the endocardium and valves so does not affect myocardium. Indeed, myocardial involvement may occur in the first episode of carditis even if it is silent (19). Especially in developing countries as endemic regions, repeated streptococcal infections could cause persistan and/or recurrent myocardial changes which are usually impossible to detect by conventinal echocardiographic methods (20).

There are several publications in the literature that suggest early myocardial involvement in RHD. Local inflammation and local secretion of proinflammatory cytokines were accused for myocardial dysfunction in rheumatic myocarditis (21). In addition, it is indicated that rheumatic myocarditis even without a severe valvuler damage could contribute to the formation of heart failure by guidelines (22). Although not existing an clinical evidence, early recognition and early prophylaxis with penicilin are believed to prevent from progression of disease especially in latent RHD patients (23,24). However, identification of these subtle myocardial changes is a challenge in clinical practice. Different stuides failed to show myocardial involvement by conventional echocardiographic evaluation (25-27). Our findings is compatible with these previous reports while we did not

find any difference in terms of EF, SF and other M Mode measurements between groups.However, significant decrement in E/A ratio reflecting diastolic impairment was observed in study group compared to controls by pulsed Doppler echocardiography in accordance with Sobhy et al (28).

Myocardial mechanics measurement by different echocardiographic methods including myocardial strain, TDI has been reported as effective in many diseases, recently. Although, there are studies that report abnormal global longitudinal strain or abnormal values of TDI systolic indexes in RHD in the literature, this is the first study of evaluating MPI in latent RHD at pediatric age (13,28).

MPI is an index which can also be measured by TDI and was first defined in a group of patients with dilated cardiomyopathy by Tei et al (29,30). The benefit of calculating MPI with TDI is that revealing systolic and diastolic myocardial performance together and the facility of calculating contraction and relaxation in the same cardiac cycle (31). Moreover, TDI provides evidence of subclinical dysfunction of myocardium earlier than other conventional echocardiographic methods. For that reason, TDI and MPI calculation is frequently used in different diseases that can cause myocardial dysfunction and TDI is reported as a prognostic indicator in many heart diseases (32).So, this study should take worthy particular attention because of the feasibility, reprocudibility and reliability of MPI rather than other methods in clinical practice.

We found higher MPI in study group compared with control while EF and SF were still normal in all cases. The result of increased left ventriculer MPI due to increased preload of LV is remarkable because of the absence of mitral stenosis in any cases. The result of increased MPI values were accompanied with lengthening of IVRT in

children with latent RHD compared to healthy controls. So, we speculated an early diastolic dysfunction in latent RHD at pediatric age. Furthermore, reported systolic dysfunction is rare than diastolic dysfunction due to neccessity of more time for systolic dysfunction. So in early stages of many diseases diastolic functions impairment is reported more common than systolic dysfunction in the literature (33-35). Thus, we did not find any correlation between follow-up interval and diastolic indexes in the present study. The explanation for this result should be the short duration of diagnosis of all cases in the study group. Prolonged valvular regurgitation and recurrent silent attacks in the absence of penicillin prophylaxis would cause myocardial dysfunction due to chronic volume overload and myocardial inflammation.

Conclusion: In the light of our data we concluded that early, subtle myocardial affection and diastolic dysfunction are present in the latent RHD of children spite of normal EF values. This finding highlighted the importance of early diagnosis and early initation of penicilin prophylaxis in endemic regions.

The limitations of this study originate from its cross sectional design and small number of patients. Long term, prospective studies with large samples should be performed for the contribution of MPI value to assess ventriculer function and its relationship with prognosis of latent RHD in children.

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Table legends :

Table 1. Demographic and clinical variables of study participants

Table 2. Valvular lesions of study group (n: 40)

Table 3. Conventional echocardiographic measurements

Table 4. Tissue Doppler echocardiographic measurements

Table 1. *Demographic and clinical variables of study participants**

	Study group(n: 40)	Control group (n:22)	p
Gender (male/female)	8/32	5/17	0.8
Age (year)	14.1±3.2	14.2±2	0.9
Systolic blood pressure (mmHg)	114±12	114.9±9.6	0.5
Diastolic blood pressure (mmHg)	66±10	65.4±10.3	0.9
Body Surface Area (m^2)	1.4±0.2	1.5±0.2	0.4

**Values are expressed as means with standard deviation*

Table 2. *Valvular lesions of study group (n: 40)*

Valvular involvement	MR	30 (75%)
	AR	3 (7.5%)
	MR+AR	7 (17.5%)
Degree of valvular involvement	Mild (Grade 1)	32 (80%)
	Moderate(Grade 2)	6 (15%)
	Severe (Grade3)	2 (5%)
Types of RHD	Borderline	21 (52.5%)
	Definite	19 (47.5%)

MR:Mitral regurgitation, AR : Aortic regurgitation, RHD : Rheumatic heart disease

Table 3. *Conventional echocardiographic measurements**

	Borderline RHD(n:21)	Definite RHD(n:19)	Control(n:22)
Ejection fraction (%)	74±4.4	73.1±6.2	73.2±5.5
Shortening fraction (%)	42.8±4.3	42.3±5.6	42.1±4.9
LVED (mm)	43.2±5.8	44.8±5.3	43.6±5
E/A**	1.5±0.1	1.4±0.2	1.6±0.2

A:late diastolic wave; E:early diastolic wave; LVED: Left ventricle end diastolic diameter

*Values are expressed as means with standard deviation

**p: 0.05 for E/A (any RHD versus control), p:0.04 for E/A (definite RHD versus control)

Table 4. *Tissue Doppler echocardiographic measurements**

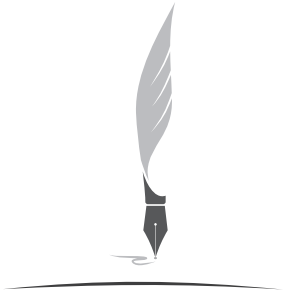
	Borderline RHD(n:21)	Definite RHD(n:19)	Control(n:22)
IVCT (ms)	66.1±7.9	68.2±8.1	65.2±9.2
IVRT (ms)**	65±8.5	67.4±8.4	58.1±10.1
ET (ms)	252.2±21.6	248.7 ±19.6	255.1±19.5
S (cm/s)	9±0.1	9.5±1.5	9.6±1.4
E'/A'	2.4±0.3	2.2±0.5	2.2±0.3
MPI***	0.52±0.04	0.54±0.05	0.48±0.06

A':late diastolic wave; E':early diastolic wave; ET:ejection time; IVCT: izovolumetric contraction time; IVRT: izovolumetric relaxation time;MPI: Myocardial performence index; S: myocardial systolic wave.

*Values are expressed as means with standard deviation

** p: 0.005 for IVRT (any RHD versus control), p:0.04 for IVRT (borderline RHD versus control),p:0.005 IVRT (definite RHD versus control)

*** p: 0.005 for MPI (any RHD versus control), p:0.004 for MPI (definite RHD versus control)



Chapter 7

BURN STASIS ZONES AND TREATMENT: ITS RELATIONSHIP WITH OXIDATIVE STRESS

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BURN STASIS ZONES

Tissue damage caused by thermal, chemical, electrical or radioactive agents is called burn (1). Regardless of the cause, the final result of the burn is protein denaturation in damaged tissues (2). Basic damage mechanisms are heat-induced tissue death, inflammatory mediators-induced damage and ischemic damage caused by thrombosed vessels with the effect of heat (2). The severity of the damage varies depending on the quantity and quality of the agent encountered, the degree of heat, contact time, environment (air-liquid), contacting body surface and skin thickness in that zone. Small-surface burns cause regional tissue damage, whereas burns covering a large body surface result in systemic response along with skin damage (2). The skin can withstand temperatures up to 40 ºC for a long time. However, tissue damage increases logarithmically at temperatures above 40 ºC. Protein denaturation at temperatures higher than 45 ºC exceeds the repair capacity of the cell (1, 3).

1. Burn and Injury Zones

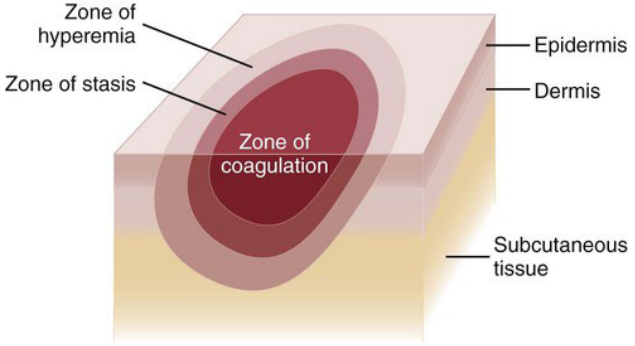
Thermal trauma causes protein denaturation at the cellular level and deterioration of plasma membrane integrity. Local coagulation necrosis develops in the zones that are directly exposed to high temperature. Temperature and contact time are the factors that determine the degree of injury. For a full-thickness burn, it is required to be exposed to a temperature of 45° C for one hour, while it is sufficient to be exposed to 69° C for one second for the formation of a burn of the same depth (4).

With burn damage, a significant loss of fluid occurs in the tissue and in the entire organism depending on the affected surface area, and numerous inflammatory mediators are released. Cytokines, prostaglandins, nitric oxide and superoxide radicals released from macrophages and leukocytes increase tissue damage. Mediators such as interleukin-1 (IL-

1) and interleukin 6 (IL-6) and tumor necrosis factor α (TNF α), whose amount increase in case of burns, are the substances that increase immunosuppressive catabolism. These mediators cause hypermetabolic status and increase vascular permeability. Thus, they cause cardiovascular and renal disorders, burn shock, disruption of gastrointestinal mucosal integrity and multi-organ damage (5, 6).

Jackson described three injury zones observed in the first 24 hours of acute burns, surrounding each other (7). The first zone is the coagulation zone exposed to direct heat. The coagulation zone located at the center of the damage is not self-renewing. The second zone is the stasis zone surrounding the coagulation zone. The third zone is the hyperemia zone located at the outermost zone of the injury, where superficial epidermal damage can be seen, but there is no dermal damage. The hyperemia zone with increased blood circulation and metabolism is a zone that can be completely healed. Stasis zone located between coagulation and hyperemia zones, is a zone that is not directly exposed to burn damage, it maintains its viability for the first 24 hours, but blood flow is reduced due to ischemia (8, 9) (Figure 2.1.).

Burn stasis zone forms the basis of the studies on the treatment of burns (8, 10). The recovery of the stasis zone prevents the increase of the depth and width of the burn (5).



Picture 1. *Burn Zones*

2. The Width and Depth of Burn

The evaluation of burn is usually done after the first emergency intervention. The severity of the burn is determined by the area of the burning surface, the depth and the location of the burn in the body. Large burns are not only a local trauma on the skin, they may cause systemic effects. The increase of the burned surface area increases mortality rates. In addition, the patient's age and associated comorbidities will also affect morbidity and mortality. Severe scars, dysfunction and disability may occur in deep burns. The burn width directly determines initial fluid resuscitation and subsequent nutritional requirements.

The skin is comprised of two layers. Epidermis and dermis. The epidermis is above the basal membrane and some of it is keratinized. The dermis is under the epidermis and above the subcutaneous adipose tissue. There are hair follicles, nerves and vascular structures, sweat and sebaceous glands inside it.

It is not always possible to accurately diagnose the depth of the burn at first sight. It may be decided to be more superficial or deeper in the clinical evaluation. The skin is thinner in children under the age of five and in adults over the age of 55, therefore the depth of the burn is usually more than predicted. In addition, in the inner surface of the arm, perineum and ear, skin is thinner than other body parts (11, 12).

While superficial burns heal by reepithelization, minimal scarring and skin discoloration may occur. There is always serious scar formation in deep burns. Moreover, if a superficial burn is not treated well or if an infection occurs, it can turn into a deep burn. Sweat glands and hair follicles play an important role in healing burns that are not full-thickness. They are of varying depth, since less sweat glands and hair follicles remain in deeper burns, the

healing time is prolonged, more inflammatory responses occur, and more severe scars are formed.

With the accurate diagnosis of burn depth, conservative treatment or aggressive surgical treatment decision will be given. Thus, the treatment results will be determined by this evaluation. Hypertrophic scar and functional impairment are usually not seen in burns that heal within three weeks. However, long-term discoloration may remain. Hypertrophic scars and functional disorders often occur in burns that are not healed in more than three weeks (11,12,13). Burn depth is classified into four degrees. First degree, second degree superficial and deep, third degree and fourth degree. In addition, it is also appropriate to group burns under two headings as superficial and deep.

2.1. First Degree Burns

In such burns, only the outer layer of the epidermis and the stratum corneum are damaged, and no damage is observed in the dermis (14, 15, 16).

2.2. Second Degree Burns

Such burns are deeper than first-degree burns and necrosis spread into the dermis. The damage infests the entire epidermis and a part of the dermis. Clinically, it is characterized by pain, erythema and bulla formation. The rate of recovery depends on the depth of skin damage and infection.

Second degree burns usually heal up spontaneously and in a short period of time unless an infection occurs. If an infection occurs in the wound, it easily turns into a third-degree burn. The systemic effects of burns and the nature of the healing are directly related to the amount of injury in the dermis. Burns in this group are divided into two; superficial and deep dermal second-degree burns (15, 17).

2.2.1. Superficial Second-Degree Burns

They are formed as a result of contact with flame or hot liquids for a short time. Burned zone looks dirty red or pink. Due to plasma-like liquid leakage in the burned zone, the surface is usually moist. The painful wound is sensitive to air contact. Bulla can occur immediately or in the first day. The earlier the bulla occur, the deeper the burn is. Healing begins with basal cells remaining in the skin or with hair follicles and sweat gland epithelia covering the burning zone. Usually less scar occurs compared to deep dermal burns. In superficial second-degree burns, usually the upper part of the stratum germinativum is damaged. Epithelialization begins from the intact sections of the stratum germinativum and skin appendages. Recovery usually results in 3-4 weeks without any scar or leaving a very slight scar (18, 19).

2.2.2. Deep Second-Degree Burns

They occur as a result of contact with flame, hot liquids or chemicals, or by exposure to high electrical current. In such burns, the epidermis is completely burned, the damage extends to the stratum germinativum and into the bottom of the dermis. Epithelial regeneration occurs only in sweat glands and hair follicles. The skin is soft, the surface appears red or pink and it is usually moist, a plasma-like liquid exudes from the injured zone. Fluid loss and metabolic effects in deep second-degree burns are similar to third-degree burns. During burning, pain in the wound is very severe and hyperaesthetic zones occur in the skin. If an infection occurs in the burned zone, the wound turns into a third-degree burn. The time required for reepithelization depends on the damage of the dermis, the amount of burned hair follicles, sweat glands, and the width of the infected zone. If the wound is properly protected, it closes within 2 months, leaving scars on the skin surface. Scar and contracture occur in zones that are

healed in more than two months. In this case, it is quite difficult to differentiate the wound from third degree burns and its treatment takes longer (20, 21).

2.3. Third Degree Burns

All structures in the skin are damaged. Dermis and subcutaneous adipose tissue have been destroyed by coagulation necrosis (22).

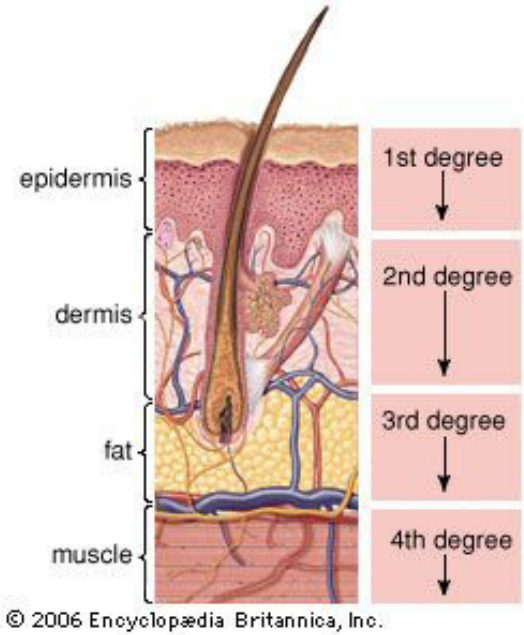


Figure 2. *Skin layers according to the degree of burn*

The skin, of which all layers are damaged, is characterized by autolysis and leukocyte infiltration at 2nd or 3rd weeks. This is usually accompanied by suppuration. If the burn comprises subcutaneous adipose tissue, healing may take much longer. The treatment of third-degree burns requires removal of the scar and grafting of the wound. In case of lack of grafting, a thick granulation

layer is formed over time and contraction develops. At this stage, reepithelization occurs only at the wound edge and it is scarce. The granulation is soft, it may be infected, and the healing of the wound may take months. Following the healing of these types of wounds, permanent deep scars occur on the skin and surgical intervention is usually required for the skin to regain its normal appearance (18, 19, 22).

2.4. Forth Degree Burns (Carbonization)

Subcutaneous fat and deeper tissues (muscle, bone, brain, etc.) are burned along with all layers of the skin. It is the carbonization of burned tissues.

3. Evaluation of Burn Depth

Many methods have been described for determining the depth of the burn. Of these, the most commonly used and standardized one is clinical evaluation. Burn is a dynamic event. Due to changes in the first 72 hours, it is often not correct to make a definite evaluation. A superficial seeming burn may become deeper after a few days. In order to plan the treatment and decide whether the patient will be treated as an outpatient or inpatient, the depth of burn should be evaluated correctly. Therefore, although clinical evaluation is currently the most commonly used and trusted method, new searches are continuing (12, 23).

4. Physiopathology of Burn

As a result of the burn injury, firstly local pathophysiologic effects occur in the burned zone and in adjacent zones, and systemic pathophysiologic effects develop as a secondary response to them. All these effects are associated with the severity of the burn (2, 24). The majority of these systemic responses develop as a result of the release of various mediators.

4.1. Local Effects

Local physiopathologic changes in the burn wound are characterized by heat-induced protein denaturation and an inflammatory process caused by the release of inflammatory mediators.

4.2. Physiopathology of Stasis Zone

Initially the burn wound has different depths in different zones. Because of the different degree of heat transfer, the burn wound consists of several tissue destruction zones. The zone in the middle, where heat transfer is the most intense and irreversible cell death occurs as a result of protein denaturation is called coagulation zone. The coagulation zone is surrounded by a zone of stasis characterized by an apparent inflammatory reaction. This zone, which has a potential of recovery, can turn into a totally destructive zone by the infection or drying of the wound. The outermost zone is the hyperemia zone with minimal cell damage and spontaneous recovery (2, 24).

In the first phase, local edema period begins mainly in the stasis zone as a result of vasodilatation of the vessels in the burned zone, elevated extravascular osmotic activity and increased microvascular permeability, within an average of 13 hours after burn. This period is followed by a second phase called the “no-reflow” phenomenon. In this phase heterogeneous reductions in tissue perfusion occur. This leads to local tissue ischemia, which causes necrosis. This disorder in microcirculation reaches its worst level in 12-24 hours after burn. In the third phase, endothelial cells, platelets and leukocytes accumulate in the vessel wall, followed by their extravasation and migration to damaged parenchymal cells and microorganisms. Circulating platelets contribute to the occurrence of different levels of hemostasis and local thrombosis. During all these or as a result of these, the

coagulation system and the complement system are activated, and some kind of reaction chains begin to form because of the release of many inflammatory mediators such as cytokines, prostaglandins, quinines, vasoactive amines, free oxygen radicals, etc. to the medium from a variety of sources, and these local effects result in a systemic pathophysiological response (24).

