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Prof. Dr. Engin ŞAHNA - Prof. Dr. Hasan AKGÜL

Prof. Dr. Zeliha SELAMOĞLU

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CHAPTER 1

ARTIFICIAL SWEETENERS[1]

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1. INTRODUCTION

Sweeteners, as defined by the Turkish Food Codex (TGK, 2023), are substances specifically employed to lend a sweet flavor to various food products or to sweeten table-top products designed for direct consumer use. Sweeteners are chemical substances that are sweeter than the same amount of sugar and contain less energy (Özdemir et al., 2014) . Sweeteners hold an important place within the food industry, which has a very large economic dimension. Sweetening agents can be broadly categorized into three main groups: naturally sourced sweeteners, sugar alcohols (also known as polyols), and artificial or synthetic sweeteners (İşgören and Sungur, 2019).

Natural sweeteners are compounds extracted from plants, specifically from components like their flowers, leaves, bark, and roots. Chemically, this category encompasses monosaccharides (simple sugars), disaccharides, and other sweetening agents with diverse structures that originate from various botanical sources. Monosaccharides commonly included in our diet are fructose, glucose, and galactose. Disaccharides are composed of two monosaccharide molecules, the most common being sucrose, lactose, and maltose (İşgören and Sungur, 2019).

Sugar alcohols (or polyols) are sweeteners derived from sugars (like sucrose) that are chemically modified. They are used in food because they are stable and contain fewer calories (15-25% less than glucose) while providing 25-100% of the sweetness of table sugar. They are only partially absorbed in the small intestine, which results in lower calorie delivery and a much smaller impact on blood sugar levels (low glycemic index). They occur naturally in fruits and vegetables but are commercially produced from sucrose and starch. When consumed in large amounts, they can cause problems such as stomach gas, bloating, and diarrhea, but they are not associated with health problems as seen with high-intensity sweeteners. They are generally non-cariogenic because they are not fermented by bacteria in the mouth (İşgören and Sungur, 2019).

Artificial sweeteners, which are frequently used as additives in the food, beverage, and pharmaceutical industries, have sweetening effects of up to hundreds or several thousand times greater than sucrose. Therefore, their use has rapidly increased in recent years (Demirhan et al., 2018). Furthermore, these additives are extensively incorporated into items labeled as "diet" or "sugar-free," covering a broad spectrum of products. These products include a variety of items such as: Baked goods, soft drinks and powdered beverage mixes, confectionery (candies), puddings, canned foods, jams and jellies, dairy products. Numerous other types of foods and beveragesAccording to the FDA (2025), consumers can confirm the presence of a specific sweetener in a product by checking the ingredient list on the product’s label for the sweetener's name.

The FDA has granted its approval for the use of several synthetic sweetening agents, namely: saccharin, aspartame, sucralose, acesulfame potassium (Acesulfame K), and neotame. Cyclamate and alitame are artificial sweeteners used in the food sector in many countries, including Europe. Although adverse results have been reported in studies regarding the reliability of these sweeteners, the evidence that they are generally not toxic and carcinogenic is greater, and they have been approved by many reliable organizations (Özdemir et al., 2014). The three main compounds widely used as sugar substitutes in America are saccharin, aspartame, and sucralose. In many other countries, artificial sweeteners and the herbal sweetener stevia are commonly used (Whitehouse et al., 2008).

The Food and Drug Administration (FDA) regulates synthetic sweetening agents under various regulations, most notably the Food, Drug, and Cosmetic Act. The agency is required to examine data pertaining to any novel substances intended for use as food ingredients. The FDA holds the authority to restrict how a food additive may be used in processed food items. The responsibility falls upon the FDA to establish whether a substance is safe or poses a risk. Once a product is introduced to the market, the FDA typically prioritizes the evaluation of chronic toxicity. This includes assessing its long-term effects on: Fertility and reproduction, fetal development (teratogenicity), the potential to cause cancer (carcinogenicity), the potential to cause genetic mutations (mutagenicity). However, the evaluation must also incorporate the assessment of acute effects on functions such as the nervous system, cardiovascular system, and other organs. (Whitehouse et al., 2008).

Furthermore, there are studies indicating that the use of artificial sweeteners can cause changes in the gut microbiota depending on time and dose. However, due to the inadequacy of studies investigating the effects of these sweeteners on the gut microbiota, it is not possible with current information to state the exact cause of the differences observed in the gut microbiota. Individuals should be made more aware of this issue (Mendeş and Arslan, 2024).

Research shows that consuming artificial sweeteners leads to an increase in an individual's desire to eat and the amount of food consumed. This is because artificial sweeteners do not stimulate the feeling of satiety. By causing effects such as less suppression of the hormone that stimulates the satiety signal and suppresses appetite on digestion, absorption, and metabolism, they motivate increased food intake. Although these findings do not yet constitute definitive evidence, experts recommend taking them into consideration and not exceeding the daily intake amounts determined by the relevant institutions (Aydın et al., 2022).

Today, the use of artificial sweeteners as additives in foods frequently consumed by both children and adults has become widespread. For this reason, it is thought that more research should be conducted with more people in different groups, and particular attention should be paid to safe consumption amounts. In the selection of low-calorie sweeteners used in food products, taste and stability characteristics are considered in addition to cost, and a mixture of two sweeteners is also used to achieve the desired characteristic (İşgören and Sungur, 2019).

There are different views, both positive and negative, regarding the effects of artificial sweetener use on human health. Food manufacturers aim to reduce the amount of sugar in foods due to its negative effects on human health, in line with consumer demands. In this sense, the industry is trying to offer consumers healthier, natural, more nutritious, and lower-calorie sweeteners (Kızılaslan, 2017). Studies are also continuing for the development of new sweeteners. In light of these facts, and considering the important role of glucose in the organism, it seems more rational for non-diabetic individuals to prefer traditional natural sweeteners in moderation instead of refined sugar and artificial sweeteners, without completely abandoning sugar, and for diabetic individuals to consume artificial sweeteners as little as possible (İşgören and Sungur, 2019).

1.1. ASPARTAME (E 951)

Aspartame has been deemed appropriate for use as a sweetening agent in various food products. This sweetener is widely recognized under the commercial names Nutrasweet®, Equal®, and Sugar Twin® (FDA, 2025). Aspartame is a laboratory-produced, synthetic dipeptide sweetening agent. It is chemically formed from the two amino acids, phenylalanine

and aspartic acid. This compound is widely incorporated into foods, pharmaceuticals, and beverages, particularly in categories like carbonated and powdered soft drinks (Choudhary and Pretorius, 2017; FDA, 2025). When phenylalanine and aspartic acid chemically link together in the correct configuration, the result is aspartame, which possesses an exceptionally strong sweet flavor. Because aspartame is not stable when exposed to heat, its sweetness is lost upon heating. Consequently, it is generally avoided in the production of baked goods (FDA, 2025). The speed at which a substance breaks down (its degradation rate) is contingent upon both the pH level and the temperature of the environment (Magnuson et al., 2007).

Aspartame use has been evaluated in the USA, Canada, European countries, Australia, and Brazil. Recent studies found the average daily aspartame consumption in the American general population to be 3 mg/kg/day in the group using aspartame 90% of the time; and 2.5-5 mg/kg/day in children aged 2-5 years. Despite differences in evaluation due to varying methodologies across countries, aspartame intake in all studies is consistent and well below the Acceptable Daily Intake of 50 mg/kg/day for the American population and 40 mg/kg/day for other countries (Öz, 2003).