4.3. Systemic Effects

Extravasation occurred as a result of all these local effects causes local and systemic edema, and consequently the effective blood volume decreases. Although cardiac output increases initially due to high metabolism rate, it may decrease up to 20% of the normal rate due to hypovolemia. As a result, tissue perfusion, which is initially tried to be kept within normal limits in the vital organs by compensation mechanisms, gradually decreases and metabolic acidosis occurs. The decrease of blood flow in the kidney causes the release of antidiuretic hormone and increases fluid resorption. In addition to these, hemoglobin and myoglobin formed by burn damage may precipitate in the tubules and may cause acute tubular necrosis. As a result, if fluid and electrolyte losses are not compensated, acute tubular necrosis and subsequent renal failure develop (2, 24). The response of the pulmonary system to large burns is hyperventilation. Ventilation doubles due to increased oxygen demand and perfusion-ventilation imbalance. In large burns, lung volume decreases, and pulmonary resistance increases because of the vital capacity increase. If it is in the gastrointestinal tract, paralytic ileus and acute gastric dilatation develop as a result of severe splanchnic vasoconstriction. In addition, 4-10% of patients with large burns may develop clinically massive hematemesis characterized by multiple small ulcers in the stomach and duodenum and curling ulcers accompanied with melena. In the liver, swelling,

hepatocellular necrosis and fat degeneration may occur due to hypovolemia and hypoxia (2, 24). As a result of burn trauma, many metabolic and neuroendocrine changes are observed. Some of these are due to the low flow rate of hypovolemia. But others occur under sufficient perfusion and tissue oxygenation. In burn patients, there is a high metabolic rate, which increases oxygen consumption above the basal level. Although the exact reason of this is not understood, metabolic rate decreases to normal with wound healing. This hypermetabolic condition is seen as protein catabolism, hyperglycemia, decrease in glucagon/insulin ratio and changes in intracellular cation. The negative nitrogen balance accompanying high metabolic rate, considerably increases the nitrogen requirement. Nitrogen requirement increases due to increased catabolism and decreased protein synthesis. Glucagon, cortisol and catecholamines were also elevated in the burn patient. These hormones are believed to play an important role in catabolic response (2, 24).

Jackson described three injury zones, observed in the first 24 hours of acute burn, surrounding each other (7). The first zone is the coagulation zone which is directly exposed to heat effect. The coagulation zone located at the center of the damage is not self-renewing. The second zone is the stasis zone surrounding the coagulation zone. The third zone is the hyperemia zone located at the outermost injury area, where superficial epidermal damage can be seen, but there is no dermal damage. The hyperemia zone, in which blood circulation and metabolism is increased, is a zone that can be completely healed. The stasis zone, which is not directly exposed to burn damage, located between coagulation and hyperemia zones is a zone where blood flow is reduced due to ischemia and continues to survive for the first 24 hours (8, 9).

This zone is exposed to oxidative stress due to ischemia - reperfusion damage occurring in the stasis zone

(9). Reperfusion injury predominantly causes apoptotic cell death and the increasing apoptosis in the stasis zone results in progressive tissue loss (4). If stasis zone with healing potential is not treated, blood circulation stops completely in time and necrosis develops (10).

Recovery of the burn stasis zone forms the basis of the researches about burn treatment (8, 10). The recovery of the stasis zone prevents the increase of the depth and width of the damage in the burn area (5).

5. Treatment

Burn treatment can be performed in different centers depending on the width of the burn. While mild burns can be treated as outpatient, moderate burns should be treated in a fully equipped hospital and severe burns should be treated in a hospital with a burn center (25).

5.1. Fluid Electrolyte Treatment in Burn

Studies on large patient groups have shown that the amount of fluid loss is not proportional to the depth of the burn, but to its width (24). In addition, it is possible to say the critical burn width, for which the loss of fluid can compensated by natural means or it is unable to compensate and tend to switch to a decompensated shock circuit. This critical area is 15% of the body surface in adults. Children have less resistance to fluid loss and can withstand only the burns of less than 10% of the body surface. Sometimes fluid electrolyte treatment may be necessary even for the babies with 8% burns (3).

Regarding severe burns requiring liquid electrolyte treatment, many formulas have been proposed for the fluid treatment to be administered during the first 48 hours, but there is no single formula that can be administered in all cases (26). All of the formulas currently used were obtained experimentally, from clinical observations (Table

2. 3). The applied formula can only give the clinician an idea about the extent of the fluid requirement. In severe burns, one should not be strictly adhered to mathematical formulas for intravenous fluid application, and the physiological response of the patient should always be considered during treatment (24).

Parkland Formula: 4 ml × body weight × burn percentage (kg) = Ringer Lactate

½ of the calculated fluid is given in the first 8 hours. ¼ of the liquid is given in the next 8 hours and the last ¼ in the last 8 hours. In the second 24 hours, half of the previously given amount is administered and the treatment of the next day is decided according to the severity and condition of the patient's burn (27).

5.2. Care for Burn Wounds

Serious problems such as burn shock and metabolic disorders in the acute phase of burns can be solved by medical treatments. However, the infection that develops on the burn surface increases the dimensions of the case and causes a second-degree superficial burn to deepen or even turn into a third degree. Burned and necrotic tissues provide an ideal medium for microorganisms. The toxins secreted by these pathogen microorganisms bred in the medium may interfere with the circulation, may cause endotoxin shock, organ failure and sepsis and may cause the death of the patient. As it is known, according to the research, sepsis (62.2%) is the most common cause of mortality due to burns (28).

Medical treatment of burn wound: Medical wound care is composed of antibiotic and non-antibiotic ointments and various dressings.

Since there is no blood flow to the eschar tissue, systemically used antibiotics do not reach the eschar tissue.

Therefore, antibacterial agents that are intended to be effective on the burn wound should be used topically (29). Normal skin is not very rich in bacterial flora. Diphtheroid and Staphylococcus epidermidis, and sometimes Staphylococcus aureus are the main microorganisms found in the skin flora. The skin flora depends on the patient's environment, as well as the topical or systemic antibiotic used. In the first days of burns, gram-positive microorganisms are dominant in the burn wound, whereas later on gram-negative microorganisms such as Proteus, Klebsiella and Pseudomonas become more common in the wound (29). By using topical antibacterial agents, bacterial colonization in the burn wound is delayed at the beginning and colonization is kept to a minimum in later periods. If topical antibiotic treatment is successful, the development of invasive burn wound infection is prevented (29).

Surgical treatment of burn wound: With the progresses in burn shock physiopathology and anesthesia, early removal of the burn eschar before the localization of the infection and immediate grafting of the defect allowed the recovery of the acute phase of the burn with a minimal complication, thus increasing the chance of survival for many burn patients. Early Surgical Excision and Grafting Method, which became the main rule, especially in the treatment of large burns in recent years, was pioneered by researchers such as Janzekovic, Jackson and Groves. The first study on this subject was published in 1968 by Janzekovic. Then, Jackson published the results of his early tangential excision using a modified humby knife in 1969 and 1972. Again in 1971, a similar study was published by Groves. Many similar studies showed that early excision and grafting have an important effect on the immune system. It was found that with the excision of early necrotic tissues and grafting; sepsis was prevented, cellular immunity was improved, the functions of

phagocytosis cells returned to normal and blood loss decreased (28).

Current approaches in wound care:

1) If full-thickness burns smaller than 20% and moderate-depth burns are treated by an experienced surgeon, the length of hospital stay is shortened thanks to early excision and grafting, thus patient cost decreases.

2) Early excision and grafting dramatically reduces the number of painful debridement needed in all patients.

3) Patients with a total body burn surface of 20-40% develop less infectious wound complications if early excision and grafting are performed (26, 30).

6. The Importance of Oxidative Stress in Wound Healing

Wound healing is a process that involves successive mechanisms after the injury of the skin or soft tissue and that is regulated by different factors. Reactive oxygen species (ROS), one of the factors affecting wound healing, are produced during normal metabolic events by Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NADPH), which is an enzyme complex system. Of these products, hydrogen peroxide (H_2O_2) is not a radical but it may cause significant damage to cells (31). H_2O_2 forms hydroxyl radicals, especially in the presence of iron and copper ions, causing severe cell damage. ROS is normally required for the defense system against pathogenic microorganisms in the wound area. Neutrophils and macrophages produce large amounts of ROS as a result of oxidation, and the resulting ROS plays an important role in the elimination of foreign organisms. On the other hand, the ROS released from these phagocytic cells may also cause tissue damage and may cause wounds in the surrounding tissues (32). The superoxide anion (O-) and

H_2O_2 produced by the inflammation of endothelial cells in the injured area stimulate the proliferation of new vessels by regulating microvascular blood flow and provide regular nutrients and oxygen for the activity of the wound area. In addition, low amount of ROS function as a means of intracellular signal transduction (33).

As mentioned before, free radicals or oxidants may cause tissue damage in the wound area and disrupt the healing process. In particular, hydroxyl radicals and O-anion may alter the adhesion, proliferation and viability of fibroblasts by degrading hydroxyproline and proline in the collagen structure. At the same time, H_2O_2 inhibits the migration of keratinocytes on one hand, on the other hand it leads to serious damage on fibroblasts by inhibiting signal communication of epidermal growth factor (EGF) (34). Especially in chronic wounds, a strong inflammatory infiltration and elevated ROS indicate the presence of oxidative stress. High amounts of ROS cause cytotoxicity and delay wound healing. Therefore, the elimination of ROS is an important condition especially in the healing of chronic wounds.

The severity of tissue damage is determined by measuring ROS in the pathogenesis of many diseases. Even though oxidants play a role in wound healing at every stage of healing, the amount of ROS was found to be highest in the inflammatory phase. For example, 8-isoprostane formed by peroxidation of unsaturated fatty acids in the cell membrane is a marker of oxidative stress and disrupts the structure and function of cell membranes. It has been reported that this marker is high in wound fluid of chronic venous ulcers (35).

In ischemic wounds resulting in insufficient blood flow, tissue leukocyte count rapidly increases and necrosis occurs. The superoxide anion generated by the neutrophils

migrating to the zone during the ischemia period and after reperfusion causes endothelial cell damage (36, 37).

Oxidants in thermal wounds have been shown to cause severe systemic and local damage. In burns, intravascular flow of neutrophils into the region increases the formation of free radicals. The resulting ROS is responsible for the increase in edema (38). Antioxidant levels also decrease significantly in local burn wounds. In animal and human studies, it has been shown that the damage caused by free radicals is significantly improved after topical application of antioxidant compounds (39, 40).

7. Biochemical Parameters Used in The Evaluation of Oxidative Stress

7.1. Superoxide Dismutase

Superoxide Dismutase (SOD) is an enzyme that catalyzes superoxide anions formed by NADPH as a result of oxidation to molecular oxygen and hydrogen peroxide; there are three types of Superoxide Dismutase (SOD) in eukaryotic cells. SOD1 (Cu/Zn SOD) is an important component of the oxidative defense system, has a molecular weight of 32 000 and it is present in the cytoplasm. SOD1, which has a dimeric structure, is Cu/Zn dependent and even though it is effective in different phases of wound healing, it is found to be more effective in the early stages of the inflammation phase (41). It has been shown to shorten the wound healing process when applied topically (42). SOD2 (Mn SOD) is Mn bound and has a molecular weight of 22 000 and is present in mitochondria. In skin injuries, it is released by the neutrophils of granulation tissue at a high level (43). SOD3 is a tetramer structure of 135000 molecular weight and is present in plasma, lymphoid tissues and cerebrospinal fluid. It is localized in the extracellular region and tends to bind to collagen type 1 and heparan sulfate. It is released

by various cells, especially fibroblasts. Its main function is to react with nitric oxide to form peroxynitrite radicals and to maintain the vasodilatory effect of nitric oxide. It has glycoprotein structure and it contains Cu and Zn ions. It catalyzes the same reactions as SOD 1 (3,5,6).

7.2. Catalase

Catalase is an important enzyme against oxidative stress, with a molecular weight of 240000, composed of tetrameric subunits. It plays a role in regulating intracellular H_2O_2 level. It is present in peroxisome and mitochondria in large amounts, and in the cytoplasm and endoplasmic reticulum in smaller amounts (44). During wound healing, the expression of enzyme in proliferation granulation tissue was found to be quite high. It plays an important role in detoxification by preventing the formation of H_2O_2 radicals in the wound area. The low level of H_2O_2 in the wound area is one of the regulatory tasks of the catalase enzyme (45).

7.3. Glutathione Peroxidase

Glutathione peroxidase (GSH-Px) is an important component of the antioxidant defense system in mammals and generally shows activity in the presence of selenium. GSH-Px enzyme, which is found in different tissues and which has five different isoenzymes, is localized in cytosol and mitochondria and it is an important radical scavenger for H_2O_2. Low concentrations of H_2O_2 are first removed by GSH-Px. This enzyme prevents the harmful effect of hydrogen peroxide in the environment where reduced glutathione is converted to oxidized glutathione by high specificity. In the reaction where reduced glutathione (GSH) is converted to oxidized glutathione (GSSG), hydrogen peroxide is reduced to water by GSH-Px enzyme. Then, NADPH is spent through the reaction catalyzed by glutathione reductase enzyme, and oxidized

glutathione is reduced again (46). These enzymes are found at high amount in the wound area during the inflammatory period. GSH-Px occurs intensively with all isoforms in the inflammatory phase of wound healing. In cases where oxidative stress is intense, it induces proliferation of keratinocytes by creating resistance against ROS products (46). In the wound healing study conducted by Steiling et al., it was shown that GSH-Px levels increased with oxidative stress in the wound tissue. In addition, it has been suggested that antioxidant enzyme expression during recovery is increased for adapting to increased oxidative stress (43).

7.4. Malondialdehyde

Malondialdehyde (MDA) is one of the toxic end products produced by the breakdown of non-enzymatic oxidative lipid peroxides. The main source of MDA is cyclic endoperoxides which are liberated in the autoxidation of fatty acids containing more than two double bonds and in eicosanoid synthesis. These lipid peroxidation products, especially aldehydes have toxic and biological effects (47, 48, 49). Their long life and ability to penetrate membranes suggest that these compounds are responsible for the effects of lipid peroxidation on target organs. These end products cause high concentrations of cell death, inhibits important functions such as mitochondrial respiration, monooxygenase system functions, and protein synthesis (50).

MDA shows the toxic effect by binding to amino groups of proteins, phospholipids or nucleic acids (51). Measurement of MDA amount is often used to determine lipid peroxide levels (52).

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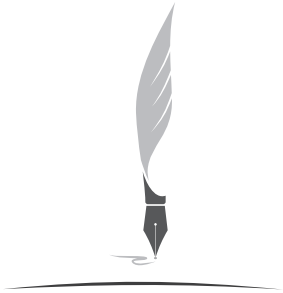

Chapter 8

PIEZOELECTRIC SURGICAL TECHNIQUE IN RHINOPLASTY

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INTRODUCTION

Rhinoplasty surgery may often require osteotomy to achieve lasting and satisfying outcomes, and the nature of the osteotomy (medial, lateral, or transverse) depends on the type of nasal deformity to be addressed (1). Following a hump removal, the dorsum tends to remain wide, leading to an open roof deformity. Lateral osteotomy enables closure of an open roof, narrowing of the bone nasal pyramid, and fixing of the nasal bones; however, it is usually the most difficult step of rhinoplasty and is associated with complications that include massive bleeding, prolonged post-operative edema, and ecchymosis, as well as asymmetrical irregularities of the nasal wall (2). Visibility may be obstructed during osteotomy, and control over the incipient fracture line may not be possible. Owing to these challenges, osteotomy is associated with various unpredictable complications in the early postoperative period, such as bleeding, ecchymosis, and edema (3).

In 1975, Horton et al. introduced piezoelectric ultrasonic vibration for gentle cutting in alveolar bone surgery (4,5). These authors reported better healing of bony fragments with piezosurgery, and this technique was subsequently applied to various surgical fields (4,6). Piezoelectric technology was described for nasal osteotomy by Robiony et al. in 2007 and by Pribitkin et al. in 2010 (7,8). The piezosurgical device comprises a platform with a powerful piezoelectric hand piece and an irrigation system for cooling. Pribitkin et al. reported that the piezosurgical device permitted safe, precise, graded bone removal that was adjustable with respect to frequency and cutting power and that did not damage the surrounding soft tissue and mucosa of the nose (8). The piezoelectric osteotomy is safe and prevents osteonecrosis and soft-tissue damage due to the establishment of selective, very delicate, and accurate methods (9).

SURGICAL TECHNIQUE

Propofol (2 mg/kg), remifentanil (0.2 mg/kg/min), and rocuronium (0.6 mg/kg/min) were administered to induce anesthesia. Desfluran (4-6%) was administered as needed for maintenance of anesthesia. Sugammadex (2 mg/kg) was administered for reversal of anesthesia. Hypotensive anesthesia was applied to achieve an average arterial blood pressure of approximately 60 mm Hg. Lidocaine with 1:100,000 adrenaline was injected into the nasal dorsum and septum 10 minutes before the first incision was made. A midcolumellar incision was made, and sharp dissection was performed to expose the nasal skeleton from the tip to the nasal bone. For patients who underwent ultrasonic osteotomy, a periosteal incision was made over the dorsum and nasal bone with a no. 15 scalpel blade, and the entire nasal bone was exposed subperiosteally by means of a periosteal elevator (**Figure 1**). Ultrasonic osteotomy was carried out with a Variosurg3 piezosurgery unit (Nakanishi Inc, Tochigi, Japan) and customized cutting tips (**Figure 2**). All patients underwent hump resection with a Rubin osteotome followed by bone roof rasping, septoplasty, and fixation of the caudal septum to the maxillary spine. Patients subsequently underwent medial oblique, low-to-high internal osteotomy with an ultrasonic device (**Figure 3**). The surgeon did not manually infracture the nasal bones; instead, the bones were carefully narrowed with the probe of the device. To prevent the bony vault from collapsing, the surgeon maintained the integrity of the inner mucosa, the transverse nasalis, and the scroll ligament. Following osteotomy, each patient underwent tip-plasty as needed (eg, tip sutures, repositioning of the lateral crura, or alar batten graft). Postoperatively, each patient received dexamethasone (8 mg). Patients received prophylactic antibiotic treatment during the first week postoperatively and were advised to apply a cold

compress for 15 minutes every hour during the first 24 hours postoperatively.

DISCUSSION

The main morbidities of rhinoplasty are postoperative edema and ecchymosis. The severity of these morbidities depends on the degree of soft-tissue injury, including vessels during subperiosteal degloving of nasal dorsum, and during osteotomies. These types of osteotomies and surgical techniques also have effect on these morbidities. Surgeons have tried various techniques, instruments, and intra- and postoperative methods and materials to diminish these uncomfortable morbidities. However, edema and ecchymosis remain problems following rhinoplasty. Piezoelectric surgery is commonly used during osteotomies to decrease the severity of morbidities. There are new studies about the advantages of piezoelectric surgery, compared with the conventional osteotomies, on postrhinoplasty ecchymosis and edema.For successful rhinoplasty, the surgeon must sculpt the bony pyramid while preserving the soft-tissue envelope. Surgical maneuvers to shape the bony structure of the nose typically are challenging and associated with complications (10). For osteotomy, the surgeon must perform a series of procedures that may include decreasing dead space, narrowing the lateral walls of the nose, reducing the dorsal hump, closing an open-roof deformity, and straightening the bony framework of the nose to create symmetry (11,12).

Many studies have addressed osteotomy techniques and instruments (13-15). In a cadaver study, Kuran et al. determined the average thickness of osteotomized segments of the nasal pyramid and found that lateral-wall thickness correlated with fragmentation rate and soft-tissue injury in lateral osteotomy (16). In a subsequent cadaver study, Harshbarger and Sullivan confirmed these

results. Kuran et al. also compared different types of osteotomes and concluded that a narrow, curved chisel was optimal to minimize soft-tissue injury (16,17). Becker et al. described a power-assisted technique to address the nasal dorsum that resulted in less tissue disruption than observed with rasping (18). Sinha et al. compared external osteotomy with internal osteotomy and found that external osteotomy caused less edema and ecchymosis (19). Therefore, our choice of internal osteotomy may be considered a limitation of our study. In addition, Nolst Trenité described the utility of micro-osteotomes to reduce postoperative ecchymosis and edema when refining the bony pyramid of the nose (20).

Osteotomy with conventional osteotomes is associated with mucosal injury and trauma to overlying soft tissue that can lead to bleeding, bruising, and edema (21,22). Lateral osteotomy can cause bleeding into the soft tissue owing to disruption of the angular artery or vein. Edema and ecchymosis develop perioperatively and may persist until the ninth postoperative day (12). Periorbital ecchymosis and edema may be exacerbated by vigorous rasping and the application of a large osteotome (23). Eyelid edema and periorbital ecchymosis may develop postoperatively and cause great concern in patients. Palpebral edema can affect vision, and periorbital ecchymosis is socially off-putting and may result in increased pigmentation (24,25).

In an observational study, Robiony et al. utilized piezosurgery to perform osteotomy and demonstrated that this technique was associated with minimal amounts of bleeding, edema, and periorbital ecchymosis (6,7). Pribitkin et al. performed sculpting of the nasal dorsum with an ultrasonic bone aspirator and noted that the technique was safe and precise (8). However, this study was observational, not comparative. In a study set in an academic clinic for facial plastic surgery, Greywoode and Pribitkin applied an ultrasonic bone aspirator to reduce

the nasal spine, deepen the glabella, sculpt mobile bone fragments and smooth bony edges after medial osteotomy, and reduce the convexity of the nasal bones (26). These authors described the aesthetic results of this procedure in terms of subjective evaluations by the patients and surgeon, and they did not compare this approach to conventional osteotomy (26). In a cadaver study, Ghassemi et al. performed osteotomy with a piezo scalpel and found it to be effective, as assessed histologically (27). In a study in 2017, Koc et al. compared piezosurgery and lateral osteotomy in rhinoplasty, and found that on day 1 after surgery, the piezosurgery group showed better results regarding edema, ecchymosis, and hemorrhage. Similarly, the results for edema and ecchymosis in the piezosurgery group were better on the 7 days (28).

Cost is a major problem of piezosurgery. The device itself is expensive, and the incisive tips generate further costs. On the other hand, the tips could be used for more than one patient as osteotomes. In addition, insufficient mobilization and the need for revision that may occur following a conventional osteotomy can be eliminated by piezosurgery, due to osteotomy under direct visualization, thereby mitigating the revision costs. Another problem with piezosurgery is that the handpiece must be used with extreme care. If metal tips touching the skin are not irrigated with water as much as necessary, it can cause skin burns, particularly during transverse osteotomy, which can lead to undesirable results.

CONCLUSIONS

Several surgical modifications to osteotomy have been described. The ideal osteotomy technique should be safe and easy to perform, should allow for accurate and precise control, and should yield a pleasing aesthetic result.

LEGENDS TO FIGURES

Figure 1. *Intraoperative view of this 25-year-old woman who presented with functional (breathing) and aesthetic concerns. The patient underwent ultrasonic osteotomy.*

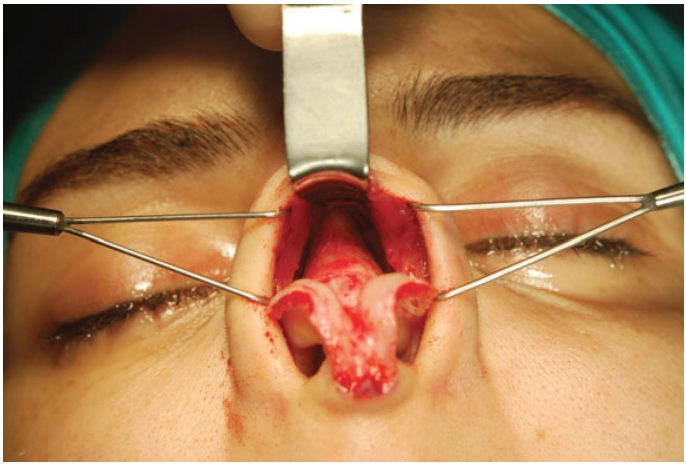


Figure 2. *The ultrasonic device applied for osteotomy in this study.*

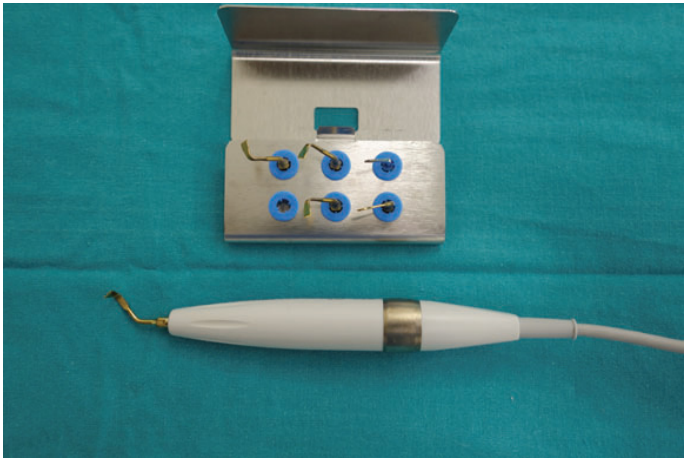
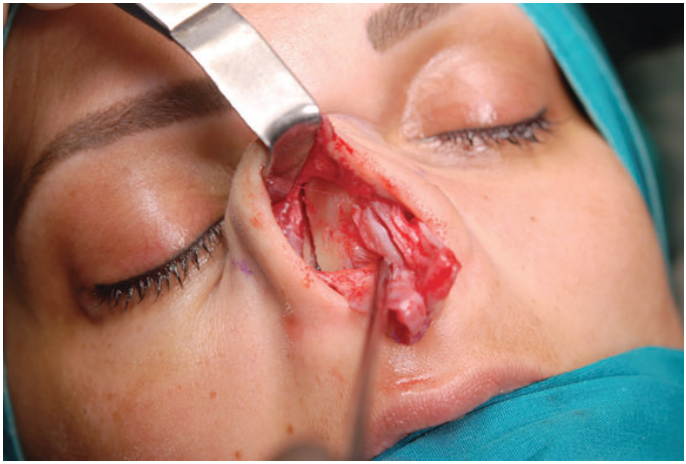


Figure 3. *Intraoperative view of this 25-year-old woman (also presented in Figure 1) who underwent ultrasonic osteotomy.*



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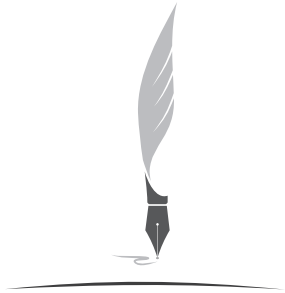

Chapter 9

IMPLANT-SUPPORTED PROSTHESES IN DENTISTRY

Tahir KARAMAN

The word implant is generally used to describe structures that replace a missing organ or tissue. A dental implant is a biocompatible apparatus that replaces a lost tooth and surrounding tissues. It is placed in or on the bone to provide support to a prosthesis (1,2). Osseointegration, which is the connection of dental implants to bone, plays an important role in the success of implants. Dental implants are an important treatment alternative for all types of toothlessness. However, some conditions may impose limitations on implant applications. Implant applications may also be contraindicated, especially in chronic, uncontrolled diseases, and in cases with abnormalities in the mucosa or jaw (1).

The planning of implant-supported prosthesis procedures directs the application of treatment. The condition of the existing oral and dental tissues, oral hygiene, compatible occlusion and excessive occlusal forces affect the success of the implant treatment. Implant prostheses are classified according to level of toothlessness, since prosthesis options will change depending on whether the patient has a full or partial set of teeth.

Misch has reported five options for implant-supported prostheses. The first three are fixed prosthesis (FP) applications (FP-1, FP-2, and FP-3), and the last two options are RP-4 and RP-5. FP-1 is the size and appearance of natural crowns. In FP-2, the crown and a portion of the root are restored, the contour of the crown is normal to the occlusal, and the gingiva is extended or over-contoured. FP-3 restores the missing gingiva, crown, and root in the toothless region; metal-ceramic crown can be used, although artificial teeth and gums are used in dentures. RP-4 is the only implant-supported overdenture prosthesis, while RP-5 is an overdenture prosthesis with implant and soft tissue support (1).

PROSTHETIC COMPLICATIONS IN IMPLANT-SUPPORTED PROSTHESES

Elimination of the aesthetic issues and functional loss caused by tooth loss are among the main aims of prosthetic treatment. Dental losses can be treated with fixed prosthesis or removable prosthesis. Meanwhile, implant restoration failures depend on various factors, such as biological, aesthetic, and mechanical complications (3-6). In the planning and implementation of implant restorations, it is very important for the clinician to apply preventive measures and the appropriate methods to eliminate possible failures.

Biological complications: These complications mainly include peri-implant mucositis, peri-implantitis, fistula formation, soft tissue hyperplasia, and implant loss (6).

Peri-implant mucositis: Similar to gingivitis in natural teeth, this is an inflammation that occurs in soft tissues around a functional implant and is reversible, if treated early (2).

Peri-implantitis: This is an irreversible condition in which inflammation of the tissue surrounding the implant occurs, along with bone destruction. Although peri-implantitis resembles periodontitis in natural teeth, it is more aggressive and difficult to control. The formation of a suitable environment for the accumulation of bacteria in the subgingival area due to dental cement overflow plays an etiological role in peri-implantitis. Bacterial interactions occur due to the cement overflows, along with allergic reactions and foreign body reactions, such as inflammation around the implant and peri-implant disease progression (2).

Fistula Formation: This occurs due to an implant abutment screw loosening or poorly compatible

infrastructure restorations. The loosened screw must be tightened with the appropriate torque, and the poor restoration should be replaced by a well-matched restoration (6,7).

Soft Tissue Hyperplasia: This occurs in the presence of predisposing factors, such as inadequate oral hygiene, poorly adapted prostheses, dead spaces under the substructure, or inadequate attached gingival. These predisposing factors need to be addressed, especially poor oral hygiene, and mechanical deposits should be removed. In some cases, surgical resection may be required for hyperplasic tissues (6,7).

Implant Loss: Early implant losses occur as a result of failure to achieve osseointegration prior to functional loading. Early implant losses are associated with bone overheating, infection, poor bone quality, and inadequate bone quality in implant placement. Late-period implant losses may occur after functional loading due to occlusal overloads, peri-implantitis, or poor osseointegration due to poor bone quality (6,8).

Mechanical Complications: These usually occur when the applied forces exceed the capacity of the implant and its components. These complications can include a fracture of the veneer or substructure material, a loss of retention, screw loosening, a screw fracture, or an implant fracture (6).

Veneer or Infrastructure Material Fracture: This is one of the most common mechanical complications of fixed implant restorations. Metal-ceramic or full-ceramic implant supported crown restorations can suffer from a fracture of the ceramic part, and a hybrid prosthesis can suffer from the fracture of acrylic resin teeth. A material fracture may be seen in the infrastructure due to inappropriate laboratory procedures, while fractures in the ceramic structures may occur due to incorrect occlusion.

The substructure material must have sufficient thickness and hardness to withstand the chewing forces. Porcelain repair materials used in the mouth can be used in small fractures, while large-scale fractures require laboratory procedures (6,10). The screw connection makes it possible to easily remove and perform laboratory procedures in cases in which the restoration needs to be renewed (9).

Loss of Retention: Loss of retention in an implant-supported fixed prosthesis typically occurs in restorations with cement retention and usually develops due to insufficient retention and resistance of the abutment. Improper abutment selection, poor abutment design, or lack of occlusal distance for the restoration may also result in a loss of retention of the implant-supported fixed prosthesis (6). Loss of retention in an implant-supported removable prosthesis occurs due to wear, detachment, or breakage of the attachments during usage. Replacing worn or corroded attachments eliminates the loss of retention (11).

Screw Looseness and Fracture: Screw loosening is a common complication of prosthetic restorations. Micro-movements in the abutment screw implant connection region cause screw loosening. Inadequate initial torque, poor infrastructure compatibility, and excessive occlusal loads are the sources of the micro-movements. Screw fractures usually occur in the presence of high torque applications, excessive occlusal loads, and restorations with poorly compatible infrastructure (7). If the abutment screw is broken, it is very difficult to remove. If the fracture is above the implant neck surface, the screw should be loosened and removed with a handpiece in the direction of loosening. If the screw fracture line is at or below the implant neck level, special kits and ultrasonic scales have been developed for removal. The removal of the fractured screw should be performed very carefully,

and the implant groove structure should not be damaged (6,12).

Implant Fracture: In very rare cases, implant fractures are usually due to excessive force or the use of an implant with a narrow diameter (4,13).

Aesthetic Complications: Reasons for the aesthetic failure of implant restorations include interdental papilla loss, gingival recession, poor restoration contours, and incompatible restoration color (14). An implant restoration will meet aesthetic expectations if the existing soft and hard tissues are in ideal condition and position. However, aesthetic expectations may not be fully met due to a high amount of resorption in the existing tissues or irregularities in the jaw relations. During the implant restoration planning stage, it is very important to evaluate the patient's oral status in detail and to know his/her expectations. To make restorations more aesthetic, the necessary surgical arrangements should be made in the existing soft and hard tissues. Gingival recession, especially in single tooth deficiencies, can be removed thanks to the use of pink-colored porcelain, although the addition of pink porcelain in the restoration provides a weak aesthetic appearance (6). In the presence of resorption, which can be due to various reasons, adequate tissue support may not be available for the implant restorations. In such cases, the base and edge extensions of full dentures will compensate for the missing tissues by providing support to the tissues. For patients using a long-term full prosthesis or an implant-supported fixed prosthesis, patient satisfaction in terms of aesthetics may not be met since adequate tissue support cannot be provided. The patient should be informed that an implant-supported removable prosthesis is a more appropriate treatment option in these cases (12).

Residual subgingival dental cement, which is one of the leading factors in the formation of peri-implant diseases, cannot be easily removed on crown and gingival groove surfaces (2,15).

IMPLANT-SUPPORTED PROSTHESES FOR EDENTULOUS PATIENTS

In the case of the loss of all teeth, chewing and speech functions deteriorate; there are also aesthetic problems and people experience a loss of self-confidence (16). The prosthetic treatment option for an edentulous patient is a complete denture prosthesis. Aesthetics, function, phonation, and comfort are successfully resolved by the complete prosthesis. Implant-supported prostheses can be considered as treatment options for elderly patients with decreased adaptation capabilities and impaired muscle-control mechanisms, as well as for those for whom complete prosthesis retention is insufficient and patient comfort cannot be provided. Prosthesis planning should consider the amount of crest and crest morphology in the implant prostheses applied to completely toothless patients (17).

Overdenture Prostheses

Patients with complete toothlessness are expected to have fixed prostheses at the dental implant treatment stage. However, in cases where bone resorption is high, the provision of lip support should be considered, and an implant-supported overdenture prosthesis should be planned. For overdenture prostheses, there must be sufficient vertical distance in the mandible, and the implants must be within the limits of the prosthetic base. In the case of implants, a vertical distance of 9-11 mm should be available, and the prosthesis should have a sufficiently thick layer of acrylic resin to withstand chewing forces without breaking (17). A minimum of two implants are

required to support in implant-retained overdenture prostheses. The lower jaw is usually placed in front of the mental foramen to provide opposite arch stabilization. If the amount of bone available is appropriate, the number of implants can be increased, and the biomechanical support plane can provide prosthesis retention and stability (16).

Overdenture prostheses utilize different types of retainers, which are placed on the implant. The holder shapes are bar connections, stud/ball head holders, and locator attachments. The applied holders contribute to prosthesis stability and retention. Since the support of the prosthesis over the implant is provided by the implant and the mucosa, long-term resorption is less than that of conventional full dentures (16).

Implant-supported Hybrid Prostheses

The application of a fixed hybrid prosthesis on the implant application between the mental foramens or the distal part is a reliable and predictable treatment approach in the case of a completely edentulous mandible. By placing dental implants between the mental foramens, it is possible to eliminate the constraints due to bone deficiencies in the posterior region of the lower jaw (1,17).

In the case of tooth loss in the upper jaw, vertical and medial resorption is observed in the alveolar crest. Accordingly, the anterior region base of the prosthesis must be extended in order to provide existing lip support. Implant-supported fixed hybrid prosthesis planning can be considered in cases where maxillary resorption is at the at a minimum and in the anterior/posterior direction (1,17).

When conventional complete dentures cannot be used, implant-supported fixed prosthesis may be an alternative treatment option, providing high levels of stability and retention (17).

IMPLANT-SUPPORTED PROSTHESES APPLIED TO PARTIALLY EDENTULOUS PATIENTS

Dental implant applications are successfully performed in the prosthetic rehabilitation of patients who have lost one or more teeth. In the case of a partially edentulous mandible and maxilla, fixed prostheses are applied, and patient satisfaction is high. Although the number of missing teeth is more or less, the prosthetic treatment of the implant differs depending on whether the toothless cavity is located in the anterior or posterior region. There are two different options: cementation of the implant over the prosthesis to the support structure or connection of the implant-crown structure with a screw (16).

Screw-retained Restorations

This is based on screwing the passively-seated prosthesis on the implant in the palatinal, lingual, or occlusal surface by means of the entry path (16). The main advantages of such restorations are the absence of problems due to dental cement overflow and the ease of repetition in cases where additional restorations need to be reconstructed. However, screw loosening and/or fracture poses a major problem. In addition, in cases where the vertical distance is insufficient, screw connections negatively affect the occlusal morphology of the implant restorations. If the dental implant margin has a subgingival area deeper than 3 mm, the removal of cement residue becomes very difficult, and restoration planning with a screw connection is the best treatment option (17).

Cement-retained Restorations

Depending on the implant placement angle, the screw opening may come to the buccal or facial surface, especially in the maxillary anterior region. In these

cases, cement-retained restoration is indicated. However, problems may occur due to dental cements, and there are other disadvantages, such as the difficulty of removal in cases where the restoration needs to be renewed. In implant-supported cement-retained crowns, the abutments should resemble the appearance of a natural tooth preparation (2,17). A better aesthetic is provided by cement-retained restorations, and less fractures occur in the occlusal material since the ceramic integrity is not disturbed by the screw entry path (16).