Ever since the FDA first sanctioned Aspartame in 1981, the scientific community has been continually discussing both the recommended safe intake levels and the long-term impact of this sweetener on various organ systems (Choudhary and Pretorius, 2017). Moreover, research has substantiated the safety of aspartame across a wide spectrum of the human population. This confirmation extends to: Healthy individuals across all age groups (infants, children, adolescents, and adults), obese individuals, diabetic patients, lactating women (breastfeeding). Individuals who are heterozygous for phenylketonuria meaning they carry one gene for the disorder, which results in a decreased capacity to metabolize the essential amino acid phenylalanine. (Butchko et al., 2002).

Current animal studies and a limited number of human studies show that aspartame and its metabolites, when consumed in quantities significantly higher than the recommended safe dosage or even at recommended safe levels, can disrupt the oxidant/antioxidant balance, cause oxidative stress, and damage cell membrane integrity, which can affect various cells and tissues, leading to the deregulation of cellular functions and, consequently, systemic inflammation (Choudhary and Pretorius, 2017).

The daily consumption of Aspartame is on the rise among health-aware young adults, extending beyond just diabetic patients. This heightened use often occurs without consumers having adequate knowledge about the substance. It is also evident that people are using aspartame irrespective of their specific clinical status or age. Furthermore, observations indicate that commercially sold aspartame products frequently lack explicit warnings or specified intake limits on their labels (Zafar et al., 2017).

While aspartame is considered safe for adults, it is not recommended for children. Although aspartame is an alternative for sweetness for people with diabetes who cannot routinely consume sugar due to health concerns related to sugar intake, strict monitoring is still required. It is important to monitor aspartame consumption, even by diabetic consumers. This is because it is not necessary to use artificial sweeteners at every meal. It should be remembered that no synthetic molecule can adapt to the human body like a natural, herbal, and non-toxic substance. Aspartame is a molecule that mimics the sweet taste for the tongue's taste buds; it is not a natural form of sugar. When consumed uncontrollably, it cannot be beneficial and safe (Zafar et al., 2017).

Individuals who have difficulty metabolizing phenylalanine due to the genetic disorder Phenylketonuria (PKU) must avoid or restrict aspartame (FDA, 2025).

A wide range of low-calorie products can be obtained when aspartame is used in combination with other carbohydrates and high-sweetness potential sweeteners such as sucrose, glucose, fructose, and saccharin. When used with other sweeteners, aspartame exhibits a synergistic effect, which also allows for a reduction in the total amount of sweetener used. A synergistic effect is observed when aspartame is used with acesulfame K, and studies on various foods have determined the most effective ratio for the aspartame/acesulfame K mixture to be 1:1 (Yılmaz, 2007).

1.2. ACESULFAME K (E 950)

Acesulfame K, also identified by the code E950, is a synthetically manufactured substance. Its precise chemical designation is 6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide potassium salt. This substance was initially named "acetosulfam," and in 1978, WHO reported that the use of the name "Acesulfame potassium salt" was deemed appropriate. Today, it is briefly referred to as "Acesulfame K" (Altuğ and Elmacı, 2001).

The FDA first granted approval for this substance's use in dry food products and alcoholic beverages in 1988. Later, in 2003, its regulatory status was expanded, and it was approved for use as a general-purpose sweetener. The ADI value is 15 mg/kg/day. It is reported that at this dose, there is no evidence of toxic or carcinogenic effects, and its degradation product, acetoacetamide, is toxic but is only reported to be risky at excessive doses. It is heat-resistant. It is included in the composition of about five thousand products in more than a hundred countries. It is generally used with aspartame, cyclamate, or sucralose to create synergy in food products to obtain a better taste and reduce the total amount of sweetener (İşgören and Sungur, 2019).

It is white, odorless, and crystalline. It is a synthetic salt. The taste of Acesulfame-K is similar to sucrose, is quickly perceived, and the taste is long-lasting. It does not leave an aftertaste in the mouth. However, when used at high intensity, it imparts a bitter, metallic taste. It has a taste 200 times sweeter than sugar. It has a wide range of uses in the food industry. It is used in the production of carbonated and non-carbonated beverages, fruit nectars, fruit juice concentrates, dairy products, ice creams, desserts, marmalades, jellies, jams, baked products, frozen desserts, chewing gums, some vegetable pickles, toothpaste, and mouth sprays (Whitehouse et al., 2008).

A study conducted by Mukherjee and Chakrabarti in mice in 1997 reported that acesulfame K caused dose-dependent mutations in bone marrow cells. However, the suitability of this study was stated to be debatable in the SCF report published in 2000, and furthermore, a second study by the same authors later proved that the acesulfame/aspartame mixture had no mutagenic effect (Mukherjee and Chakrabarti, 1997; Mukhopadhyay et al., 2000; Özdemir et al., 2014).

Acesulfame K is not metabolized in the body and is excreted unchanged in the urine . Pharmacological studies have shown that 95% of consumed acesulfame K is excreted unchanged in the urine, and thus provides no energy because it is not metabolized, and at the same time, acesulfame K consumption does not affect potassium intake into the body (Yılmaz, 2007).

1.3. SACCHARIN (E 954)

Saccharin, identified as the pioneering artificial sweetener, has been regulated as a food additive by the FDA since 1977. Its utilization is currently approved across a broad spectrum of applications, including beverages, fruit juices, and various nutritional bases or mixes. Additionally, it serves as a sugar substitute in culinary practices and as a tabletop sweetener, as well as an ingredient in processed foods under specific preparatory conditions (FDA, 2025). Beyond its primary sweetening function, saccharin is also employed for distinct technological objectives. In its dry state, the compound exhibits an extensive shelf life and demonstrates significantly higher stability compared to liquid formulations of aspartame or acesulfame K (Prakash et al., 2008).

Marketed under commercial labels such as Sweet and Low®, Sweet Twin®, Sweet'N Low®, and Necta Sweet®, this sweetener possesses a potency roughly 200 to 700 times that of sucrose (table sugar) while maintaining a zero-calorie profile (FDA, 2025). A paramount characteristic of saccharin is its robust resistance to thermal processing and low pH environments. Regarding safety parameters, ADI level established by the FDA is specified as 5 mg/kg body weight per day (İşgören and Sungur, 2019).

The first studies on saccharin reported an increase in bladder tumors in male rats, which brought up the issue of the potential carcinogenic effects of artificial sweeteners, primarily saccharin. Saccharin was banned by the FDA in 1977, but subsequent studies showed that such an effect did not occur in humans, and it was re-approved in 1991. It is thought that the initial study led to this result due to the administration of hundreds of times the normal human dose of saccharin to the rats, and the bladder structure and urine chemistry specific to rats. Animal studies have shown that the mechanism by which saccharin causes cancer in rats cannot be applied to humans, and existing human studies have found no evidence of a link between saccharin use and cancer (Özdemir et al., 2014).

1.4. SUCRALOSE (E 955)

Sucralose is recognized as a highly effective sweetener suitable for diverse applications within the food industry. Marketed under the brand name Splenda®, this compound possesses a sweetening potency approximately 600 times greater than that of sucrose (table sugar). The FDA regulates sucralose as a food additive, having initially approved its use across fifteen distinct food categories in 1998. Subsequently, in 1999, its regulatory status was expanded to that of a general-purpose sweetener, permissible in foods under specified conditions of use (FDA, 2025).