Different designs have been realized by clinicians in order to prevent peri-implantitis due to cementation in cement-retained implant restorations. Various combinations of screw and cement-retained restorations have been performed (18-20).

In order to prevent peri-implant diseases due to the presence of cement—which is one of the disadvantages of the cemented-retained prosthesis used in single crown applications on the implant— as well as prevent the occurrence of screw fractures in screw restorations, screw-cemented (screwmentable) crown applications have been introduced. Since the abutment is hexagonal in single crown applications, screw loosening and cement dissolution due to the rotational forces on the crown are prevented.

Application of Screwmentable Crowns

Depending on the clinical experience of the physician, the impression can be performed either by standard or open tray methods. If the implant placement angle is in the normal position, the standard hexagonal abutment selection is performed, and the model obtained for the formation of the crown substructure is sent to the dental laboratory (Figure 1). A metal substructure is obtained such that the abutment screw entry path is open, whose

metal substructure is considered to be ceramic crown work.

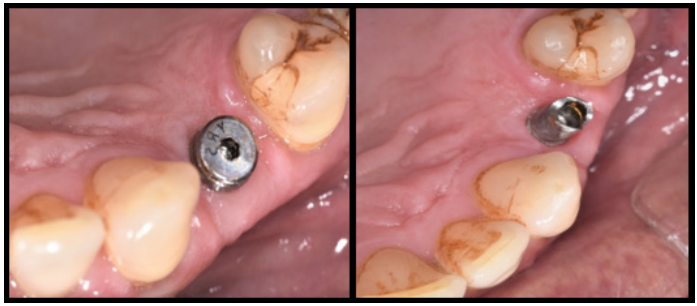

Figure 1: *The appropriate implant angle and abutment height should be available for single-implant restorations.*

The standard metal infrastructure fitting stages are performed in cement-retained restorations, and the infrastructure is sent to the laboratory for the addition of porcelain. When the porcelain application is performed, the abutment entry path is left open, as in the metal infrastructure. As a result of evaluations, such as the relationship between the restoration and periodontal and adjacent teeth, occlusal harmony, and aesthetic appearance, the appropriate restoration is sent to the laboratory for glazing.

After the final restoration is placed in the mouth and checked, the abutment screw and its cavity must be covered with a temporary filling material, teflon, or cotton so that cement cannot escape into the cavity after the cementation process is performed. The cementation material starts to harden, and the cement on the abutment screw inlet path and the overflow in the plastic phase are cleaned. In order to remove cement overflowing in the cervical region and the subgingival area, the restoration must be removed again (Figure 2), At this stage, it is necessary to wait long enough for the cementation material to fully harden in order to avoid decementation of the crown. The abutment screw is loosened, and the crown is removed as

a whole with the cemented abutment. After the cement overflowing in the cervical region and the subgingival area is thoroughly cleaned, the crown on the implant is put in place and torque is ratcheted at the appropriate value, as specified by the implant manufacturer.

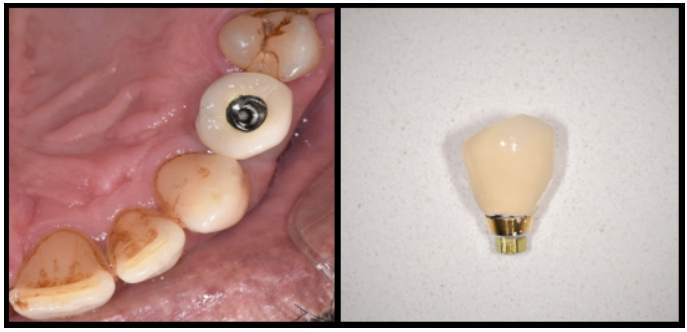

Figure 2: *The abutment screw entry path should be left open, and the crown abutment should be removed in one piece after the cement hardening is completed.*

After the final torque application, the abutment screw entry path is closed with teflon or a temporary filling material, and occlusal porcelain, a flowing composite resin material of a suitable color, is used to cover the part left open. After the polymerization of the composite resin material, occlusion control is performed again. The patient is then informed about oral care and controls (Figure 3).

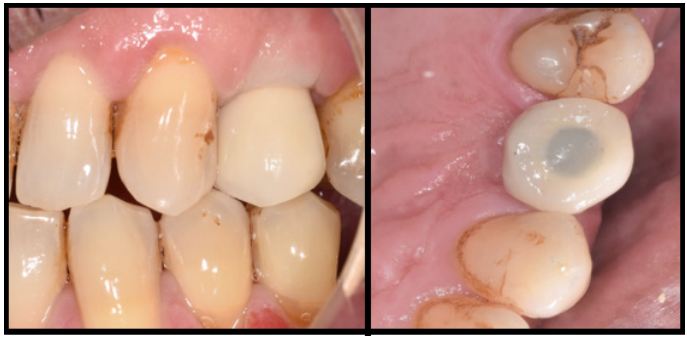

Figure 3: *Final restoration is completed.*

In the screwmentable type of implant-supported crown-bridge applications, if the placement angles of the implants are in a normal position, no-hex abutment selection is performed, and the model obtained for the formation of the crown substructure is sent to the dental laboratory. The subsequent steps are the same as those performed in single crown restorations.

There are many important advantages to screwmentable restorations, such as cleaning the cement, easily finding the abutment-screw entry path, and easily removing and renewing the crown. However, it has disadvantages, such as being easily decemented in cases where there is insufficient abutment height, discolorations in the dental porcelain-composite resin junction due to the closure of the abutment inlet path with a flowable composite, and fractures in occlusal surface ceramics.

CONCLUSION

Dental implants are used extensively in the field of dentistry. In order to ensure the success of the applied treatment, a good analysis of patient expectations, appropriate indications, and correct surgery and prosthetic treatment options are extremely important. The implants must be placed in the proper and correct position for the prosthesis, and the distance to adjacent teeth or implants and the implant outlet profiles must be considered. To reduce the failures seen in implant-supported prosthesis, the factors causing these failures must be eliminated. In cases where cemented restorations should be used, the difficulty of removing the cement in subgingival areas should always be borne in mind. In order to prevent peri-implantitis caused by cement and reduce failures, screwmentable restorations, which combine the advantages of cemented and screw restorations, can be used.

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Chapter 10

ELECTROMAGNETIC FIELDS AND CANCER

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INTRODUCTION

With the development of technology, the use of electrical energy in the society and the interaction with the devices that produce electromagnetic field are increasing.[1] Concerns about extremely low-frequency electromagnetic fields are increasing. Research report are suggesting a possible relationship between electromagnetic fields and childhood cancers. Although the frequency ranges of ELF-EMF fields vary between 1-300 Hz, most of the electrical devices in daily life operate between 50 or 60 Hz.[2] With the rapid increase in mobile phone technology, accessibility in society is increasing. They form an electromagnetic field in the range of 900-2100 MHz. The use of mobile phones by individuals at home, at school and at work continuously has effects on human health and living organisms. [3]

RF-emitting devices are widely used in industry, telecommunications, medicine and everyday life.[4] In 2011, the International Agency of Research on Cancer (IARC) commission classified radiofrequency electromagnetic fields as carcinogens for humans (2B). In the light of epidemiological studies and scientific information, this committee re-evaluated the recommendations regarding the cancer risks of RF radiation in 2019. IARC has proposed an update to the "probable" that RF recipients may be potential (Group 2A) cancer agents.[5]

When a new electromagnetic source is planned or installed, the health impact should be minimized as far as possible according to ALARA (As Low as Reasonably Achievable) principles.[4]

This chapter focuses on the effects of man-made electromagnetic fields, which have been increasing in our environment in recent years, on humans. These biological effects are of great concern in society.

Biological effects of electromagnetic fields

The mechanisms of interaction between EMF and biological systems have been studied for most of this century. RF fields induce torques on molecules. Biophysical modelling approaches contribute to the understanding of radiofrequency interactions at the cellular and molecular levels.[11] 900, 1800 and 2100 MHz RF fields have been reported to cause oxidative DNA damage in brain tissue of rats.[3] The workers working in the hairdressing salons constantly interact with the hairdryer. It is stated that the electromagnetic field created by this device reduces the total antioxidant level and increases the oxidant level in the blood serum of the employees.[8] High frequency EMF waves have more energy than low frequency waves and therefore tend to be more harmful. In general, according to the literature review, although there is no relationship between ELF-EMFs or RF-EMFs and childhood cancers, they state that these results have short-term exposures. To obtain definitive answers, long-term studies are needed.[9] RF fields may alter the transmission of Na and K ions in the cell membrane.[11] There are scientific reports that long-term occupational exposure to ELF-MF can increase the risk of Alzheimer's disease and dementia in men.[12] Static and ELF-EMFs on living organisms are altering free radical activity in the cell. However, chronic exposure leading to the excessive and persistent presence of free radicals can cause oxidative stress and should be avoided.[17]

In cell culture studies, ELF-EMF exposures (50 Hz, sinusoidal, 1–24 h, 20–1,000 microT, 5 min on/10 min off) may cause single and double-strand DNA breaks depending on dose and time.[13]

Electromagnetic fields and cancer

Mobile phone users who use more than the long period of 10 years, glioma, acoustic neuroma, and has been reported

to increase the risk of intracranial tumor.[4] Long-term exposure to radiofrequency electromagnetic fields, even below the limits (0.04 and 0.4 W/kg SAR) for humans, has been shown to increase significantly in the number of tumors in the lung and liver of animals compared to the control group.[6] There is a thermal or non-thermal mechanism that supports tumor growth of the biological process underlying a possible relationship between exposure to mobile phones and cancer risk.[10] Some epidemiological studies have shown that exposure to ELF-EMFs may pose an increased risk in certain types of adult and childhood cancers, including leukemia, central nervous system cancer, and lymphoma.[13] In Denmark, the incidence of cancer has been investigated in people with occupational exposure to electromagnetic fields. In the study, it was emphasized that there is an increased risk of leukaemia in some occupations working in the electricity business. Besides, a slight excessive risk for male breast cancer in these areas has been proposed, but has not been confirmed by a coherent increase among women.[14] Studies on cytogenetic damage and increased cancer risk in human cells are important and needed.[15]

Some studies Show that RF fields are not related to cancer formation.[11] Nevertheless, studies linking ELF-EMF to cancer are weak. More and better research is needed.[16]

However, the options of cancer treatment in medicine have been investigated. Although electromagnetic fields (EMF) in medicine are used for therapeutic or diagnostic purposes, the use of non-ionizing EMF for cancer treatment is an emerging concept. Radiofrequency radiation by clinical oncologists has been used as a hyperthermia approach at high temperatures.[7]

Antioxidants against EMF fields

The use of ganoderma and melatonin has been reported to protect oxidative damage caused by electromagnetic

fields.[1] Vitamins E and A play a role in reducing oxidative stress caused by cell phone exposure to testes.[18] Different doses of ionizing radiation were used on E.coli bacteria. Carob, basil, ginger, rosemary, yarrow and cumin showed a protective effect against the effects of radiation in the study.[19]

Research methods used

Proliferative cell nuclear antigens (PCNA), TUNEL assay, histological, histopathological and various microscopic imaging method are used to determine the effects of cells exposed to electromagnetic fields.[1] The effects of electromagnetic fields on learning and memory, the behavior of the experiment animals are made with the Morris water tank.[2] The Comet assay method is used to detect DNA damage at a single-cell level.[3]

Result

With the development of technology, electromagnetic devices are increasing in our environment. Especially in developing countries and around the world, the interaction time with these devices increases and raises concern. In recent years, accessibility to mobile phones has been increasing. At the same time, occupational electromagnetic field exposures also make workers uneasy. Therefore, scientific studies on electromagnetic fields are increasing and warnings are made about their effects on the biological system. Research reports on the relationship between cell phones and other electromagnetic field exposures to cancer are increasing. In addition, although there is not a complete consensus in the scientific studies, international commissions are working on these areas. Longer electromagnetic field exposures and more reliable data are needed.

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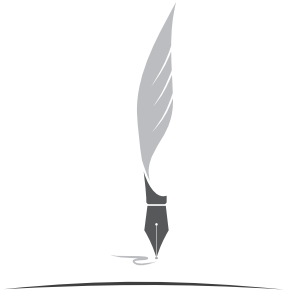

Chapter 11

HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY IN OVARIAN CANCER

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Despite advances in the field of surgery and chemotherapy in parallel with the developing science and technology, ovarian cancer is a life-threatening malignancy that has a significant place in gynecological oncology. Although it is the fifth most common cancer in women, it constitutes 3% of the cancers seen (1). It has the highest mortality rate among gynecologic cancers. In 2011, it accounted for 6% of deaths related to women's cancers (2). In 2009, itwas seen in 6.9 individuals per one hundred thousand people (3). 1.4% of women in the general population are at risk of ovarian cancer. This risk increases in women with inherited genetic anomalies. In women with breast cancer susceptibility (BRCA) mutation, this possibility increases up to 40 percent (4).

Symptoms of ovarian cancer include; persistent inguinal and abdominal pain, increased abdominal circumference, bloating, difficulty in eating, and quick saturation, increased need for urination, and / or urgency(5). Therefore, usually,diagnosis is made at Stages III or IV., and the survival rates are low. According to the International Federation of Gynecology and Obstetrics, it has four stages. Stage 1; cancer is limited to ovaries only with an incidence of 20% and survival rate of73%. Stage 2; cancer has spread out of the ovaries and grows within the pelvic-confined area with an incidence of5% and survival rate of 45%.Stage 3; cancer has spread into the abdominal cavity with an incidence of 58% and survival rate of 21%. In stage 4, cancer metastasizes to other body organs away from the ovary with an incidence of17% and the survival rate less than 5% (6). The five-year survival rate in advanced stage is 37.6%. Therefore, additional treatment with surgical treatment and chemotherapies are required along with standard treatment. High-grade serous type with rapid spread constitutes 60-80% of ovarian cancers (7).

Treatment of tumors spreading to the intraperitoneal region, such as the later stages of ovarian cancer, is a critical problem in oncology. As standard treatment for this type of cancer, appropriate surgical staging and cytoreduction should be performed, and platinum-basedchemotherapy should be used.70% of ovarian cancers are sensitive to chemotherapeutic agents. For chemotherapy to be effective, the tumor must have a small volume. To achieve this aim,cytoreductive surgery is performed. Survival rate increases with surgical procedures performed. Asstaging surgery,retrieval of abdominal irrigation fluid, type 1 extrafascial hysterectomy, bilateral salpingo-oophorectomy, appendectomy, pelvic-paraaortic lymph node dissection, omentectomy, and abdominal biopsies are performed.Besides cytoreductive surgery, bowel resection, diaphragmatic peeling, splenectomy, distal pancreatectomy, liver resection, resection of porta hepatis, and other procedures may be performed (8). Standard primary ovarian cancer surgery involves the removal of internal genital organs, omentum, and peritoneal tumor implants, while bowel resection is required in 25-35% of thepatients for optimal eradication of the tumor. At the same time, in 74% of the cases, pelvic and paraaortic lymph node metastases, which is parallel with the stage areseen (9).

After cytoreductive surgery complications such as pleural effusion, pneumothorax after resection of the diaphragm, and formation of enterocutaneous fistula after small bowel resection can be seen. Due to their proximity to gynecological organs, the risk of complications such as vascular injury, ureter damage, bladder perforation, bowel injury increases. Injury to organs close to the surgical site may result in partial or complete organ damage and failure, such as acute kidney damage. After vascular injuries, severe blood loss may cause problems ranging from organ perfusion disorder to death. Anesthetic

agents decreaseurine output, glomerular filtration rate, renal blood flow and the excretion of electrolytes (10). The aim of this type of intervention with greater number ofpotential complications is not to leave any macroscopic tumors in the area. However, tumor cells may remain in patients with peritoneal carcinoma. Intraperitoneal chemotherapy is also being used to eliminate residual cells and malignant cells spilled into the peritoneal cavity during cytoreductive surgery.

Administration ofintraperitoneal chemotherapy at high temperature [Hyperthermic Intraperitoneal Chemotherapy (HIPEC)] in addition to cytotoxicity increases drug efficacy as it causes vasodilatation and increased peritoneal blood flow (11). Systemic effects are minimal because the drug is directly in contact with tumor cells on the peritoneal surface. Therefore, side effects due to chemotherapy are rarely seen.HPEC also increases the sensitivity of tumor cells to radiotherapy andchemotherapeutic agents. It provides the distribution of heat and the medication on all surfaces of the abdomen andpelvis. Since it is applied under general anesthesia, nausea and vomiting are not seen. Time elapsed during HIPECallows the correction of functional parameters,*i.e.*body temperature, coagulation, hemodynamics, etc. Also, tumor nodules onthe bowel serosa and mesentery can be excised (12).

Epithelial ovarian tumors spread and contained primarily in the peritoneal cavity due to their biological behavior and natural course. Intraperitoneal administration of the drug results in formation of high drug concentrations directly on the tumor. Three large randomized trials have shown that the use of intraperitoneal chemotherapy in the first-line treatment of ovarian cancer provides an advantage in terms of survival (13). The significant theoretical benefit of intraperitoneal normothermic chemotherapy is the ability to result inlowersystemic toxic side effects while achieving high drug concentrations on

the tumor. Although persuasive data are available, it still has not been included in the routine treatment modalities because it causes postoperative intraabdominal adhesions, and possibly loss of fertility in individuals whose fertility potential should be preserved, and italso increases morbidity (14).

The favorableeffects of intraperitoneal hyperthermia can be listed as follows (15):

• Increases drug penetration into tissue.

• Increases cytotoxicity of selected chemotherapy agents.

• The heat has an antitumoral effect.

• Increased heat on the peritoneal surfaces increases the cytotoxicity of systemic chemotherapy on small tumor nodules.

•Intraoperative chemotherapy ensures the distribution of heat and the drug all over surfaces of the abdomen, and pelvis.

• Renal toxicity of chemotherapy can be avoided by careful monitoring of urine output during HIPEC.

• Since it is applied under general anesthesia, nausea and vomiting are not seen.

• The time elapsed during HIPEC allows the correction of many functional parameters (body temperature, coagulation, hemodynamics, etc.).

• During HIPEC, tumor nodules in the bowel serosa and mesentery can be excised.

The most commonly used chemotherapeutic agents in HIPEC are; cisplatin, doxorubicin, mitomycin C, oxaliplatin, and melphalan. Because of the high molecular weight of these agents, they can remain in the abdominal cavity for a long time and show their cytotoxic effects on

tumor cells with their active pharmacological forms more effectively when compared with the intravenous route (16). Most studies related to this approach in epithelial ovarian cancers have been performed retrospectively with a small number of patient groups and include mostly cisplatin-, and carboplatin-based therapies.

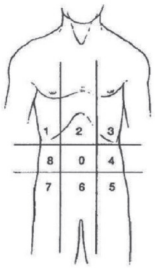
Beforesurgery, prior surgical score (PSS), which is a quantitative prognostic indicator, is calculated in cases withperitoneal surface malignancies. As shown in Figure 1, the abdomen is divided into nine regions, and the number of sites undergoing surgical dissection is recorded (17).

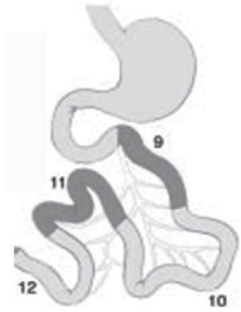
• PSS-0: no macroscopic tumor infiltration

• PSS-1: 1 tumor infiltration in the peritoneal region

• PSS-2: Tumor infiltration in 2-5 peritoneal regions

•PSS-3: Tumor infiltration in the peritoneal region 5-9. Complete cytoreduction, and survival rates are decreased in patients evaluatedas PSS-3.





Regions: *0. Central; 1. Upper right; 2. Epigastric; 3. Upper left; 4. Left flank; 5. Lower left; 6. Pelvis; 7. Lower right; 8. Right flank; 9. Proximal jejunum; 10. Distal jejunum; 11. Proximal ileum; 12. Distal ileum.*

Lesion size:*LS-0 no tumor; LS-1 tumor ≤0.5 cm; LS-2 tumor ≤5 cm; LS-3 tumor> 5 cm.*

Peritoneal cancer index (PCI) is another prognostic scoring system calculated at exploration performed at the beginning of the operation (18). In exploration, scoring is done according to the size of tumor nodules.

• LS-0:Lack of macroscopic tumor focus

• LS-1: tumor nodule smaller than 0.5 cm

• LS-2: tumor nodule is between 0.5-5 cm in size

• LS-3: tumor nodule larger than 5 cm

** If tumor nodules are combined, then all of these nodules are given a score of 3. (LS: lesion size)*

Abdominopelvic regions are used to score the distribution of disease on the peritoneal surface. As shown in **Figure 1**, the abdomen is divided into 13 regions, and the lesion sizes within each region are scored, and the total score is calculated. The maximum score is 39 (13x3). Patients with unresectable tumor infiltration in risky anatomical sites have a poor prognosis, even if the peritoneal cancer index is low (e.g. common biliary duct).

Another prognostic indicator is the complete cytoreduction score (CC), which determines the amount of residual tumor tissue remaining.

• CC-0: Absence of anymacroscopic residual tumor tissue remaining aftercytoreduction.

• CC-1: Total residual tumor tissue less than 2.5 mm after cytoreduction.

• CC-2: Total residual tumor tissue after cytoreduction is between 2.5 mm-2.5 cm.

• CC-3: Non-resectable tumor nodules larger than 2.5 cm.

In high-grade tumors, the ideal cytoreduction score should be CC-0. In less invasive malignancies such as pseudomyxoma peritonei, the complete cytoreduction score should be CC-0 and CC-1.

In summary, cytoreductive surgery for HIPEC including peritonectomy and visceral organ resection is planned to be combined with peroperative intraperitoneal chemotherapy. Surgery should aim to reduce the residual tumor tissue to a microscopic level. Thus, chemotherapeutic agents administered through intraperitoneal and systemic routes can be combined with hyperthermia to eradicate residual tumor tissue.

HIPEC can be applied usingthree different techniques. These are the Coliseum (closed) method, the closed method, and the semi-open method (**Table 1**).

Mortality and Morbidity in HIPEC

The mortality rate withinthe first 30 days after primary cytoreductive surgery in epithelial ovarian cancer was found to be 3.7% on average in community-based studies (19). The mortality rate after cytoreductive surgery in recurrent epithelial ovarian cancer is 1.4% (20). In a review of 19 studies, Chua et al. found a mortality rate between 0% and 10% after the application ofcytoreductive surgery combined with HIPEC in epithelial ovarian cancer. In the HYPERO study performed on 141 patients who had undergonecytoreductive surgery and HIPEC, the mortality rate was found to be 2.1% (21). When the meta-analysis including 13 studies and a total of 256 cases was examined, the mortality rate after application of cytoreductive surgery and HIPEC in recurrent epithelial ovarian cancer was found to be 3.9% (21).

The **Common Terminology Criteria for Adverse Events** classification established by the *International Cancer Institute* was used to standardize morbidity after

cytoreductive surgery and HIPEC.This classification includes postoperative complications that require re-intervention for their resolution. It is classified from 1 to 5 according to the severity of postoperative morbidity.

• Grade 1 mild; asymptomatic or mildly symptomatic; clinical or diagnostic observation is sufficient; intervention is not required.

• Grade 2 moderate; requiring minimal, local, or noninvasive intervention.

• Grade 3 is severe or significant, but not creating an immediately life-threatening condition; conditionsrestrictingpatient's self- care and requiring hospitalization.

•Grade 4 life-threatening outcomesrequiring urgent intervention

•Grade 5 conditions leading to death.

After optimal cytoreduction of 41 platinum-sensitive recurrent epithelial ovarian cancer patients in a single-center, severe complications developed in 34.8% of the patients who received oxaliplatin-based HIPEC treatment and 14% of them required re-operation. The most crucial surgical complication is bleeding. Bleeding occurred in 16.3% of the cases in this study. Five patients had hemoperitoneum, 3 had rectal bleeding, and 1 casehad hemoperitoneum and rectal bleeding. Other major complications were subphrenic abscess developed after pancreatic fistula requiring open drainage, large abscess focus requiring 2 hospitalizations, 1 portal vein thrombosis and 1case of sepsis. Pleural effusion rate was found to be 19.5% (22).

In a review of 19 studies where cytoreductive surgery together withHIPEC was appliedin epithelial ovarian cancer, grades 1,2,3, and 4morbidities were seenin 6-70%,

3-50%, 0-40%, and 0-15% of the patients, respectively (23). The most common postoperative complications were ileus, pleural effusion, infection (wound site), anastomotic leakage, fistula, bleeding, transient hepatitis and thrombocytopenia.

Although HIPEC has been used more widely in the recurrent treatment of epithelial ovarian cancer, HIPEC has also been used in the treatment of primary disease. In 26 prospectively designedmulticenter study of advanced stage epithelial ovarian cancer cases in Italy, HIPEC and cytoreductive surgery were used in primary treatment, and five-year, anddisease-free survival rates werefound to be 60.7% and 15.2%, respectively (24). Chi et al., applied maximal cytoreductive surgery and adjuvant systemic chemotherapy in 210 advanced ovarian cancer patients, and foundpostoperative five-year, anddisease-free survival rates as 58.2% and 31%, respectively (25).

Between 1991 and 2008, the data of 246 platinum-resistant and sensitive patients with recurrent epithelial ovarian cancer who had undergone HIPEC and cytoreductive surgery in two centers in France were retrospectively analyzed, and 62 platinum-resistant and 184 platinum-sensitive patients were detected. In this study, HIPEC,and also using open and closed technique cisplatin alone or with combination of doxorubicin and mitomycin C was applied in 95.5% of thecases. If we examine the morbidity and mortality rates in the study; one patient exited onpostoperative day 12 due to anastomotic leakage, and grade 3 leukopenia was detected in 8, intraabdominal hemorrhage in 6, and anastomosis leakage in 12 patients. At the end of the follow-up period, the mean life expectancy was 48.9 months. The mean disease-free survival was 12.8 months (25).

In the study of 53 patients with epithelial ovarian cancer performed by Roviello et al. the patients who

received cytoreductive surgery and HIPEC followed by systemic chemotherapy (45 primary, 8 recurrent) were followed up for an average of 27 months and complete cytoreduction (CC-0) was achieved in 70% of the patients. In this study, the 5-year survival rate was 71% in patients with CC-0 and 44% in patients with CC-1. The risk of cumulative recurrence in 5 years was found to be 54% in cases in whomCC-0 could be achieved (26).

In another study conducted by Fagotti et al.in Italy,the survival data of patients with platinum-sensitive recurrent ovarian cancer after applications of cytoreductive surgery and HIPECorcytoreductive surgery and systemic chemotherapy were compared. Thirty patients in the HIPEC group and 37 patients in the control group were followed up for at least 24 months, and in the HIPEC group, HIPEC was appliedwith oxaliplatin administered at a dose of 460 mg / m2 using closed technique for 30 minutes at 41.5ºC.The intestinal anastomosis was performed after HIPEC. Mortality was not observed in the patients who underwent HIPEC, and the rate of severe toxicity was 34%, and systemic chemotherapy could be started on the 40th day after HIPEC. Recurrence rate was 66.6% in the HIPEC group and 100% in the control group. During the follow-up period, the mortality rate was 23.3% in the HIPEC group and 62.2% in the control group. Response time to secondary treatment was 26 months in the HIPEC group and 15 months in the control group (27).

When we examined the prospectively designedphase 2 study performed in Italy, 39 primary and recurrent epithelial ovarian (30 recurrent, 9 primary) cancer patients were prospectively examined. HIPEC was administered by deliveringcisplatin + paclitaxel in 11, cisplatin + doxorubicin in 26, paclitaxel + doxorubicin in 1, and doxorubicin in 1 patient. HIPEC was applied for 90 minutes at an average temperature of 41.5ºC. The mean

operation time was 10.9 hours, PCI was<15 in 27 and ≥15 in 12 patients. Microscopic complete cytoreduction (CC-0) was achieved in 35, macroscopic cytoreduction (CC-1) in 3, and macroscopic tumor cytoreduction (CC-3) in 1 patient.The average hospital stay was 23.8 days, and intensive care unit stay was 4.97 days. Postoperative complications occurred in 7 patients (18%), and three patients required reoperation because of development of colon ischemia, abscess, hemorrhage. One patient died duringpostoperative period because of peritonitis and septicemia. Systemic postoperative chemotherapy was given to 27 patients. The averagefollow-up period was 19.8 months. Recurrence developed in 23 patients and average time torecurrence was 14.4 months (28).

In a multicenter study conducted by Deraco et al. between 1995 and 2005 in Italy, the data of 56 patients who had undergone HIPEC after secondary cytoreductive surgery were retrospectively analyzed. Cisplatin + doxorubicin or cisplatin + mitomycin-C agents were applied with HIPEC closed technique in 4-6 liters of liquid for 90 minutes at an average temperature of 42.5ºC. The averageoperation time was 563 minutes. The averagehospital stay was 27.6 days and the averagePCI score was 15.2. In 47 patients CC-0, in 7 patients CC-1, and in 1 patient CC-2 cytoreduction could beachieved. Major complications developedin 15 patients and the procedure-related death occurred in 3 patients. The averagefollow-up periodwas 23.1 months, while average overall(25.7 months),disease-free (10.8 months), 5-year (23%), and disease-free 5-year (7%) survival times were as indicated (29).

In conclusion, in consideration of all these studies, we think that HIPEC treatment will find a wide range of applications in the futurein ovarian tumors and peritoneal superficial carcinomatosis, either asprimary or secondary treatment modality.

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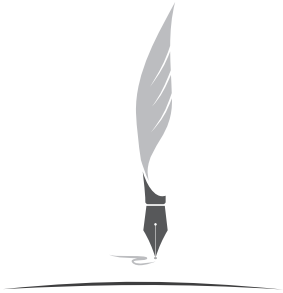
Tables

Table 1. *HIPEC application techniques.*

	Coliseum (open) Method	**Closed Method**	**Semi-open Method**
Efficacy	Surgery may proceed during application of chemotherapy	Surgery should not be maintained during application of chemotherapy	Surgery should not be maintained during application of chemotherapy
Environmental hazard	Environmental dissemination of aerosols may affect the individuals who are applying this method	It is safe	It is safe
Distribution	Adequate distribution over all surfaces	Poor distribution	Adequate distribution over all surfaces
Pressure	It does not increase intraabdominal pressure	It increases intraabdominal pressure and penetration into tissue	It does not increase intraabdominal pressure
Perforation of the diaphragm	Chemotherapeutic drug penetrates into pleural spaces, and its intrapleural distribution may be curtailed	Diaphragmatic perforation is repaired prior to HIPEC, and thus its therapeutic impact on pleura is avoided.	Diaphragmatic perforation is repaired prior to HIPEC, and thus its therapeutic impact on pleura is avoided.
Management of the catheter-related technical problems	It can be easily relieved	Re-opening of the abdominal incision may be required	Closed method is good, but not as good as open method
Detection of occult intestinal perforation	It can be easily detected, and can be repaired during HIPEC	It is very hard to detect, and HIPEC should be terminated for its repair	It is not difficult to detect, and HIPEC should be terminated for its repair
Prevalence	Worldwide use for years	Worldwide use for years	It is gaining popularity in Europe. It is not available in the USA



Chapter 12

FOOD STORAGE METHODS

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INTRODUCTION

When the historical developments related to the inclusion of chemical substances in food are examined, it is seen that salt and wood incense are the oldest known methods of using additives. The use of food dyes dates back to ancient Egypt in 3500 BC. B.C. In 3000 BC, salt was used to store meat products. Both salt and wood incense were used as food storage methods in 900s BC. In the Middle Ages, besides salt and wood incense, nitrate was added to the meats to prevent botulism and the color of the meat appeared to be healthier (Baysal et al., 1990; Çalışır et al., 2001). The development and recent innovations in food and health services have eliminated some of the problems encountered in the past but brought some new problems. It is stated that, after the transition to settled life order, people continued to use a significant part of the food production and storage techniques and spread to the world starting from Anatolia which has been the cradle of several civilizations. Turkish tribes brought their culinary culture together when they came to Anatolia while also being influenced by the cultures of the communities living there. As a result, Turkish culinary culture was formed. In this study, the traditional food preservation methods from Central Asia to Anatolia were examined in a systematic manner by taking food groups into consideration and it was aimed to reveal their continuity and differences (Koşay, 1982; Baysal et al., 1993; Çalışır et al., 2001). Conservation methods have been included in the scope of the research, while the methods applied based on regions have been excluded. The use of food additives (GKM) in the food industry is increasing day by day as a result of the production techniques brought by the technological development and the diversification of the consumer expectatitons. Efforts are underway to develop new methods that reduce losses in food production, extend shelf life and ensure food

safety. Food irradiation, which is one of the methods that have been studied intensively in recent years, is a method that can meet these expectations and its usage is becoming more widespread. Food irradiation provides several benefits for manufacturers, consumers, and sellers. The most important function of this method in terms of public health is the ability to destroy pathogenic microorganisms in foods. The main methods of food preservation are heat application, cooling, drying, freezing, fermentation, adding chemicals, preservation in controlled and modified atmosphere, and radiation (Ünüvar, 2007; Altuğ, 2001; ADA, 2001; American Council., 2003).

1. Heat Application

In a narrow sense, it is preservation by conservation. In this method, food items in conservation containers (cans, jars, bottles, etc.) are subjected to pasteurization or sterilization processes to neutralize microorganisms and enzymes and the can container is hermetically sealed (closing the can so as to prevent any leakage from inside to outside or vice versa) to prevent contamination. Thus, the food can be stored for a long time.

Pasteurization: The process of killing microorganisms by keeping food items with a pH below 4.5 (acidic food) in hermetically sealed containers at a temperature of 85-100° C for a certain period of time. Tomatoes and tomato products, fruits and products made of fruits are pasteurized as they are acidic.

Sterilization: The process of killing microorganisms by keeping food items with pH above 4.5 (low-acid food) in hermetically sealed containers at a temperature over 100° C for a certain period of time. Low acid foods such as vegetables, meat, fish and milk are sterilized as the microorganisms called Clostridium botulinum, which causes “botulism” poisoning, can be destroyed

only at high temperatures. Sterilization of milk by heat is a particularly remarkable process. Milk a very suitable nutrient environment for microorganisms both in terms of nutrients and pH. On the other hand, it can easily undergo changes in terms of taste and the nutrients it contains. Therefore, many methods are used to make milk more durable.

1. ⇥ Short time Heating: At 71-74° C for around 30-40 seconds

2. ⇥ High temperature pasteurization: Heating at 85° C for 5-10 seconds, then cooling to +5° C

3. ⇥ UHT Sterilization: Heating at 135-150° C for 2-3 seconds, then cooling to +5° C

4. ⇥ Classical Sterilization: Heating at 110-120° C for 20-40 minutes

5. ⇥ Continuous Heating (Heating at low temperature for an appropriate period): Heating at 62-65° C for 30 minutes (Ünüvar, 2007).

2. Cooling

Cold storage means that foods are stored at 0-6° C and by selecting the appropriate temperature for each food. Microorganisms cannot be killed by cooling. By controlling chemical and enzymatic reactions, the durability can be extended for days and even months without damaging the quality. In addition to fruits and vegetables, meat and soil oil are particularly suitable for cold storage. Although we store our food in our homes in the refrigerator without any classification, the characteristics of the food must be taken into account to avoid the loss of quality during storage and cooling of commercially large-scale food. In this respect, before cooling the food in the cooling chamber, the suitable

temperature and relative humidity of the food must be calculated.

The following cooling methods are applied in the cooling of food items.

1. Cooling with high airflow in a special cooling tunnel for some fruit and vegetable varieties (strawberries, cabbage) and meat

2. Emptying the total package by evaporation of water (cooling of vegetables such as spinach, parsley)

3. Cooling of melon, asparagus, watermelon and other vegetables with ice water

4. Cooling with crushed ice pieces used for fish cooling

Appropriate conditions must be met when storing food. Some vegetables such as lettuce, parsley, spinach and celery require more than 90% relative humidity. Optimum storage conditions of some chilled vegetables and storage period (Skrede,1996; Rickmann et al., 2007).

3. Freezing

Although the cold storage period for food is limited, frozen food can be stored for a long time without deterioration. Microorganisms cannot function in frozen foods, but enzyme activity continues, albeit slowly. Foods are stored below -10° C by freezing. Frozen fruits and vegetables lose their vitality and defrosted food deteriorates rapidly. In this respect, frozen foods should be brought to the kitchen in a frozen state and kept that way without defrosting. Food should be frozen quickly. If the freezing process is slow, the ice crystals become larger and the ice crystals form in the intercellular spaces. This causes the cell water to go out and damage the cell wall. Thus, the food becomes watery and deformed when

defrosted, deteriorating more quickly. The storage time and degree vary depending on the type of food and the degree of freezing. Boiling reduces the number of bacteria by up to 90%. Boiling can be done with steam or hot water. Boiled and frozen vegetables also do not require long cooking time after they are defrosted (Skrede 1996; Rickman et al., 2007).

4. Drying

Some food items, such as flour, semolina, noodles, are traditionally found in dry form and thus have optimum durability. On the other hand, foods such as milk and eggs are also dried for longer storage. Making food more durable by drying under the sun is the oldest known storage method. A certain amount of water must be present in the food for microorganisms to be active. The purpose of drying is to reduce the water level in foods to a level that limits the activity of microorganisms. Drying can be done by leaving the food under the sun for a while as well as by using artificial dryers called dehydrators. Reducing water activity in food is important to prevent microorganisms that spoil food. In addition to milk and eggs, fruit, vegetables, potatoes and meat can also be dried. There is a lot of semi-finished and finished food products such as coffee and dried soup.

Numerous methods have been developed to properly remove water, as food often reacts too much to temperature. In order to maintain color and quality, sulfur dioxide has long been used for apples, apricots, pears, peaches, and sometimes figs and grapes. To do this, the sulfur strip is burned to produce sulfur dioxide fumes and the fruit is held in the smoke from 30 minutes to several hours. Sun-drying breaks the carotene in grapes, figs, and other fruits. There is little loss of carotene in drying by artificial heating under controlled temperature, humidity, and airflow. Riboflavin is less damaged by both drying

methods. Vitamin C breaks down in both ways in the drying of non-sulfuric fruits. The loss of nutrients during the storage of dry substances depends on the drying process and the temperature of the storage. When the amount of sulfur dioxide is less than 0.5%, many dehydrated fruits darken, their flavor deteriorates, apricots quickly lose their ascorbic acid (Koşay, 1982; Baysal, 1990; Ünüvar, 2007).