As a general-purpose sweetener, sucralose is integrated into a wide variety of commercial products, including beverages, chewing gums, gelatins, frozen dairy desserts, and baked goods. A critical functional attribute of sucralose is its exceptional thermal stability; it retains its sensory profile even when subjected to high temperatures during industrial or domestic cooking processes. This heat-stable nature renders it an ideal sugar substitute specifically for baked goods and other heat-treated food products (FDA, 2025).

Sucralose, like sucrose, is not metabolized in the body, so it provides no energy. Furthermore, because it is not perceived by the body as sugar or carbohydrates, it does not affect blood sugar and insulin levels. Due to this property, it can also be used in diabetic foods (FDA, 2025; Yılmaz, 2007). The ADI value is 5 mg/kg/day (İşgören and Sungur, 2019).

Saccharin is slowly absorbed when taken into the body. However, it is not metabolized and is eliminated from the body unchanged by the kidneys. JECFA (Joint Expert Committee on Food Additives) determined that the consumption of saccharin at an ADI of 0–2.5 mg/kg per body weight would not increase the risk of cancer in humans, and its use in foods was permitted (Yılmaz, 2007).

A recently published study showed that a preparation containing sucralose along with the carbohydrates dextrose and maltodextrin reduced beneficial gut microflora in rats, increased fecal pH, and increased the expression of some cytochrome P enzymes. Based on this, the authors suggested that this sucralose-containing preparation might affect the bioavailability of orally administered drugs (Özdemir et al., 2014).

1.5. NEOTAME (E 961)

Neotame is classified as a high-intensity sweetener suitable for various food applications and is commercially available under the brand name Newtame®. Distinguished by its extreme potency, it is estimated to be approximately 7,000 to 13,000 times sweeter than sucrose. The FDA authorized neotame as a general-purpose sweetener in 2002, marking its formal entry into the regulated food market (FDA, 2025).

The safety profile of neotame is defined by specific quantitative limits established by leading regulatory bodies. The FDA has set ADI value at 18 mg/person/day; conversely, JECFA determined an ADI of 2 mg/kg body weight per day in 2004. These benchmarks ensure regulated consumption levels across different international frameworks (İşgören and Sungur, 2019).

Neotame is a novel non-nutritive sweetener. NEOTAME is a derivative of aspartame. Neotame has a clean sweet taste and a good flavor profile. It is a high-potency sweetener ; it is 6,000-10,000 times sweeter than sucrose and 30-60 times sweeter than aspartame. Neotame is a calorie-free, non-cariogenic sweetener . Neotame has a long shelf life under dry conditions. It is stable under dry storage conditions; its stability in aqueous solutions varies with pH. In aqueous food systems, it exhibits the same functions as aspartame in acidic environments, but it is significantly more stable in neutral environments. Consequently, neotame is a functional sweetener in baked products. Neotame is compatible with reducing sugars and aldehyde-based flavorings . It has flavor-enhancing properties (Nofre and Tinti, 2000).

In 2002, the FDA authorized neotame as a multi-functional agent, serving both as a general-purpose sweetener and a flavor enhancer across various food categories, with the specific exclusion of meat and poultry products. Its application is subject to defined regulatory conditions that govern its use in the commercial sector. One of its most significant technical properties is its robust thermal stability, which allows the compound to maintain its sensory integrity even when exposed to high temperatures (FDA, 2025).

This heat-resistant profile is particularly advantageous for the food processing industry, as it ensures that neotame retains its sweetening capacity throughout rigorous cooking and heating cycles. Consequently, it is recognized as an effective and reliable sugar substitute in the production of baked goods and other thermally processed items (FDA, 2025).

Neotame is rapidly and completely metabolized in the body and is eliminated from the body through normal biological pathways. Neotame does not affect glucose and insulin concentrations in diabetic patients. Therefore, these individuals can safely add neotame to their food. Products containing neotame also do not require labeling for patients with

phenylketonuria (Yılmaz, 2007). Although neotame contains phenylalanine, the amount used in the body is very low due to its high-intensity sweetening property, and the amount released in the body is negligible (Nofre and Tinti, 2000).

1.6. ADVANTAME (E 969)

Advantame is a high-intensity sweetener recognized for its extraordinary sensory profile, being approximately 20,000 times sweeter than sucrose (FDA, 2025). Regarding clinical safety limits, ADI has been established at 5 mg/kg body weight per day (İşgören and Sungur, 2019). In 2014, the FDA granted approval for its use as a multi-purpose agent, functioning as both a general sweetener and a flavor enhancer across various food groups, with the specific exclusion of meat and poultry products (FDA, 2025).

A primary functional attribute of Advantame is its robust thermal stability, which allows the compound to maintain its sweetening properties even under the stress of high-temperature processing. This heat-resistant nature ensures that the additive retains its sensory integrity during cooking and industrial baking cycles. Consequently, Advantame is highly suitable for use as a sugar substitute in baked goods and other thermally treated food products (FDA, 2025).

2. CONCLUSION

Especially in recent years, changes in lifestyle have led to changes in eating behaviors, which, in turn, have caused an increase in the likelihood of non-communicable diseases (e.g., obesity). To mitigate this effect, the consumption of natural and artificial sweeteners, which are lower-energy alternatives that provide a sweet taste, has increased. Products where sweeteners are used instead of sugar attract the interest of consumers. The use of non-nutritive natural and artificial sweeteners instead of part or all of the sugar in these products will also increase the consumption of the products.

In products where sweeteners are used, the flavor provided by sucrose and the flavor provided by the sweetener are similar, but the taste differs in both systems: some desirable flavor characteristics disappear, while some undesirable flavor characteristics may emerge. Therefore, the type of sweetener to be used in the product must be appropriate for the product, and there is a need to determine the appropriate doses and compatible combinations to prevent the metallic or bitter taste that appears after the consumption of some sweeteners. The issue that needs to be emphasized in such products is that a product of the same quality cannot be obtained by using sweeteners instead of sucrose, but a different product can be developed with a new formulation. Informing consumers about natural and artificial sweeteners and their daily usable limits will contribute to the conscious consumption of such products (Günaydı and Ayar, 2021; Talas and Özyürek, 2022; Yılmaz, 2007).

The effect of artificial sweeteners on human health and their effects on diabetes are controversial. Institutions that supervise/approve foods, such as the FDA, approve products containing the sweeteners aspartame, acesulfame K, and saccharin when consumed under the rules they recommend. Although artificial sweeteners are found in many products, there is concern that their long-term effects emerge gradually, and they may not be a healthy alternative to natural sweeteners. Especially, the search for natural alternatives to sweeteners whose negative effects on the biggest problems of our age, namely cancer, obesity, and diabetes, have been discovered, continues (Budak and Tezcan, 2019). The frequent use of

sweeteners in the food sector has raised questions about their effects on health, and experimental studies have begun . Although the number of studies has begun to increase in recent years, the studies are still very insufficient, and clear results cannot be presented (Pehlivan and Köksal, 2020).

Specifically, data linking the consumption of sweetened beverages to body weight gain, disturbances in the metabolic profile, and other adverse health outcomes have led to concerns about the health consequences of artificial sweeteners. Furthermore, there is a significant debate regarding scientific data showing that sweetener intake can affect nutrition, metabolism, and the gut microbiota through various mechanisms in the body. More comprehensive research is needed to detail the work of authorities to reach definitive conclusions on health effects (Talas and Özyürek, 2022).