5. Fermentation

Fermentation is a biochemical phenomenon that is widely used and is of increasing interest to scientists. Because of the protective properties of lactic acid formed by fermentation, it is known that lactic acid fermentation has been utilized throughout history in preserving and stabilizing fruit and vegetables. Storing by fermentation is possible only for some foods. Fermented foods completely lose their natural properties and a new product with different properties is produced. In this method, acids such as milk acid, vinegar acid, tartaric acid, citric acid are added to the environment (or by promoting the activities of acid-producing microorganisms) and the activity of microorganisms in the neutral environment is prevented. Typical examples of this type of storage are pickle and pickled olives. The activity of some unwanted microorganisms is prevented by adding salt to pickles or brine. Only a 4-5% salt solution is sufficient for dill pickles. They should be stored in a cold environment. Pickled cabbage is made by fermentation in approximately 2.5% salt solution of cut cabbage. This must be stored in the cold or canned (Türker, 1974).

6. Adding Chemicals

The most important chemicals used for protection are sugar, salt, acids, nitrite, and sulfur dioxide (Quattruocci and Masci, 1992; Bağcı, 1997)

Salt: Microorganisms cannot function if 15-25% common salt (NaCl) is added to food. The salt has an antiseptic effect on microorganisms after certain concentrations. It also binds some of the water in food as in sugar, creating an unfavorable environment for the activity of microorganisms. Preservation by salting has been used for canning meat and vegetables since the ancient times.

Sugar: Sugar is effective in canning food due to its significant water-binding ability. For this reason, when the amount of sugar to be added to the food is increased above 40%, it can have a conservation effect. The ratio of water to the product plays a decisive role in determining the ratio of sugar concentration. Accordingly, although 40% of sucrose is sufficient for canned plum, 50-55% sucrose is required for jams and up to 60% sucrose for syrup. The preserving property of sugar is also supported by the fruit acids used (Koşay, 1982).

Acids: Since most microorganisms cannot develop in acidic environments, organic acids can be used to conserve foods. Sorbic acid is particularly effective against mold. It acts by inhibiting dehydrogenase enzyme to molds. It is used in the ratio of 0.1-0.2% (as potassium sorbate) in cheese, pickles, bread etc. Caprylic acid is also effective on molds. It is especially applied to paper wrapped on cheese. Benzoic acid is used as sodium benzoate or pyrohydrolysis benzoic acid at a rate of 30.1% (Charvalas et al., 2001; Boğa and Binokay, 2010).

Sulfur dioxide: Either directly used as $S0_2$ gas or as sulfur salt (sulfides) that produce $S0_2$ gas when decomposed. Sulfuric acid (H_2S0_4) is formed when $S0_2$ dissolves in water. This acid has very high antimicrobial properties.

It is very effective against all microorganisms in highly acidic foods. It is used in Na_2S0_3, $NaHS0_3$, $Na_2S_20_5$,

and $S0_2$ forms for dried fruits, syrups, and purees in a dose of less than 200 ppm. Sulfur dioxide does not only have an antimicrobial effect but also inhibits both enzymatic and non-enzymatic browning (Maillard reaction) reactions. Sulfides prevent yeast, mold, and bacteria. $S0_2$, which is widely used in the food industry, is harmful to health as a substance. Therefore, $S0_2$ should be used consciously, its dosage should be well adjusted and research-based regulations should be introduced for this purpose (Taylor and Bush, 1986; Türkyılmaz et al.,2013; Pundir and Rawal, 2013)

Nitrite and Nitrates

Although Na and K nitrites are mainly used in meat and meat products, they have recently been restricted or completely banned in many countries claiming to be harmful to health. Nitrite can then be converted to nitrosamines, a carcinogenic substance in the organism. It has an antimicrobial effect especially in the presence of NaCl. It is more effective at acidic pH. This group of substances is sold in solid and powder form, used in the control of taste, odor, color and microbial stability in cured meat products and fish. Nitrates and nitrites are the most effective on Clostridium botulinum, C. butyricum and C. sporogenes microorganism. Since there is not a better substance that prevents growth and toxin formation of C. botulinum in food, nitrates and nitrites are still used in some foods, although it has some drawbacks. The use of nitrate and nitrite in curing makes the product microbiologically resistant and gives meat products a characteristic color and flavor. Nitrates and nitrites used in meat and meat products for this purpose combine with amines or other nitrogenous compounds in food or stomach and become carcinogenic nitrosamines. However, it should be remembered that nitrosamines are taken from different sources, synthesized, and accumulated in the

body. Nitrates should not be added more than 500 ppm and nitrites more than 200 ppm in foods, even these amounts should be further reduced. Nitrates are naturally found in some green leafy vegetables and water. This situation is of great importance in areas where fertilization methods are applied incorrectly (Yıldırım, 1979; Özçelik, 1982; Bayraktar et al, 1998; Palatoğlu and Sarıçoban, 2012; Hermann et al., 2015).

Sorbic Acid:

Sorbic acid and Na and K salts are effective on mold and yeast. Unsaturated organic acid that is allowed to be used as antimicrobial in food is sorbic acid only. It has little effect against bacteria. Its effect depends on pH and can be used up to pH 6.5. Sorbates are used in the food industry in various cheese varieties, cereals, wine, jam, jelly and marmalade, sauces, ketchup, ready-made salad and salad dressings, fruit cocktails, margarine, dried fruits, meat and fish products, pickles and brine, and syrups. It is most commonly used in the cheese industry. They are the most successful and stable mold inhibitor for kashar cheese (Charvalas et al., 2001; Kıvanç, 1991).

Propionic Acid:

Propionic acid is rarely used in the food industry because it is corrosive and pungent. Thus, Na and Ca salts are preferred more often. They are mostly used as mold and "rope" inhibitors in bakery products and as mold inhibitors and emulsifiers in cheese technology. Ca salt is added to strengthen the bread dough (Ouattruocc, and Masci, 1992).

Benzoic acid

Benzoic acid is used in the food industry mostly in the form of salt. The widespread use of sodium benzoate is due to the fact that its solubility in water is higher than

that of benzoic acid. Benzoates are effective on yeast and mold and less effective on bacteria. Benzoic acid is a solid material and is sold in granular form. Potassium benzoate may be used where sodium has a detrimental effect. Benzoic acid and its salts can be used as calcium benzoate in pickles, various sauces and ketchup, table olives, margarine, jam, jelly and marmalades, cocoa products, biscuits, wafer and cake creams, soft drinks, fruit cocktails, fruit juices and various syrups against molding. Benzoic acid (0.05-0.1%) is mainly used in combination with other preservatives (Ouattruocc, and Masci, 1992; Boğa and Binokay, 2010).

Acetic Acid:

Acetic acid is especially effective for bacteria and yeasts. It is added to ketchup, vegetable pickles, bread and bakery products in the form of free acid or sodium and calcium salt. It is obtained by dehydration of ethyl alcohol. Artificially produced by oxidation of acetaldehyde and butane. Vinegar production is mostly carried out by alcohol fermentation of apple juice, grape juice, sucrose, glucose and malt followed by oxidation of alcohol to acetic acid (Erkan, 2010).

7. Controlled and Modified Atmosphere Storage

The tendency to consume food freshly increases. The most convenient and effective method for preserving food fresh by delaying spoilage is the cold preservation technique. However, nowadays, in addition to cold storage applications, the application of controlled atmosphere storage and modified atmosphere packaging techniques has found an increasing application area in preserving the freshness of foods for a longer period of time. The storage conditions created by adjusting the $C0_2$ and 0_2 ratios in the storage atmosphere in the fresh storage of foods are

called the controlled atmosphere. In this environment, the oxygen content in the atmosphere is generally reduced while the carbon dioxide content is increased, and the rate of gases present in the storage atmosphere is kept constant throughout the storage period. In the modified atmosphere, the air in a package that is impermeable to gas or has a certain degree of gas permeability can be removed by vacuum. After the vacuum packing or the air in the package is removed by vacuum, the package is filled with N_2, $C0_2$ or a certain ratio of these two gases. The main factor in the storage of food in a controlled atmosphere or modified atmosphere is the gas composition in the storage or packaging atmosphere. However, the gas composition to be applied in a controlled and modified atmosphere varies according to the chemical, microbiological, and physical properties of the foods. Other factors that are important in the storage of food in a controlled or modified atmosphere are temperature, relative humidity, and pressure, as well as the composition of gases in the atmosphere. Temperature is a critical factor in a controlled atmosphere and a modified atmosphere. In general, the lower the storage temperature, the slower the tissue and microbial respiration rate. As a natural consequence, the rate of deterioration of the product slows down (Akkaya et al., 2017).

8. Radiation

Some rays (infrared, ultraviolet, x-ray, gamma, and cathode rays) have a lethal effect on microorganisms. Atoms of some substances break down continuously and emit ionized rays to the environment during this reaction. Substances that break down in this way by emitting ionized light are called radioactive substances. Elements such as uranium are naturally radioactive substances. Some elements are artificially converted into radioactive material as a result of unique methods

and processes. Elements such as Co60 or Cs137 are examples of artificially radioactive substances, called radioactive isotopes (radionuclides). Alpha, beta, gamma or x-rays emitted by radioactive materials cause the formation of electrically charged ions in the material they strike. Therefore, these rays are called "ionized rays" or "ionizing rays." Although it has been known for many years that ionized rays have mutagenic and lethal effects on living cells, their use in irradiated storage of foods has been possible after the realization that radioisotopes such as Co60 and Cs137 producing gamma rays can be used for this purpose. Economically feasible beam sources are needed to use ionizing rays in the irradiation of food at the industrial level. Today, two different sources are used for this purpose. These are electron accelerator devices and artificial radioactive substances. Although these two methods are different from each other, the rays produced from these two sources have the same effect on microorganisms and insects found in foods (Lacroix and Quattara, 2000; Cleland and Stichelbaut, 2013).

Gamma Rays

Gamma rays are the most widely used ionizing beam in food preservation. Gamma rays are high energy electromagnetic rays with a short wavelength. Co60 or Cs137 is used as a beam source to produce gamma rays. They do not give radioactive properties in foods. They can also be used to irradiate packaged foods. It can be used to prevent germination in vegetable products such as potatoes, onions, and garlic, to kill insects and larvae in spices and cereals, and to protect fruits such as strawberries against molding. Gamma rays are the most economical method of irradiation for food preservation.

In the irradiation process, the amount of radiation absorbed by the substance, i.e. the irradiation dose, is important for the purpose, the quality of the irradiated

food, and human health. In order to define the radiation dose, it is necessary to specify the units in question. The definitions of these units are given below. 1 Gray (1 Gy): 1 joule of energy per 1 kg of a homogeneous substance under the influence of ionizing radiation.1 Gy= 1 J/kg. In many sources, the radiation dose is also given as rad (radiation absorbed doses), (Cleland, 2013; Alkan, 2008).

CONCLUSION

Today, industrial food types are increasing to meet the increasing food needs. However, various food storage methods have been developed to prolong the life of foods. Some food additives are still a cause of concern because of their advantages and disadvantages. In some studies, it is concluded that processed meat may increase the risk of colon and stomach cancer. It is thought that nitrite and nitrate in processed meat may cause this. Smoked meat with high salt content is also considered as a cause of cancer. It has been suggested that nitrite, nitrate and similar compounds used for food storage may increase the risk of breast cancer and colorectal cancer.

Today, the effect of each substance used as a food additive on human health is examined in detail, and the use of those at significant risk to human health is not allowed. In the last 30 years, there has been a complete explosion of the additives used in foodstuffs, especially in developed countries. There are around six thousand additives, most of them used for aroma/flavoring. As the consumption of these substances increases, there is also evidence of links to certain disorders. The most common ones are eczema, asthma, headache, allergic itching, gastric disorders, diarrhea, hyperactivity (especially in children), and hypersensitivity.

However, it should be noted that unconscious nutrition and increased consumption may lead people to

consume more additives and as a result, have negative effects on health. As a conscious consumer, we need to take into account the content of food items, the stages through which they pass, and their shelf life. Otherwise negative effects will occur. As a result, in addition to balanced and adequate nutrition, we should take care not to consume ready-made foods excessively for our health.

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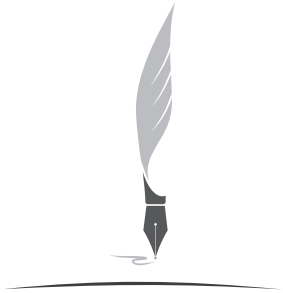

Chapter 13

MIDWIFERY SERVICES IN RURAL AREAS*

Özlem DURAN AKSOY[1]

* *This study was presented as a speech text at the 6th International 10th National Congress of Midwifery Students*

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INTRODUCTION

Midwifery is one of the oldest professions in the world. The profession of midwifery, that arised from social needs, is called with definitions partially differentiating in terms of content (Altay, 2017). According to the World Health Organization (WHO), "A midwife is a person who is trained to provide the necessary care and counseling to mothers during pregnancy, childbirth and postnatal periods, to carry out vaginal births under her own responsibility and to provide newborn care." (WHO, 2019). According to International Confederation of Midwives (ICM), "A midwife is a person who participates in regular training programs, has the necessary midwifery qualities, is legally licensed to practice the art of midwifery, cares for women during childbirth and postnatal periods, and provides newborn and infant care." (Crozier et al., 2007; ICM, 2017). According to the Ministry of Health, "A midwife is a person who graduated from a school registered by the Ministry of Health, conducts maternal and child health services, helps women to give birth, provides services during and after childbirth." (Beydilli, 2007; Çiçek, 2009).

Midwives play a key role in achieving the ideal of healthy generations and healthy society with the goals of protecting, developing and maintaining maternal and child health. WHO reports that maternal and infant mortality, cesarean delivery rates decrease and birth intervals extend in countries where midwives actively work. Midwifery practices, which primarily serve women and children, have a direct impact on public health (Altay, 2017). In this respect, the quality of midwifery services offered in rural areas and the obstacles encountered in the provision of services gain importance.

Midwifery Services in Rural Areas of Turkey

In the past, midwifery services were carried out in rural areas by people who were called "ebe nine" or "ebe anne", these were elderly, soft spoken, mannerly, charming, warm-hearted people trusted in their experience and dexterity in villages and towns. As these folk midwives were usually old and fat, they were called with the Turkish saying *"Karnı burnunda olursa gebedir, burnu karnında olursa ebedir."* which meant that midwives were usually fat but pregnant women were fat because of their pregnancy. The tasks of the folk midwives were to make the necessary preparations for the birth, psychological relief of the woman who were about to give birth, helping women to give birth, caring for the puerperal women and newborn, termination of pregnancy, making recommendations to infertile women and preparing some herbal mixtures (Yeşil & Yeşil, 2018). In time, the transfer of knowledge, manners and experience in midwifery were moved to the traineeship stage (1843-1846, 2-year midwifery courses at Mekteb-i Tıbbiye), but midwifery services in rural areas continued to be carried out with folk midwives as the midwives who were trained in these courses mostly served in urban areas (Altay, 2017; Beydilli, 2007; Kaya & Yurdakul, 2007; Köker, 1997; Sogukpinar et al., 2007). The Ministry of Health dealt with this issue in 1925 under the title of compulsory health problems, focusing on preventive health services, taking the health organization to the villages and raising midwives. Although there was no statistical data on whether maternal and infant deaths occurred during childbirth was due to the error of midwives without diplomas or other reasons, it was considered that increasing the number of midwives with diplomas would be a solution (Altay, 2017; Öztürk, 2011a). Thus, with The Law on the Practice of Medicine and Medical Sciences entered into force in 1928 (Article 47: To be able to practise midwifery in Turkey,

it is needed to be Turkish citizen and to have a diploma from midwifery schools and to certify and register this diploma by the Ministry), midwives who did not get any special education (without diplomas) were prohibited from assisting childbirth and this act was considered as a crime (TR Official Gazette, 1928). In the Public Hygiene Law, which was enacted two years later, municipalities were required to employ midwives for assistance during childbirth (Article 20), midwives were charged with the notification of epidemics (Article 61), and the government, municipal doctors and midwives were obliged to assist in the delivery of poor women (Article 154) (TR Official Gazette, 1930). According to the researches conducted in the following years, some of the medical problems that occurred before, during and after the birth were caused by the wrong practices of the midwives even if maternal age, consanguineous marriage, the interval between the two births, diarrhea and pneumonia were thought to be causes of maternal and infant deaths. In order to solve this problem, a legal regulation was brought in 1936 and the organizational structure was reorganized with the Law on Organization and Officer of the Ministry of Health and Internal Affairs, and village midwives were added to the village health organization. In order to increase the number of midwives with diplomas and to meet the needs of the midwives of the villages, it was decided to form a system to raise the midwives in the villages in Balıkesir and Konya in the same years (two years of education for middle school graduates, employment in provinces and towns, one year of education for primary school graduates, employment in villages). As of 1937, health schools have been opened in different cities with different education durations (9 months, 1 year, 1 and half year, 2 years and 3 years) (Başer, 1997; Beydilli, 2007; Kaya & Yurdakul, 2007; Sogukpinar et al., 2007). With the Directive on the Duties and Employment of Village

Midwives prepared in 1937, village midwives were made obliged to serve for four years in their own villages or nearby villages with a population of not more than 4 thousand, to visit pregnant women at least once a month from six months, to prepare a place for birth, to deliver the baby, to continue to follow up during the postpartum period, to record births and to report to the government doctor about the problems in mother and baby during childbirth (Altay, 2017). The regulations on the inclusion of village midwives in the organizational structure were reorganized by the law enacted in 1943, and providing birth assistance in the village and village groups where they were civil servants, monitoring the health status of pregnant women, performing child care works and handling other health care activities were defined as the duties of village midwives (TR Official Gazette, 1943).

The importance of midwifery education was explained by Dr. Besim Ömer Akalın in his book called "Türk Çocuğu Yaşatılmalıdır" as *"According to the latest number, the female population in our country is close to half of our entire population, and when it is thought that most of these women are married and give birth, it is understood how important it is to take into consideration the midwife issue during the population policy period."* Because in this period, as approximately eighty percent of the country's population lived in villages, midwives who were raised in modern style were thought to be more effective in order to realize the transformation in villages. Therefore, the efforts to exclude the traditionally raised midwives from the health system, the lack of qualified midwives and the lack of financial resources for midwifery education, have largely hindered the services provided in rural areas (Atay, 2017).

In 1943, a midwifery branch was established in the Ministry of Education Village Institutes in order to deliver midwifery services to rural areas, and this education

service ended when the Village Institutes were closed in 1954 (Başer, 1997; Kaya & Yurdakul, 2007; Sogukpinar et al., 2007). According to the National Health Plan prepared in 1946, health centers were designed to serve a population of approximately 20 thousand (for 40 villages). 10 patient beds, two physicians, one midwife, one health officer and one visiting nurse were planned to be employed, and the integration of preventive and therapeutic services was planned to be carried out in these centers, but they were not fully implemented. In addition, a midwife and a health officer were provided for every ten villages. The number of health centers opened in this period was 1 and 16 more centers were opened until 1950 (Öztek & Eren, 2006; Öztürk, 2011a; Sülkü, 2011).