It is assumed that artificial sweeteners and their metabolic by-products or components do not harm humans when used at normal levels. Although not proven by controlled studies involving a sufficient number of people, some sweeteners have been associated with certain symptoms. There is a need for long-term and comparative research on this subject (Kızılaslan, 2017).

Research has reported that the adverse health effects of artificial additives used as sweeteners remain minimal, provided that the consumption amount does not exceed the Acceptable Daily Intake (ADI value) for humans. However, it has been suggested that adverse effects may arise if the consumption amount exceeds the ADI value (Yılmaz, 2007).

Laboratory animal models indicate that the consumption of artificial sweeteners may disrupt energy homeostasis and is frequently correlated with various metabolic syndrome markers, such as abdominal adiposity, insulin resistance, and impaired glucose tolerance. Additionally, adverse impacts on hepatic enzyme activities and oxidative stress markers have been documented. However, the existing literature presents a dichotomy; while certain studies highlight increased glucose intolerance and negative shifts in liver enzymes and oxidative stress, others have yielded no statistically significant findings (Pehlivan and Köksal, 2020).

In contrast, certain investigations regarding natural sweeteners suggest a more favorable physiological profile, reporting enhanced insulin sensitivity, improved glycemic control, and a reduction in both oxidative stress parameters and liver enzymes. Nevertheless, scientific consensus remains complex, as independent studies have observed that natural sweeteners may have no significant effect on these specific metabolic variables (Pehlivan and Köksal, 2020).

Consequently, a clear conclusion cannot be reached in the research conducted on laboratory animals because the type and gender of the animal used for the research, the duration of the research, the type and amount of sweetener administered, and the parameters to be evaluated at the end of the research differ. Therefore, more long-term and experimental research is needed to determine the health effects of artificial and natural sweeteners, which are used so frequently today (Pehlivan and Köksal, 2020).

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CHAPTER 2

PSEUDOMONAS AERUGINOSA VIRULENCE FACTORS AND DETECTION OF ELASTASE ACTIVITY IN *P. AERUGINOSA ISOLATES*[1]

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INTRODUCTION

Pseudomonas aeruginosa is a gram-negative bacterium belonging to the family Pseudomonadaceae, genus *Pseudomonas*. *P. aeruginosa* was first discovered in 1882 by Carle Gessard, who named it *Bacillus pyocyaneus*. *Pseudomonas* species are commonly found in soil, water, plants and animals, decaying organic matter, vegetation, and the aquatic environment [1,2].

P. aeruginosa is a bacterium that is resistant to environmental conditions and can thrive in various environments both as a harmless saprophyte and as an opportunistic human pathogen [3]. In hospitals, it can be found in environments such as food, sinks, toilets, floor mats, respiratory therapy and dialysis equipment and even in disinfectant solutions [4].

P. aeruginosa, which is classified as a "high-priority" pathogen by the World Health Organisation, occupies a leading position in antibiotic resistance [5]. It is an opportunistic pathogen that causes various infections, especially in immunocompromised patients [6].

MORPHOLOGICAL CHARACTERISTICS

P. aeruginosa is a gram-negative, non-fermenting, 1.5-3 μm long, 0.5-0.8 μm wide, straight or slightly curved, non-spore-forming bacillus. The outer surface of the cell contains a capsule-like glycocalyx layer and is generally motile with unipolar or multipolar flagella [7,8].

P. aeruginosa is an obligate aerobe with an optimal growth temperature of 37°C. It causes beta hemolysis on blood agar and forms lactose-negative colonies on lactose-containing media such as Eosin Methylene Blue agar (EMB) and McConkey agar [9,10].

P. aeruginosa cannot ferment carbohydrates. It differs from the Enterobacteriaceae family in that it is oxidase-positive [9]. It does not produce indole and H_2S. They produce catalase and L-arginine dihydrolase [7].

EPIDEMIOLOGY

P. aeruginosa is an important pathogen worldwide with high morbidity and mortality, especially in hospitalised patients. This situation is particularly relevant due to its role in hospital-acquired infections, ventilator-associated pneumonia and severe infections caused by various sepsis syndromes [11,12].

In 2017, multidrug-resistant (MDR) P. aeruginosa caused an estimated 32,600 infections and 2,700 deaths among hospitalised patients in the United States [13]. While Pseudomonas spp. is responsible for approximately 3-5% of nosocomial pneumonia, P. aeruginosa is particularly common in ventilator-associated cases [5].

ANTIBIOTIC RESISTANCE MECHANISMS

It is known that *P. aeruginosa* develops resistance to most classes of antibiotics, including beta-lactams, aminoglycosides and quinolones. Several mechanisms have been described for antibiotic resistance in Pseudomonas, including intrinsic antibiotic resistance, efflux systems and antibiotic-inactivating enzymes. Intrinsic antibiotic resistance is the ability to limit membrane permeability to antimicrobial agents. Efflux systems allow the bacterium to efflux harmful or toxic compounds from the cell membrane [14-16].

According to the 2024 IDSA Guidelines for the Treatment of Antimicrobial-Resistant Gram-negative Infections, "Difficult-to-Treat Resistant (DTR)" *P. aeruginosa* is defined as resistant to all of the following antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin [17].

PATHOGENESIS

Although *P. aeruginosa* is widespread in nature, it is an "opportunistic" pathogen that rarely causes disease in healthy people [18]. Factors predisposing to *P. aeruginosa* infections include disorders of skin and mucosal integrity, the presence of intravascular catheters or endotracheal tubes, neutropenia, hypogammaglobulinaemia and complement deficiency [6].

Infection Stages

The pathogenesis of *P. aeruginosa* infection proceeds in three phases: bacterial colonisation, invasion and systemic spread [8]:

1. Bacterial Colonization

For an infection to begin, the bacterium must first adhere to the surface [8]. *P. aeruginosa* has a single flagellum, which is necessary for cell adhesion and biofilm formation. Type IV pili are projections composed of pilin polymers that allow the bacteria to move on surfaces and play an important role in biofilm formation and adhesion of airway epithelial cells [8].

2. Tissue Invasion

Extracellular enzymes and toxins secreted by *P. aeruginosa*, which colonises by adhering to the epithelial surface, play an important role in the invasion of tissues to overcome natural barriers [6]. In this way, it disrupts physiological barriers, damages the host through cell destruction and impairs organ function. Histologically, Pseudomonas infections show widespread tissue necrosis, formation of microabscesses, deterioration of vascular structure and haemorrhage [18].

3. Systemic Spread

Following adhesion, colonization, and invasion of epithelial barriers, bacteria and their produced toxins enter systemic circulation, resulting in widespread systemic infection symptoms. *P. aeruginosa* is resistant to phagocytosis and other systemic bactericidal defense mechanisms in the bloodstream [6].

CLINICAL SYNDROMES

P. aeruginosa can cause infections in almost any part of the body, but does not usually lead to infection in a healthy host [4]. It can cause a variety of clinical manifestations:

Respiratory System Infections

P. aeruginosa is a gram-negative, aerobic, non-spore-forming rod that can cause various infections in both immunocompetent and immunocompromised hosts [4]. Its tendency to cause infections in immunocompromised hosts, its extreme versatility, its antibiotic resistance and its broad dynamic defence spectrum make it an extremely challenging organism to treat in modern medicine [4].