With the Law No. 224 on Socialization of Health Services adopted in 1961, it was planned to establish health centers on the basis of population instead of government physicians. The practices started in Muş for the first time in 1963 and expanded each year in a few more provinces, expanded in 45 provinces in 1979 and in 53 provinces in 1982. With an arrangement made in 1983, a health organization in compliance with this law was built throughout the country (Öztek & Eren, 2006; Öztürk, 2011b; Sülkü, 2011). The basic principles of socialization of health services were listed as being equal, continuous, integrated, gradual, preferential, participatory, team-based, supervised, appropriate and population-based services (Öztek & Eren, 2006; Öztürk, 2011b; TR Official Gazette, 1961). In the organizational model prepared in accordance with the principles of socialization, health centers providing primary care services for 5-10 thousand people and health houses serving 2000-2500 people in places far from health centers were built (TR Official Gazette, 1961). In the creation of the model, considering the distribution and size of villages and hamlets, road conditions, direction of travel for public operations, a

health house for 3-4 villages and a health center for 3-4 health houses were planned to be built. It is possible to say that these institutions are midwife-centered institutions since it is expected to expand the provision of maternal and child health services in these institutions that form the basis of socialized health services. In addition to the services offered in these institutions, midwives carried out a significant portion of primary health care services by making home and village visits. As the midwives stayed in the same building as the health house, they could be reached out 24 hours a day. In this respect, midwives have been in close relationship with the society due to both the health center and health house service model and the characteristics of midwifery services, and have undertaken important duties and responsibilities in community development and in social areas (Yücel, 2016; Yücel, 2018).

The increase in the number of health centers and health houses increased the need for midwives, and new village midwife schools were opened to meet this need, but health personnel could not be trained at the desired level, and there were problems in training and employment processes according to the characteristics of primary health care services. Health Schools that were carried out under different names, periods and programs until 1961, continued their education and training under the name of Health College which was structured with a new program (Başer, 1997; Beydilli, 2007; Kaya & Yurdakul, 2007). Although the model was successful in rural areas, the model could not be made effective in urban areas due to not adopting socialization by managers and health workers, lack of supervision, inadequate distribution of health manpower, alienation of health personnel and society to each other, inadequate budget and material support (Öztek & Eren, 2006; Öztürk, 2011b; Yücel, 2016).

In the 1970s, due to the political changes in the world, the period of privatization of health services was started, various models were proposed for the financing and organization of services and a continuous reform was sought (Fişek, 1991; Şişman, 2010). In 1978, Health Colleges were terminated and they were transformed into Health Vocational High Schools. In 1985-1986, two-year midwifery associate degree programs were started within the Vocational Schools of Health Services. Bachelor's degree in 1996, master's degree in 2000 and doctoral degree in 2013 have started in midwifery (Başer, 1997; Kaya & Yurdakul, 2007; Koçak et al., 2017; Sogukpinar et al., 2007). Health system was restructured with the transformation in health and Family Practice was introduced in primary health care provision. With the endorsement of the Pilot Law of Family Practice Implementation in 2004, the first application was initiated in 2005 in Düzce. In 2010, the Regulation on Family Practice Implementation was endorsed throughout the country. In this model, health centers were converted into family practice centers (serving 3500 people on average), and this system was composed of a family physician working together with a family health worker (midwife, nurse, health officer and emergency medical technician). Midwives working in health houses were asked to work under the family physician(s) to whom the region was connected and to work under the community health center in administrative terms (TR Official Gazette, 2010).

According to the system, health houses are institutions created to facilitate accessibility to villages and providing multi-faceted services in areas other than the settlements where family health centers are located (TR Official Gazette, 2015). According to the Turkey Statistical Institute data, health housed can be opened in towns, villages and hamlets where the total population is over 500. More than one town, village and hamlet can

be connected to a health house (TR Ministry of Health, 2017). One midwife often works in health houses, and these midwives mainly provide maternal and child health services, they are responsible for detection, monitoring, delivery and control of pregnant women, monitoring and control of puerperal women and children, vaccination services, infectious diseases, family planning, nutrition and health education, environmental health (TR Official Gazette, 2015; Öztek & Eren, 2006). In the Public Health Center and Affiliated Units Regulation published in 2015, responsibilities of midwives and nurses working health houses were described as carrying out preventive health services with the family physician, supporting mobile health services, keeping records about health, evaluating health criteria and identifying problems and priorities, identifying risky pregnant women in the region and taking action to include them in the scope of guest mother practice, identifying individuals in need of home health care services and reporting them to family physician and coordination center, taking part in pre-marital counseling services, taking part in infectious disease control programs, monitoring chronic patients, taking part in obesity counseling services, taking part in school health services and public education (TR Official Gazette, 2015).

Quality and Barriers to Midwifery Services in Rural Areas

The services provided by midwives and nurses working in health houses in our country are very important for public health especially in rural areas that are away from the family health center. Therefore, the effects of changes on the provision of services in rural areas along with the changes experienced in the health system and barriers encountered in the provision of services have been evaluated together with the results of researches.

In the studies conducted in our country, there were differences in the roles and responsibilities expected from midwives working in health houses according to the socialization practice and there was a decrease in the satisfaction levels of midwives. More than half of the employees in the health houses received in-service trainings about their duties and responsibilities, half of them had undergraduate-graduate degree and their duration of working in the health house was less than one year. Less than half of them did home visits, did trainings to improve school health, did also outpatient clinics and gynecological cancer prevention trainings, birth preparation training and premarital counseling training within the scope of mobile health services (Akbaba et al., 2018; Çiçeklioğlu et al., 2013; Duran Aksoy et al., 2017). Although these results show that midwives perform some independent roles at a lower level, the factors affecting the results are thought to be related to lack of experience, problems in health manpower employment and midwifery roles not being made visible in the system.

Barriers that affect the provision of health services in rural areas and reduce quality include inadequate employment of health workers, unfavorable working conditions and transportation problems (Filby, McConville, & Portela, 2016). Inadequacies in health houses are listed in the researches as materials, safety, reaching to the population and dispatching, physical characteristics of the working environment, and it is stated that the provision of adequate working environment and appropriate housing can be motivating for working in health houses (Ageyi-Baffour et al., 2013; Akbaba et al., 2018; Belaid et al., 2017; Crowther, 2016; Duran Aksoy et al., 2017; Rehman et al., 2015).

Another obstacle faced by health workers in rural areas in the provision of services is related to knowledge and competence. In a study conducted in our country,

80.7% of midwives and nurses working in health houses stated that their knowledge was sufficient to work in health houses and 57.9% stated that they received in-service training about their duties and responsibilities in health houses (Duran Aksoy et al., 2018). In a study conducted in Indonesia, it was found that 94-95% of midwives working in rural areas attended some in-service training courses to improve their capacity to recognize and manage complications (Makowiecka et al., 2008). In this respect, health workers working in rural areas should participate in in-service training activities that will increase their knowledge and competencies. Because it is difficult to provide quality midwifery care and to provide access to care for women and families unless there are midwives equipped in terms of knowledge and skills in the society (Pettersson, Sherratt, & Moyo, 2006).

Since health professionals working in rural areas work alone, unlike their peers working in urban areas, they need to be competent in decision-making and referral in emergencies (Miller et al., 2012). The most basic skills needed for rural midwifery practices are confidence and expertise in decision making and when to refer (Hundley et al., 2007). Knowing when the "normal" situation changes is a basic skill no matter in which environment the midwife works. At the same time, midwives should be aware and able to manage the geography, climate and the preventive effect of the rural area (Gilkison et al., 2018).

In the National Action Plan on Empowering Women in Rural Areas in Turkey, it is stated that there are important opportunities and access inequalities in rural and urban areas in terms of health opportunities and this situation affects the active participation of women in all areas of society and private life in rural areas. It is reported that there are economic, social, cultural, environmental and gender inequalities on the basis of the health problems experienced by women in rural areas and they are reported

to face some health problems more. Therefore, it is aimed to increase the general health level of women living in rural areas by improving the conditions of access to health services and the quality of services provided (TR Ministry of Food, Agriculture and Livestock, 2012).

CONCLUSION

Changes in the structure of midwifery services provided in rural areas have changed over time and this has affected the quality of services by combining the characteristics of health workers, the difficulties of working in rural areas, the characteristics of the areas served and the obstacles encountered in service provision. In line with these results, it is recommended arranging job descriptions of health workers working in rural areas within the framework of roles and responsibilities that emphasize midwifery-specific functions, making midwifery services visible in the system, providing in-service trainings on the subjects they need, providing awareness about the importance of home visits and encouraging and improving healthcare facilities.

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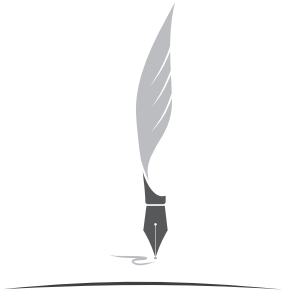

Chapter 14

MECHANISM OF POSTOPERATIVE INTRAABDOMINAL ADHESIONS

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INTRODUCTION

Fibrous bond which develops between two or more adjacent tissues or organs are defined as “adhesion” (1). Abdominal operations and intraabdominal infections are the most common causes of intraabdominal adhesions (1). Adhesiolysis performed either with sharp or blunt dissections leads to novel intraabdominal adhesions. An abdominal surgical intervention which is a peritoneal trauma is the most important stimuli for fibrous band formation. Ischemia of abdominal wall and intraabdominal organs were detected to lead to intraabdominal adhesions in animal tests. Particularly magnesium silicate derivatives of foreign bodies, surgical glove powder, pieces which adhere from textile products like gauze and compress lead to intraabdominal adhesions. Desquamation of peritoneal mesothelium may lead to intraabdominal adhesion.

Parietal peritoneal surface which covers the inner surface of intraabdominal walls and visceral peritoneal surface which covers intraabdominal organs are coated with mesothelial cells. While peritoneum opens to external environment through tubae in females, it is a closed space in males. Mesothelial cells have a good regeneration capability (2). Serous-mesothelial layer is a connective tissue with a well-developed vascular structure and rich from collagen and elastic fibers. Normally, about 50 ml of free fluid with properties of transuda may be present in intra-peritoneal space. This physiologic fluid is continuously secreted and absorbed. Absorption to lymphatic vessels is drained to systemic blood circulation via ductus thoracicus (3). Defects in various sizes which develop due to surgical trauma recover within 3 or 5 days concurrently. Mesothelial cells which are the main factors of this recovery usually originate from migration of particularly sub-mesothelial stem cells (4). Although this migration results rapidly and without adhesion formation,

likelihood of adhesion would increase with the increasing severity of the trauma.

Peritoneal wound healing

Responses occur at biochemical, physiologic and cellular level as response to the inflammation following trauma which impairs the physical and chemical structure of the tissue, and insufficient perfusion (5). Angiogenesis and epithelization phases continue following coagulation and inflammation phases. Tissue healing terminates with fibroplasia, matrix deposition and wound contraction. During this cellular and biochemical events, initially platelets and leucocytes take place in inflammatory phase and afterwards macrophages take place in clearing of necrotic tissues and foreign cells. Mainly fibroblasts, vascular endothelial cells and epithelial cells also play a role in new tissue formation (6). These cells pass into extra-cellular field with the effect of growth factors and cytokines. Growth factors are related with wound healing and cytokines like interleukin and tumor necrosis factor are message molecules which target leukocytes (7).

In peritoneal trauma, the initial response is vasoconstriction of local vessels as in all tissues. Peritoneal fluid increases with decreased vascular tone, both inflammatory and pro-coagulant factors from neighboring mesothelial tissues increase at trauma site. Platelets release many molecules through binding to the matrix which emerges with tissue damage. Platelets release epinephrine, serotonin, insulin-like growth factor, transforming growth factor and platelet-derived growth factor. They are chemotactic for these bioactive substances and the other inflammatory cells, lead to fibrin clot formation. This event is defined as “coagulation”. Fibrin clot is formed between wound surfaces which are separated due to trauma. These temporal fibrin bridges are the first step of adhesion formation. Fibrin matrix begins

to form. Plasminogen is the most important enzyme of this step. Fibrinolysis step of which plasminogen is the key protein stops following trauma, particularly at the first 48 hours (8).

In inflammatory process, number of poly-morphonuclear cells increases at wound site at 12nd hour and these cells are especially effective against bacteriae. Macrophages become predominant at wound site at 24th hour (6). Macrophages play a role in clearance of pathogens and necrotic tissues. Inflammatory cells settle on these fibrin bands if they are not disrupted. On day 4, each inflammatory cell which comes to wound site increases metabolic need. Oxygen supply and oxygenation impair due to local circulation disorder as micro-vascular system is also damaged. Tissue carbon dioxide level increases when oxygen level decreases and lactate accumulates together with the increased respiration (8). Lactate is critical for wound healing. Fibrin leads to release of various cytokines, chemo-attractants and growth factors from various inflammatory cells, mainly macrophages (5). Fibroblasts begin to become dominant at wound site at 5th day. So wound healing progresses to proliferation phase from inflammatory phase (7).

In proliferation phase, while extracellular matrix with high collagen content is formed under fibroblast predominance, inflammatory exudates with fibrin disappears (7). Both formation and re-modeling of matrix are under the influence of many enzymes and factors. Physical barriers under surgical use for reducing intraabdominal adhesions target this phase of wound healing (9). Activity of fibroblasts and mesothelial cells increase during matrix re-modeling. So collagen proportion at wound site also increases. While stronger and flexible macro-molecules like proteoglycan increase at wound site, the molecules like vitronectin and glucose-amino-glycan decrease. While these macro-molecules

preserve wound tension, they also enable to keep wound margins together. Collagen converts to a more stable and stronger structure by creating a lysyl-lysyl bridge with di-oxygenase and lysyl hydroxylase enzymes between procollagen molecules. Ascorbate and sufficient oxygenation are quite important for this stage. Also water absorption occurs at wound site and this leads to tighter adhesions during wound healing (8).

Angiogenesis is an important stage of wound healing. Angiogenesis begins with new capillary formation from the venula around the wound as response to chemo-attractants released 24-48 hours after the occurrence of wound. Angiogenesis becomes more prominent at the end of fourth day (8). Capillaries at both sides of the primary healing wound come together in a short time and thereby blood supply can be provided along with the wound. In secondary healing wounds, new capillary network joins only with the close capillary at the same direction and granulation tissue begins to develop. Angiogenesis develops as response to hypoxia and local energy insufficiency. Chemo-attractant peptides are released from macrophages for proliferation and migration of endothelial cells in case of increased lactate following hypoxia and increased anaerobic metabolism (6).

Epithelial cells which are required for epithelization are also sensitive to chemo-attractants like fibroblasts and capillary endothelial cells. The cells at wound side are seen as a few lines as the result of mitosis at the beginning of epithelization and newly formed cells begin to migrate towards the center of the wound. This movement particularly occurs with the effect of growth factors and cytokine, a new wound margin is formed with cells located at the region without epithelium. However transforming growth factor is stimulated as oxygenation of new epithelial cells. So maturation of newly developing

epithelial cells stops and mitosis accelerates again. This procedure is repeated until wound closure (8).

Maturation, lysis and contraction of collagen fibers are important for wound healing. Particularly the fibroblasts in the wound change temporal fibrin matrix with collagen monomers. These collagen monomers are polymerized in the wound however they are weaker at the early stage as they are polymerized less regularly compared to normal. This explains why wound healing is weak in the early period. The weak matrix tissue would convert to a stronger and stable matrix thereafter. Conversion and strong organization of this new matrix is very important for healing. Collagenases are released from platelets and leukocytes. Collagen degradation slows down later while it is faster at the beginning. A successful healing can be achieved as long as the new matrix could meet the accompanying lysis. If collagen synthesis is insufficient, the wound stays much weaker due to the current lysis. Under normal conditions, the wound gains strength and durability during this conversion but still vulnerable against contraction and tension (10). The receptors in fibroblast membranes bind to collagen molecules. It becomes more stable through various cross links between these fibers. Both open (secondary healing) and closed (primary healing) wounds tend to become contracted if they are not exposed to this power. This phenomenon is best seen in closures with more than 90% contraction in superficial wounds of the skin. While this is useful in back, thigh and neck regions, it leads to esthetic and function disorders in mobile regions like face and joints. This undesired outcome is defined as “stricture development” (7). Tension develops in the wound if proportion of tension exceeds contraction during maturation. This event explains loose scar formation in injuries of connective tissue of joint and increased incisional hernia rate in obese patients. If wounds are re-injured when strained,

contraction and fatigue are long standing and may lead to wound site problems. Despite remodeling, the clear consequence of healing is scar formation. The wound continues to gain strength for two years despite the absence of an increase in collagen amount after 42nd day. Wound strength depends on cross links between the molecules of collagen fibers and fiber netting (11). Gaining strength begins with suturing the wound in surgical incisions. In primary healing, strength gain begins at fourth day through increased collagen fibers, continues at a constant rate during the first four months and at a slower rate during the following eight months (12). Growth factors are quite effective and controlled in wound healing. Wound healing terminates at a proper time particularly with local hypoxia and decreased lactic acidosis (12).

Adhesions develop from fibrin matrix being organized at the beginning of wound healing. Platelets, erythrocytes, inflammatory cells, endothelial cells, epithelial cells and surgical remnants are found in fibrin matrix. While fibrin can initially close the traumatized peritoneal surfaces, they may also be closed with gastro-intestinal lacerations, omentum and neighboring organs. The fibrin in intraabdominal field is normally degraded with plasmin, a fibrinolytic enzyme. However fibroblasts regionally proliferate as fibrinolytic system would become inactive in presence of prolonged inflammation. This condition leads to permanent adhesion at wound site. Ischemia which is commonly seen together with surgical traumas leads to insufficient blood supply and decreased tissue oxygenation. This condition significantly decreases fibrinolytic activity, fibrin and proliferative formation become substantial and adhesion formation increases (13).

Adhesion pathogenesis

Platelets and fibrin contact basal membrane just after wound formation. Fibrin polymerization begins at this

stage. Mesothelial cells which have normal oxygenation under normal conditions produce plasminogen activators. Fibrin products degraded with fibrinolytic activity following acute trauma. Fibrous adhesions organize with hypoxia and this condition is a very potent stimulant particularly for angiogenesis and collagen synthesis. Fibrous adhesions begin to be seen 10 days after peritoneal trauma and are maximum 2-3 weeks later. This explains why a second operation required within 2-4 weeks following an acute event is challenging. Difficulty is separating the tissues due to adhesions or causing a novel trauma in the organs during adhesiolysis procedure. Remodeling occurs also in these fibrous adhesions as in normal wound healing. Fibrous adhesions lose their strength progressively. While mortality rate is about 20% in the operations performed due to a severe complication like intraabdominal entero-cutaneous fistula between days 10 and 120, it is about 10% in the operations performed earlier than 10 days or later than 120 days (4). In wound healing, while fibrin is degraded with the enzymatic activity, formation also continues. This fibrinolytic predominant period prevents adhesion development in wound healing. Physical, chemical or ischemic traumas impair fibrinolytic activity which prevents adhesion and thereby likelihood of intraabdominal adhesions increases. These traumas decrease plasminogen activator level in the tissues. Level of plasminogen activator inhibitors which suppress fibrinolytic activity also increase. Fibrinolysis develops with decreased fibrinolytic activity and fibroblast cell count increases (14). The novel mesothelium which develops within 2 or 3 days later is completed within one week. Histamine and vaso-active kinin release increases with mast cell activation leading to an increase in adhesion. Histamine and vasoactive kinins increase capillary permeability and lead to serous-angionous fluid accumulation. Fibrous bridges develop between adjacent

intraabdominal tissues when this fluid accumulation is not absorbed or removed (15).