P. aeruginosa is a gram-negative bacterial pathogen and a common cause of nosocomial infections, especially pneumonia, infections in immunocompromised hosts and infections in people with structural lung diseases such as cystic fibrosis [8]. The unique pulmonary environment in patients with cystic fibrosis promotes chronic infections in which the organism has a characteristic mucoid phenotype [6].

Skin and Soft Tissue Infections

The skin manifestations associated with *P. aeruginosa* can range from less severe localised primary skin infections such as green nail syndrome, interdigital infections and hot-tub folliculitis to more severe forms such as ecthyma gangrenosum in immunocompromised patients and subcutaneous nodules usually associated with bloodstream infections [5].

Bacteremia and Sepsis

During the COVID-19 pandemic, the overall incidence of bloodstream infections (BSI), including those caused by *P. aeruginosa*, increased [5]. *P. aeruginosa* BSIs in COVID-19 patients generally increased due to multiple factors such as prolonged hospitalization, high-dose systemic corticosteroid treatment for acute respiratory distress in intensive care units, and immunomodulatory treatments such as tocilizumab or baricitinib [5].

VIRULENCE FACTORS

Virulence refers to the degree of pathogenicity of microorganisms that can cause damage to host cells and disease. The virulence of pathogenic microorganisms involves the infection of the host and the induction of clinical symptoms by various factors that contribute to bacterial adhesion and colonisation. The microbial factors responsible for events such as invasion of the host and disruption of tissue integrity, suppression of the host immune response and evasion of the immune response, and depletion of host nutrients are termed virulence factors. Pathogenic microorganisms possess various virulence factors that help to evade host defences, invade host cells and immobilise or destroy host cells.

P. aeruginosa virulence factors are divided into three main groups:

- **Membrane-Associated Factors:** Type 4 pili, Flagella, Lipopolysaccharide, Alginate
- **Secreted Factors:** Pyoverdine, Pyochelin, Alkaline protease, Elastase A and B, T3SS effectors, Exotoxin A, Lipase A, Pyocyanin
- **Bacterial Cell-to-Cell Interaction:** Biofilm, Quorum-sensing

Table 1. Virulence factors of *P. aeruginosa*.

Categories	Virulence Factors	Functions
Membrane-Associated Factors	Type 4 pili	Binding to host cells, twitching motility, and biofilm formation
	Flagella	Motility, biofilm formation, and bacterial adhesion
	Lipopolysaccharide	Stimulation of host inflammatory response and phagocytosis
	Alginate	Biofilm formation, immune evasion, and bacterial adhesion
Secreted Factors	Pyoverdine	Chelates iron, promotes bacterial growth and contributes to bacterial virulence
	Pyochelin	ıelates iron, promotes bacterial growth and contributes to bacterial virulence
	Alkaline protease	Regulation of quorum sensing and protection of bacteria from host defense
	Elastase A and B	Degrades proteins in host tissues causing tissue damage
	T3SS effectors	Disruption of host actin cytoskeleton and induction of host cell apoptosis
	Exotoxin A	Inhibition of protein synthesis resulting in cell death
	Lipase A	Damages host tissue
	Pyocyanin	Disrupts epithelial cell function
Bacterial Cell-to-Cell Interaction	Biofilm	Evasion from host immune responses and antibiotics
	Quorum-sensing	Regulation of virulence factor production

ELASTASE ACTIVITY

General Characteristics

The elastase produced by *P. aeruginosa* is one of the most important virulence factors of the bacterium and plays a decisive role in pathogenesis. *P. aeruginosa* produces two types of elastase: LasA (staphylolysin) and LasB (pseudolysin). These enzymes are among the most important virulence factors as they degrade host tissue and immune components [20,21].

LasB Elastase (Pseudolysin)

LasB elastase is the major protease secreted by P. aeruginosa and is a metalloprotease with a molecular weight of 33 kDa. This enzyme:

- **Substrate specificity:** Degrades elastin, components of the complement system, serum α1-proteinase inhibitor, immunoglobulin A (IgA), immunoglobulin G (IgG), mucins, fibrin, collagen, surfactant proteins A and D.
- **Cellular Effects:** Increases epithelial permeability by breaking down the tight junctions in the epithelium of the airways and causes an increase in the neutrophil count at the site of infection.
- **Role in Pathogenesis:** Causes damage to lung parenchyma and hemorrhagic lesions (ecthyma gangrenosum) in elastin-containing tissues [22].

LasA Elastase (Staphylolysin)

LasA is a zinc metalloprotease that:

- **Functional Properties:** Enhances elastolytic activity and high staphylolytic activity, causing rapid degradation of *Staphylococcus aureus*.
- **Synergistic Effect:** LasA does not directly degrade elastin but significantly enhances the elastolytic activity of LasB elastase.
- **Clinical Significance:** Antibodies against LasA and LasB enzymes develop in chronic *Pseudomonas* infections [23].

Importance of Elastase in Pathogenesis

Elastase activity plays a fundamental role in *P. aeruginosa* adaptation and survival in challenging environments, including the infection site.

Elastase:

1. **Tissue Invasion:** Enables bacterial penetration into deep tissues by degrading host barriers
2. **Immune Evasion:** Disrupts immune response by degrading complement system and immunoglobulins
3. **Nutrient Supply:** Provides amino acid sources to bacteria by degrading host proteins
4. **Colonization:** Provides competitive advantage by eliminating other microorganisms.

Regulatory Mechanisms

Elastase production is regulated by quorum sensing systems. LasR and RhlR regulatory proteins control the expression of elastase genes (lasA and lasB). Additionally, elastase activity depends on the presence of zinc and calcium ions [23,24].

METHODS USED FOR DETECTION OF ELASTASE ACTIVITY

ELASTIN-CONGO RED (ECR) METHOD

The Elastin-Congo Red method is the most widely used quantitative method for detecting the elastase activity of *P. aeruginosa* and is suitable for cultures with medium to high elastase production [25,26].

Method Procedure:

- 20 mg insoluble elastin-Congo Red is mixed with 1 mL PBS [26]
- Filtered culture supernatant (1 mL) is added
- Incubated at 36-37°C for 24 hours (with 200 rpm shaking)
- Insoluble elastin is removed by centrifugation at 16,000 g
- Absorbance is measured at 495 nm

Cigana et al. (2020) analysed the elastolytic LasB activities of the reference strain PAO1 and the clinical isolates RP45 and RP73 using the ECR method and found that the PAO1 strain produced about 1.4 times more LasB than RP45 [27]. In a study on the phenotype of elastase deficiency in P. aeruginosa isolates from canine otitis externa cases, 11 isolates were shown to have stable low elastase activity as measured by elastin condorot [28].

In the improved method developed, sensitive measurement can be made in amounts as low as 1.65 µg for purified elastase EI within 1 hour, providing 10-100 times more sensitive results than previous methods [29].

Advantages and Limitations:

- Provides quantitative results and relatively simple procedure
- Not sufficiently sensitive for low elastase activity and may give falsely high values in the presence of LasA [30]
- The difference between 0.01-0.50 OD values is considered critical for test positivity [31]

ELASTIN-NUTRIENT AGAR PLATE METHOD

This qualitative method is used to determine the presence of elastase activity and is ideal for screening at colony level [30].