In laparoscopic total extra-peritoneal approach, dissection of peritoneum from abdominal wall leads to intraabdominal adhesions. İntraabdominal adhesion formation is explained with the ischemia and inflammation resulting from the dissection of peritoneal tissue and impairment of vascular structure (16).

Fibrinolytic system is the most important natural defense mechanism against adhesion formation. Tissue plasminogen activator activates the inactive plasminogen. Plasmin enables fibringel matrix to be separated to fibrin and thereby fibrinous adhesion formation is hindered. Fibrinous adhesiolysis occurs and normal tissue healing is completed. Permanent adhesions develop if local fibrinolysis is insufficient. The direct relationship between decreased fibrinolytic activity and increased intraabdominal adhesions was proven also with experimental studies (15).

General principles of preventing intraabdominal adhesions

İntraabdominal adhesion formation may be decreased with interventions to various stages in pathogenesis. These interventions include surgical techniques, pharmacologic agents and physical barriers. Ratio of intraabdominal adhesion formation is smaller in minimally invasive surgical interventions including laparoscopic and robotic interventions than in open surgical methods (17). However, no method or pharmacologic agent is available today to excellently prevent postoperative intraabdominal adhesion formation despite the modern technology.

A five-item approach including reducing peritoneal trauma, preventing coagulation, lysis of fibrin, separating neighboring tissues and preventing inflammatory reaction

was recommended for reducing intraabdominal adhesions in 1942 (18).

Minimizing trauma level and contact with foreign bodies is the main rule for preventing intraabdominal adhesions in abdominal surgery (19). For this purpose, a proper surgical technique should be used, laparoscopic and robotic minimally invasive surgical methods should be generalized, unnecessary fibrin formation should be prevented through sufficient hemotasis, blood or other inflammatory fluid should be cleared from the intraabdominal region at the end of the operation, manipulation should be avoided, ischemic tissue or foreign body should not be left in the abdomen, tissue repair should be achieved without tension, excessive or unnecessary suture material should not be used, infection protection should be provided and the intestines should be avoided to stay dehydrated during the operation (19).

The pharmacologic agents which are used to prevent intraabdominal adhesions influence one or more of wound healing stages. These agents may be used systemically or locally at wound or trauma site. Systemic agents would not sufficiently penetrate into the tissue if adhesion site is ischemic. For the local agents, the durable peritoneal tissue's rapidly and effectively absorbing the agent is an important problem. Corticosteroids, anti-histamines, non-steroid anti-inflammatory drugs and fibrinolytics are the most commonly used agents. Vitamin E, calcium antagonists, interferon, anti-coagulants, progesterone, estrogen, taurin, halofuginone, L-arginine and pentoxyphilin are also used (20, 21). Although adhesion development was shown to be able to be reduced with the use of these agents, it is accepted that these approaches have a limited benefit, increase complication risk and they are not completely useful (21).

İntraabdominal adhesions may also be prevented by using physical barriers (22). An ideal physical barrier should not influence wound healing, be non-reactive for the tissues, not stimulate fibrosis formation at wound site, prevent adhesion formation and rapidly metabolized following wound healing, should protect adhesion, be easy to use, bio-resorbable, prevent bacterial colonization and be economic (22). Physical barriers may be classified in two groups as liquid and synthetic solid barriers. Liquid barriers include crystalloids, dextrane 70 and hyaluronic acid. Solid barriers include hyaluronidase and carboxy-methyl cellulose-containing bio-resorbable membrane, cellulose and poly-ethylene glycol-containing solid barriers.

Conclusion

Peritoneal trauma like surgery is the most important factor for intraabdominal adhesion formation. In clinical practice, ischemia and presence of foreign bodies also increase adhesion formation (9). Minimally invasive techniques have been developed for reducing intraabdominal adhesion formation and also novel materials and molecules in different configurations made with different materials have also been introduced for the use in modern surgery. In current approaches, peritoneal trauma should be minimized as possible, the surfaces that could lead to adhesion should be cleaned, measures should be taken for reducing inflammatory response, excessive coagulation should be prevented and fibrinolysis should be induced. Although these measures decrease adhesion frequency, no excellent surgical technique or pharmacologic agent is available to completely prevent intraabdominal adhesions.

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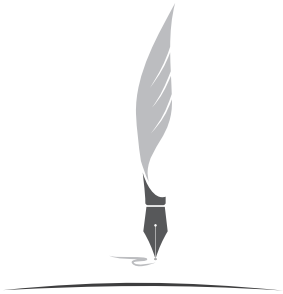

Chapter 15

GLOMERULAR FILTRATION BARRIER

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INTRODUCTION

Kidney diseases are an important health problem all over the world. It is estimated that around 10% of adults worldwide have some form of kidney damage. The prevalence of chronic kidney disease in Turkey is 15.7%. The distribution of the causes of chronic kidney disease varies by country, race, age and gender. Currently, chronic kidney disease risk factors include diseases such as diabetes, hypertension, urinary system stones, urinary tract infections, advanced age, low socioeconomic status and education level, and smoking [1].

Renal diseases, such as many organ diseases, adversely affect quality of life. For this reason, the underlying causes of the diseases have mostly become the focus of research studies and new studies are continuously added to the treatment studies. The main function of the kidneys is the formation of urine by filtration of the blood, it also has functions such as waste removal, homeostasis, hormone production, and absorption. The filtration barrier, which provides its basic function, is the focal point of various metabolic disorders and drug toxicity, this mechanism is investigated at the molecular level. Therefore, the filtration barrier structure is important in kidney disease studies. The protection of adhesion molecules, podocyte cells and proteins that form the slit diaphragm of the glomerular basement membrane constituting the filtration barrier is important in the fulfillment of the function. In order to understand the structure of the filtration barrier, the main proteins incorporated into the structure will be discussed in this section.

Kidneys

The kidneys are located in the retroperitoneal space of the large, bean-like, red, posterior abdominal cavity on both sides of the vertebral column. Its surface is

surrounded by a connective tissue capsule. The kidneys consist of a darker cortex on the outside and a lighter medulla on the inside. The cortex is composed of curved tubules and flat tubules of the nephron, collection tubules, collection tubes, and a dense vascular support along with renal corpuscle [2]. Nephron is the basic functional unit of the kidney. This critical to kidney regulatory roles, including filtration, homeostatsis, and hormonal regulation [3]. Each human kidney contains approximately 2 million nephrons. Nephrons are responsible for urine production. The nephron consists of a renal corpuscle and a tubule system. The renal corpuscle consists of the glomerule and the surrounding renal capsule or bowman capsule. Bowman's capsule is the initial part where the blood passing through the glomerular capillary undergoes filtration to form the glomerular filtrate. The renal corpuscle glomerular endothelium consists of the glomerular basement membrane (GBM) and the visceral leaf of the bowman capsule [2].

Filtration Barrier Structure

The main function of the kidneys is the removal of products generated by cell metabolism accumulated in the blood. Blood is filtered through the renal corpuscle (glomerular and bowman's capsules), water and valuable solutions are reabsorbed in the renal tubules, concentrating urine and regulating blood volume. The glomerular filtration barrier consists of the endothelium with phenestr, GBM and podocytes. Water and soluble substances are retained by GBM and podocytes [4]. The filtration barrier must maintain the integrity of the structure in order to fulfill its task. The components of this structure have been the main subject of the studies from past to present. Integrins are the cell-surface proteins mediating the extracellular matrix.

Integrin α3β1 is the main protein that provides adhesion to podocyte of GBM, which is expressed on the podocyte cell surface [5, 6]. In GBM, laminin and type 4 collagen are also required for cell-matrix adhesion [4]. Laminin α5β1γ1 (laminin-511) was found in the developing GBM, while laminin-521 was found in mature GBM [6]. The basement membrane is constructed by type IV collagen with laminin. Type IV collagen is important for strength and stability of the basement membrane, and its indispensable [7]. Integrin α3β1 is one of the laminin binding integrins [6, 8]. Genetically inactivated α3 [9, 10] and β1 [9, 11, 12] integrins have been reported to cause renal failure and disappearance of podocytes in neonatal mice. Thus, integrin α3β1 plays a critical role in the development and maintenance of glomerular filtration [9]. In addition, in vivo and in vitro studies have shown a decrease in the level of integrin α3β1 expression in kidney tissues of diabetic rats compared to the control group [13-16]. Also integrin α3β1 as well as laminin-binding integrins include α1β1, α2β1, α6β1, α10β1, and α7β1. Collagen-binding integrins are α1β1, α2β1, α10β1, α11β1, and αXβ2 [8].

Glomerular podocytes are usually the main target of kidney research [17]. Podocytes are large cells responsible for the protection of the filtration barrier, establishing specialized cell-cell contacts between their connections, called slit diaphragm (SD) [18]. Multiple protein complexes between podocytes and SD contribute to the glomerular filtration barrier [19]. The role of podocytes is important in understanding the metabolic disorders such as diabetic nephropathy and the damage caused by inflammatory and toxic agents to the kidney tissue [20]. Podocyte damage or loss of podocytes has been associated with the pathogenesis of proteinuria and has been reported to cause glomerulosclerosis [5, 21]. SD forms a filtering barrier between the pods of neighboring podocytes that

prevents serum proteins from leaking into the urine from the glomerular capillary. Thus, the molecular structure of SD plays an important role in understanding proteinuria [18, 22, 9].

SD consists of proteins nephrin, CD2-associated protein (CD2AP), zonula occludens (ZO) -1, FAT, podocin and Neph1 [23]. Nephrin is the transmembrane component of the immunoglobulin superfamily, this constitutes the structural component of SD [18, 22]. Nephrin is located in the area between the podocytes foots in the lateral and apical faces of the podocytes. Decreased nephrin expression has been reported in experimental models of glomerular diseases and other proteinuric syndromes [24-26]. Type 4 collagen and laminin expressions are normal in nephrin knockout mice, the structure of GBM has shown to be normal. The expression of ZO-1, P-cadherin and FAT in the structure of podocytes has not been reported in nephrine knockout mice. In α3 collagen type 4 knockout mice, there was no change in GBM structure up to 4 weeks, but then ıt has been reported changes in the laminin level of the GBM structure and lack of type 4 collagen chains. At the same time, molecular changes weren't observed in the SD structure up to 4 weeks. However, after 5 weeks, in α3 collagen type 4 knockout mice have observed a significant decrease in nephrin expression, disappearance of podocytes, and onset of proteinuria. Thus, the decrease in nephrine expression appears to be an important protein for maintaining glomerular filtration integrity [27-29]. Cytoplasmic scaffolding proteins of tight junctions complexes form ZO proteins (ZO-1, ZO-2, ZO-3). ZO-1 interacts with actin filaments, cingulin, afadine, microtubules and transmembrane proteins of the tight junctions complex [30]. Among the epithelial cells of the ZO-1 renal glomerulus, podocytes are also found, SD contributes to tight connection [31]. FAT is a member of the cadherin family, located in the glomerular

wall of the glomerular capillary. The cytoplasmic domain of FAT is colocalized with ZO-1 in SD [32]. Neph-1 is expressed in kidney and is homologous to nephrin [33]. Podocin localizes in SD and interacts with nephrin. This interaction is necessary for effective signal formation through nephrin and its associated proteins [34].

Nephrin and podocin are two important proteins that combine and strengthen SD. These molecules bind to the cell skeleton by CD2AP [35]. The CD2AP plays a role in regulating the actin cytoskeleton and endocytic traffic, allowing nephrin and podocin to bind to actin [36]. CD2AP has been shown to be expressed only in podocytes in the glomerulus, but also in the collecting channel and in some proximal tubule cells [27, 37]. In mice lacking CD2AP were observed glomerulosclerosis leading to renal failure and disappearance of podocyte foot processes [38]. In a study of diabetic KK-Ay mice were observed thickening of GBM and enlargement of mesengial matrix, podocyte lesion and podocyte foot injury. In the same study was reported a decrease in nephrin, CD2AP and integrin α3β1 expression levels [19]. Disappearance, rupture, hypertrophy and apoptosis in podocyte foot processes cause dysfunction of the filtration barrier and cause proteinuria [39, 40]. Increased permeability of glomerular against proteins in glomerular diseases, results in proteinuria. As long as glomerular permeability is maintained, proteins do not appear in the urine. When glomerular damage occurs, proteins such as albumin, high molecular weight proteins and electrical charge albumin-like proteins excreted into the urine [41]. Proteinuria may be used as a marker in the determination of kidney damage from the filtration barrier.

As a result, maintaining the integrity of the GBM and SD structure is critical in performing the normal function of the kidney. Changes in the molecular mechanism of these structures lead to the emergence of metabolic

disorders and diseases. Therefore, it is important to maintain the integrity of the filtration barrier structure and function. Expressions of these protein molecules are therefore important in maintaining the integrity of the filtration barrier structure and function.

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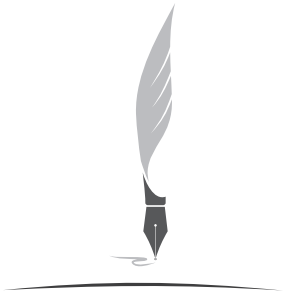

Chapter 16

EVALUATION OF HYGIENE CHARACTERISTICS OF PRE-ADOLESCENT STUDENTS

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INTRODUCTION

Adolescence is the general name of the period that starts at the age of 10-11 and lasts almost twenty years, including physical, mental and social changes.The period between the ages of 10-13 years of this process is called pre-adolescent period. Physical, cognitive, emotional and social changes occur during adolescence. The most obvious and the most challenging of these changes physıcal changes. The hygiene characteristics and requirements of the child may also change with changing height, weight and body mass (1,2,3,4).The aim of this study was to determine the hygiene characteristics of children in pre-adolescence period.

METHOD

The study was planned in analytical type between November 1, 2018 - May 1, 2019 with 540 students who already accepted to be held before the attendance at the 6th, 7th and 8th place in four secondary schools in Zonguldak province. Before the study, ethical compliance was obtained from the Human Research Ethics Committee of Zonguldak Bülent Ecevit University, institutional permissions were obtained from the schools where the study will be conducted through the Zonguldak Provincial Directorate of National Education and written consent was obtained from the students. The data were collected by using the information form in which hygiene characteristics were questioned and analyzed by percentage and frequency.

RESULTS

Table 1. Frequency of Hygene Characteristics Adolesence

		n	%
How many days do you take a bath?	Once a week	114	(20,7)
	Two to three times a week	**374**	**(68)**
	Everyday	62	(11,3)
How many days do you cut your nails?	Once a week	**279**	**(50,7)**
	Two a week	168	(30,5)
	Fortnightly	103	(18,7)
When do you wash your hands during the day?	First thing in the morning	453	(82,4)
	Before dinner	394	(71,6)
	After dinner	447	(81,3)
	before the toilet	153	(27,8)
	after the toilet	500	(90,9)
	From outside	**457**	**(83,1)**
How often do you change your laundry?	Everyday	**306**	**(55,6)**
	Every two-three days	213	(38,7)
	Weekly	31	(5,6)
How often do you change your socks?	Everday	**435**	**(79,1)**
	Every two-three days	98	(17,8)
	Weekly	17	(3,1)
How many times a day do you brush your teeth?	Nothing	83	(15,1)
	Once or twice	**378**	**(68,7)**
	three and more per day	89	(16,2)
Do you have your own toothbrush?	Yes	**515**	**(93,6)**
	No	35	(6,4)
How often do you change your toothbrush?	Contamınated	242	(44)
	Every two to three months	**243**	**(44,2)**
	In 6 months or more	65	(11,8)
How often do you see a dentist?	When my tooth aches	**374**	**(68)**
	Every six months	106	(19,3)
	Once in a year	70	(12,7)
What should we do to keep our teeth from rotting?	Use dental floss	33	(6)
	Brush our teeth	**454**	**(82,5)**
	Go to a regular dentist	95	(17,3)
	Consume plenty of water	43	(7,8)
	Not eat sugar	370	(67,3)

% 68 (n=374) of the students bathed 2-3 times a week, % 50.7 (n = 279) cut their nails once a week, %

71.6 (n=394) before eating,% 90.9 (n=500) wash their hands after the toilet, % 55.6 (n = 306) change their laundry and % 79.1 (n=435) change their socks every day. % 68.7(n=378) of the students go to the dentist 1-2 times a day, only % 19.3 (n=106) every six months and % 12.7(n=70) every year. To prevent tooth decay, % 82.5 (n=454) think that teeth should be brushed and % 67.3 (n=370) think that sugar should not be eaten. Oral and dental health is an important element for body health. In the research conducted by Önsüz and Hıdıroğlu in İstanbul, tooth brushing rates were found to be % 80.5. Pelen and Gunay (2013), found that %70.8 of students brushed more than once a day and in Arat (2013) found that % 80.6of students brushed their teeth. Çetinkaya et al. (2005) in the studyconducted with three primary school students with different socioeconomic status in Sivas province, it was determined that students with moderate and highsocioeconomicstatuswashtheirhandshigher than the low socioeconomic level students before theyleavethe toilet and washtheirhands before eating, Güleç et al. (2000), students from two different schools in different districts with different socioeconomic levels were found to have washed their hands the most after leaving the toilet (5,6,7,8,9). Similar results were obtained with field writing. Şimşek (2010), ın the study found that %61.5 of male students bath twice a week and female students 3-6 times a week (10). Kaya et al. (2006) the studyfound that female students bath more often than male students (11).

Tablo 2. Comparison of Sociodemographic Data and Hygiene Score Average (n=540)

		n	Hijyen (Med)	Test değeri; p
Gender	Girl	271	4,04±1,34	[b]t:3,065
	Male	269	3,70±1,32	p:0,002**

[a]*Tek yönlü varyans analizi* [b]*Bağımsız gruplar t testi*

[c]*Pearsonkorelasyon analizi* ***p<0,01*

It was found that there was a statistically significant difference in hygiene scores according to the gender of the students.(p=0,002).It was found that female students have higher scores than male students.The fact girls are more rigorus in cleaning than boys and the rol model of mother is considered to be the reason for the high score. It is thought that the reason why the boys have a hygienic score is that they have more attitude to play on the street, more sweating, and the male gender of this age group is more insensitive about the male gender's shake and hygiene behaviors.

CONCLUSIONS

It was determined that the students shows positive hygiene characteristics in general, but that girls attach edit more importance to their hygiene than male students. It is thought that girls have a cleaner, more meticulous and maternal role model, and boys and more sweating cause the gender difference to occur in this age group hygiene behaviors. Primary school students and their parents are advised to provide trainings on individual hygiene using both the narrative and brochure method.

KEY WORDS: Pre-adolescence, hygiene characteristics

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