Method:

- Bacterial colony or culture is inoculated on elastin-containing nutrient agar plates
- Incubated at 37°C for 24-48 hours
- Clear zones formed as a result of elastin degradation are observed

FLUOROGENIC SUBSTRATE METHOD

A fluorescence-based method for the sensitive detection of low elastase activity has been developed using novel substrates containing synthetic peptide substrates with edans (5-(2-aminoethylamino)-1-naphthalenesulfonic acid) and dabsyl (4-(dimethylamino)azobenzenesulfonic acid) [32].

Methodology and Substrates: Z-Ala2-Phe-AlaNH2 proved to be the best substrate (kcat/KM = 8600 $mM^{-1}.s^{-1}$) and the catalytic ratio of alkaline protease activity was shown to be 1000 times lower [33]. In studies with the substrate aminobenzyl-Ala-Gly-Leu-Ala-p-nitrobenzylamide, fluorescence was monitored for 20 minutes at 30-second intervals [34].

Clinical Applications: In sensitivity tests, 1 nM elastase (0.13 μg) can be easily and accurately detected (SD, 3.8% for 10 measurements) and the enzymatic activity can be measured in culture supernatants of clinical strains [33].

Advantages:

- Very sensitive (1 nM elastase can be detected), continuous measurement capability, and minimal cross-reactivity with alkaline protease [30]

IMMUNOCHEMICAL METHODS

A. ELISA (Enzyme-Linked Immunosorbent Assay)

Sensitive sandwich ELISA systems have been developed for three pathogenicity factors of P. aeruginosa - alkaline proteinase (aeruginolysin), elastase (pseudolysin) and exotoxin A [35].

Methodology: The maleimide pyridyl disulfide method was used to label rabbit anti-antigen IgG with horseradish peroxidase (HRP) and the conjugates were used as secondary antibodies in ELISA systems [35].

Performance characteristics: The detection limits for alkaline proteinase, elastase and exotoxin A were determined to be 18 pg/ml, 34 pg/ml and 22 pg/ml, respectively. The intra-assay coefficients of variation for alkaline proteinase, elastase and exotoxin A were reported as 3.4-5.0%, 1.9-3.5% and 1.3-5.4%, respectively [35].

Clinical study results: In a comparative study of 45 P. aeruginosa strains isolated from cystic fibrosis patients, ELISA measurements were 3-5 times higher than other tests, while RIA results were 2-5 times lower [36]. Immunochemical methods were developed to quantify LasA, alkaline protease and elastase in clinical strains, and the levels of the three proteases were determined in each culture supernatant of P. aeruginosa strains from 30 cystic fibrosis patients [37].

Advantages of the method: These ELISA systems have good inter-assay precision, no cross-reactivity, dilution linearity and recovery properties [35].

B. Radioimmunoassay (RIA)

Radioactively labelled antibodies were used for measurements with the RIA method and detection was carried out with ^{14}C-elastin [36].

Characteristic Features:

- Very sensitive method
- Gives 2-5 times lower values than other methods [36]

CONDUCTIMETRIC ASSAY

A conductometric assay was developed for the elastase activity of P. aeruginosa. Synthetic peptide (tetra-alanine) or unmodified insoluble elastin can be used as substrate [38].

Methodology and calibration: Using purified elastase for calibration, the activities of 45 culture supernatants from cystic fibrosis patients were determined and a linear relationship with a slope of 0.98 was found between the two assays [38].

Technical details: For pancreatic elastase, conductance measurements in the microgram range are sensitive and reproducible and provide a good picture of elastolytic activity within 30 minutes

[39]. The sensitivity of the conductometric method has been demonstrated by the ability to easily and accurately detect elastase down to 1 nM (0.13 μg) [33].

Comparison of substrates: Although elastin is a more selective substrate (not degradable by the alkaline proteinase of P. aeruginosa), tetra-alanine is a very suitable substrate when routine and/or rapid tests are required [36,38].

^{14}C-ELASTIN ASSAY

Methodology:

- ^{14}C-labeled elastin is used in microtiter plates
- Radioactivity released as a result of elastolytic activity is measured

Performance Properties:

- Simple and sensitive method
- Effective in determining elastolytic activity
- Shows good correlation with ELISA [36]

AZOCASEIN ASSAY

Methodology: Using sulfanilamide-azocasein as a substrate, the total protease activity is measured in cell-free culture supernatants and the azo dye released as a result of proteolysis is detected spectrophotometrically [40].

Application Areas:

- Suitable for total protease activity
- It has been shown that this substrate is ineffective in neutrophil elastase detection because *P. aeruginosa* elastase cleaves succinyl-Ala3-p-nitroanilide between the first and second alanine residues [40]

MOLECULAR METHODS

Recombinase Polymerase Amplification-Lateral Flow Strip (RPA-LFS)

Primer-probe sets for the elastase B (lasB) gene have been developed that allow amplification with RPA at 37°C in 30 minutes and visual detection with lateral flow strips in 10 minutes [41,42].

Performance characteristics: Detection limit of 3.05 CFU/reaction, high specificity (98.26% concordance) and concordance rate with the culture biochemical method on 574 clinical samples was 98.26% with a kappa index value of 0.9433 [41,42].

Clinical evaluation: This method was tested for specificity in 29 P. aeruginosa strains (1 reference strain and 28 clinical isolates) and 23 other bacterial pathogens (9 respiratory diseases, 14 food/waterborne diseases) [41].

CURRENT DEVELOPMENTS AND CLINICAL APPLICATIONS

Relationship to mortality: Elastase activity has been detected in P. aeruginosa isolates from 238 intensive care patients, and high elastase activity has been shown to be associated with 30- and 90-day mortality [43].

Inhibitor research: The inhibitory effect of compounds based on 1,10-phenanthroline-5,6-dione on the elastase of P. aeruginosa was investigated and the proteolytic activity was measured with specific fluorescent peptide substrates [34,44].

Modelling chronic infection: In a model of chronic colonisation of the lungs of mice, it was shown that elastase plays a role in the establishment of chronic infection and the modulation of the immune response [27].

METHOD COMPARISONS AND RECOMMENDATIONS

Four different methods (RIA, ELISA and two enzymatic tests) were compared in P. aeruginosa strains derived from cystic fibrosis patients. It was found that the enzymatic tests using elastin gave higher values than the tests using tetra-alanine and that the presence of the LasA protein caused an increase when pre-incubated with elastin, whereas this increase was not observed with the tetra-alanine substrate, so the use of synthetic substrate was favoured [36].

It is stated that the use of fluorescent substrates is recommended for more sensitive measurements of elastase activity, that the elastin nutrient agar plate method is ideal for qualitative screening at colony level and that the quantitative elastin Congo red test is suitable for cultures with medium to high elastase production [30].

CONCLUSION

Elastase activity is an important determinant of the virulence of *P. aeruginosa*. The choice of test method depends on the purpose of the research, the level of sensitivity required and the resources available. While the elastin-Congo red assay is practical for routine use, fluorescent substrates are favoured for the detection of low activity levels. Molecular methods are being developed for rapid and specific diagnosis.

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CHAPTER 3

METABOLOME PROFILES OF CARDIOVASCULAR DISEASES

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1. Introduction

Cardiovascular diseases are a group of diseases with the highest mortality rate in the world. There are many factors that contribute to the development of the disease, such as individual lifestyles, nutritional habits, stress, and genetic predisposition. Early diagnosis and treatment are crucial for the treatment of cardiovascular diseases.

Metabolomics attempts to identify and interpret changes in metabolites occurring in cells. It examines the profiles formed by metabolites by monitoring biochemical processes in living organisms. Significantly altered metabolites and the metabolic pathways they play a role in help illuminate the development and progression of diseases. Consequently, the identified metabolome profiles serve as important guides in the diagnosis, prevention, and treatment of diseases. A review of numerous studies reveals that highly sensitive analytical methods can identify even the smallest changes in metabolite levels resulting from disease, revealing significant differences. These studies also highlight the metabolic pathways involved in metabolites. Thus, metabolomics studies using analytical methods have yielded important information regarding the diagnosis, monitoring, prevention, and pathogenesis of cardiovascular diseases.

1.1. Cardiovascular diseases

Cardiovascular refers to the heart (cardiac) and blood vessels (vascular). The cardiovascular system is the system that pumps blood in the body. This system comprises the heart, blood vessels, and a network of vessels that facilitate blood transfer. The cardiovascular system functions as a transport and delivery mechanism, supplying oxygen and nutrients to the body's cells while eliminating waste materials. (Lopez et al., 2023)

Cardiovascular disease (CVD) is a term encompassing a broad group of diseases affecting the heart and vascular system. This group includes heart disease, diseases affecting the brain and kidneys, and peripheral vascular diseases. While the causes of cardiovascular illness differ, atherosclerosis and hypertension are the most common. Cardiovascular disease is the number one cause of death worldwide. Cardiovascular disease death rates have decreased in high-income countries during the last two decades, but they have risen very quickly in low- and middle-income countries. (Gaziano et al., 2006; Lopez et al., 2023)

1.2. The most common heart diseases

• Pulmonary heart disease: Coronary heart disease (CHD) is the most common cardiovascular disease caused by blockage of the coronary arteries. It causes heart problems due to reduced blood flow to the heart muscle, leading to various heart diseases.

• Cardiomyopathy: Diseases of heart muscle are called cardiomyopathy. They are usually caused by external factors, including alcohol abuse, hypertension, and fatty deposits.

• Atherosclerosis: A form of vascular obstruction resulting from plaque accumulation produced by fatty or lipid deposits on the endothelial surfaces of blood vessels.

• These atherosclerotic plaques in the blood vessels rupture and form blood clots, blocking the blood vessels supplying the heart muscle.

• Congestive heart failure: A condition in which the heart fails to pump adequate blood to all parts of the body due to an abnormal condition.

• Heart valve disease: Problems with the heart valves.

• Hypertension: A persistent ailment caused by elevated arterial blood pressure.

• Acute myocardial infarction: The medical term for a heart attack. It is a dangerous condition that causes tissue damage due to the sudden interruption of blood flow to the heart muscle.

• Angina: It is a feeling of pain or tightness, usually felt in the chest area, that occurs when a certain part of the heart muscle cannot reach sufficient blood and oxygen.

• Endocarditis: Infection of the endocardium, the inner lining of the heart.

• Cardiac arrhythmias: Changes in the rhythm of the heartbeat. These occur as a result of the heart beating faster or slower.

• Myocarditis: Inflammation of the heart muscle.

• Ischemic heart disease: It is a clinical condition that occurs when the accumulation of fatty plaque in the coronary arteries over time and the accompanying inflammation cause narrowing of the vascular lumen, thus restricting blood flow to the heart muscle.

• Pulmonary embolism: A condition caused by the sudden blockage of one of the arteries that supply blood to the lungs. (Kordalewska & Markuszewski, 2015)

1.3. Causes of cardiovascular diseases

Many factors such as smoking, hypertension, high cholesterol levels, obesity, oral contraceptive use, lack of physical activity, diabetes, unhealthy diet and chronic stress increase the risk of developing cardiovascular diseases. In addition to other changes in blood circulation responsible for cardiovascular disease, they also facilitate the accumulation of lesions and fatty plaques in blood vessels, called atherosclerosis. (Battineni et al., 2021; Münzel et al., 2022)

1.4. Main symptoms

The symptoms of cardiovascular disease vary and often depend on the type of disease affecting the individual and the organs most affected. Symptoms can range from shortness of breath, chest pain, fainting, changes in heart rate, leg swelling, dizziness, nausea, palpitations, and numbness in the left arm. Symptoms typically only appear when the disease is already present, making it difficult to prevent. (Jurgens et al., 2022; Lu et al., 2015)

1.5. Precautions that can be taken

The risk of cardiovascular disease can be reduced or prevented by taking precautions such as quitting smoking and alcohol consumption, exercising regularly, changing one's diet, trying to reduce stress in daily life, ensuring that one's weight is in line with one's body mass index, and keeping values such as blood pressure, cholesterol, and blood sugar at normal levels. (Lee et al., 2023; Pagliaro et al., 2015)

1.6. Diagnosis

There are instrumental and laboratory diagnostics for cardiovascular diseases. These diagnostic tests and devices are frequently used in hospitals. These diagnostic tools are listed below:

• The first step is the electrocardiogram (ECG). The ECG is fundamental and primary in cases of suspected heart disease or vascular damage. An ECG provides physicians with a detailed picture of the heart rate and rhythm. The results determine whether the patient has an arrhythmia, the amount of blood flowing to the heart muscle, and whether the detected volumes are sufficient to maintain normal performance.

• Blood work measures substances that indicate cardiovascular health, such as cholesterol, blood sugar levels, and specific proteins. A blood test may also be used to check for blood clotting problems.

• The ankle-brachial index (ABI) is a test used in the evaluation of peripheral artery disease and allows the determination of vascular stenosis by comparing blood pressure readings in the ankle and arm.

• Ambulatory rhythm monitoring helps detect arrhythmias by recording heart rate and rhythm over long periods with portable devices.

• Doppler ultrasonography uses sound waves to assess blood flow in the leg or neck veins and is used to identify flow changes and vascular occlusions.

• Cardiac computed tomography (CT) provides detailed three-dimensional images of the heart and coronary arteries using X-rays and computer technology.

• Cardiac magnetic resonance imaging (MRI) is an advanced method that provides high-resolution imaging of heart tissue and functions using magnetic fields and radio waves.

• MR angiography and CT angiography are versions of these imaging techniques adapted for detailed examination of vascular structures and are effective in evaluating vascular pathologies in the head, neck, and legs.

• Stress tests allow for the assessment of cardiac performance by analyzing the heart's response to increased workload through exercise or medications and can be applied in conjunction with ECG and imaging techniques.

• Cardiac catheterization is an invasive diagnostic procedure that allows direct measurement of pressure and blood flow in the heart chambers through a thin catheter inserted into the heart via a vein. (Mangla et al., 2017; Shi et al., 2020)

1.7. Treatment method

Treatment for cardiovascular disease must be prescribed by a cardiologist, and the primary goal is to prevent the condition from worsening. Treatment options vary depending on the type and severity of the condition. Medications may be used to control blood pressure, cholesterol levels, blood sugar, and heart rate. In some cases, surgery may be necessary to treat serious cardiovascular conditions such as heart attack, stroke, or heart failure. (Leong et al., 2017)

1.8. Omics concept and omics technologies

The concept of omics is a technology that enables detailed analysis of biological systems. Omics studies identify information about the structure and functioning of organisms, cells, and organs. (Banerjee et al., 2021)

1.9. Omic analysis methods developed with advances in biotechnology

• Genomics: The study of all genes encoded in organisms and their interactions with the organism, each other, and the environment to ensure the error-free operation of all systems used to define the structural and functional functions of organisms. This study aims to identify and control all genes and their interactions with the organism, each other, and the environment.

• Transcriptomics: The study of mRNA transcripts generated by the transcription process in cell genomes.

• Proteomics: The study of the structures, functions, and abundances of proteins found in specific regions within organisms over a specific period of time, their interactions with each other and with other molecules, and their locations.

• Metabolomics: The analysis and identification of metabolites formed by lipids, proteins, vitamins, hormones, and various compounds in organisms' cells, organs, or tissues over specific time periods using developing technological techniques. (Sohag et al., 2021)

1.10. What is metabolomics?

Metabolomics is a branch of biotechnology that examines the chemical fingerprints left behind by specific cellular-biochemical processes, particularly the profiles of all small molecules (metabolites) that are the products of these processes. Metabolomics is the measurement and analysis of metabolites emerging from lipids, carbohydrates, vitamins, hormones, and other cellular components in an organism's cells and physiological fluids over specific time periods. Metabolomics uses molecular tools to study and uncover cellular pathways. Numerous and diverse protein libraries are created. First, the genome map is completed. In subsequent stages, the expected proteins are analyzed using bioinformatics. Based on their similarities, families are predicted, and clusters are created. Examples include transporters, mitochondrial proteins, lipid metabolism-related proteins, channel proteins, and membrane sorting proteins. (Rhee et al., 2020)

Changes in the metabolome are the organism's ultimate response to disease-related, genetic, and environmental changes. Metabolomics applications allow the study of metabolic pathways using non-invasive biological methods. Like proteomics, metabolomics aims to identify metabolites that are disease markers or enable treatment control. It provides dietary recommendations for a patient by examining their genetic makeup and metabolic profile. While genomics and proteomics provide insights into what might be happening, metabolomics reveals the actual occurrence. Quantitative and detailed measurement of all metabolites is the most effective method for examining the phenotypic effects of toxic agents and diagnosing diseases. Metabolomics attempts to detect increases and decreases in metabolite levels based on the underlying condition. For example, if high cholesterol is detected, it can predict the possibility of a coronary heart problem, but if this is supported by other markers, it can also indicate the cause of this condition. In other words, metabolomics analysis not only provides information but can also provide explanations about the current situation. (Kordalewska & Markuszewski, 2015)

1.11. Biological materials used in metabolomics

Metabolomics studies can utilize a wide variety of materials, including urine, saliva, cerebrospinal fluid, pancreatic and lymphatic fluids, and cell and tissue samples. Metabolomics analyses are divided into two categories: targeted and untargeted. In targeted metabolic analyses, standards are established for metabolites, calibration curves are generated, and quantification is performed. Untargeted analyses attempt to identify markers for phenotypes through quantitative analysis of metabolites. Untargeted studies attempt to identify as many metabolites as possible. Targeted analyses are more costly. (Kordalewska & Markuszewski, 2015; McGranaghan et al., 2021)

Table 1: Comparison of targeted and untargeted metabolomics analysis methods

Not targeted	**Targeted**
Numerous metabolites	Limited metabolite
affordable cost	high cost
Low selectivity and sensitivity	High selectivity and sensitivity
General parameters	Specific parameters
Complex data analysis	Easy data analysis
New biomarker diagnosis	Quantification of knowns
Simple steps in sample preparation	Complex steps in sample preparation

1.12. Analytical methods used in metabolomics

Metabolomics is a multidisciplinary science encompassing biology, physics, chemistry, and mathematics. It requires analytical techniques such as chromatography, molecular spectroscopy, and mass spectrometry combined with various data analysis methods. Due to the large number and diversity of metabolites found in biological fluids, no single analytical technique is used. Different analytical techniques are used in metabolomics analyses, depending on the quantity, volume, and sensitivity of the metabolite to be resolved. Separation techniques such as capillary electrophoresis (CE), gas chromatography (GC), and liquid chromatography (LC) are widely applied, along with nuclear magnetic resonance (NMR) and mass spectrometry (MS), for the detailed characterization of metabolites. Sample preparation processes for metabolomics analyses generally exhibit similar characteristics in terms of the stationary and mobile phases used in targeted and untargeted approaches. Sensitive analytical methods are required for small molecular weight metabolites in complex biological systems. NMR is the most fundamental analytical technique for metabolomics analysis. The MS and NMR are the primary methods for studying metabolites in chromatographic methods. These techniques enable the simultaneous profiling of numerous metabolites in biological matrices with high throughput.(Zeki et al., 2020)

•Capillary electrophoresis (CE): In this method, metabolites are separated by electrophoretic differences between themselves, based on the size and charge of the analyte. It is a very suitable technique for charged and polar molecules. It is less preferred due to limitations in sensitivity and reproducibility.

• Gas chromatography (GC): This is a widely used, high-capacity method. It is primarily preferred for the analysis of volatile and stable samples. While derivatization is required for polar molecules, nonpolar substances can be analyzed directly. While its

advantages include high resolution and reproducibility, its disadvantages include the need for derivatization for many molecules and its inability to work with unstable metabolites.

• Liquid chromatography (LC): This technique separates metabolites through interactions between a liquid or solid stationary phase and a mobile liquid phase. It analyzes a wide variety of metabolites. It is a highly reproducible, rapid, and reliable method.

• Nuclear magnetic resonance (NMR): It can analyze numerous metabolites simultaneously. NMR spectroscopy identifies metabolites based on chemical shifts at their resonance frequencies when exposed to a magnetic field. Metabolites in a sample can be identified by measuring all frequencies. Advantages of NMR include robustness, high reproducibility, low cost, minimal sample preparation, and non-destructiveness. It does not require separation for the determination of compounds. It is challenging to identify low-concentration samples and has lower sensitivity compared to MS.

• Mass spectroscopy (MS): It has very high sensitivity and selectivity. For this reason, it is widely used. It ranks ionized molecules in metabolites according to their mass-to-charge ratio and determines their concentrations. (Aderemi et al., 2021; Soga, 2023; Zeki et al., 2020)

1.13. Metabolomics in cardiovascular diseases

Cardiovascular diseases are among the leading causes of mortality on a global scale. To reduce this risk, more specific diagnostic methods are needed. Metabolic disorders in the cardiovascular system lead to cardiac pathology and accelerate the deterioration of cardiac function. Therefore, investigating and examining these pathomechanisms is crucial. Metabolomics focuses on the analysis of metabolites formed within specific timeframes in the body's biological fluids and cells. It enables the investigation of molecules that contribute to the development of cardiovascular diseases. Advanced metabolic systems allow us to create a metabolomic fingerprint using various measurements of individual metabolites. As the heart is the most metabolomically complex organ in our body, it's understandable that disturbances in energy metabolism can lead to a variety of damage and diseases. Additionally, it can alter energy metabolism, leading to consequences related to cardiovascular disease. With advances in omics technology, including genomics, transcriptomics, and proteomics, broad predictions can be made about how cellular and functional changes in cardiovascular systems affect metabolism. Recent metabolomics technologies allow us to measure thousands of metabolites in biological fluids and biopsies. By taking snapshots, we create a metabolomic fingerprint. Snapshots help us in a wide range of areas, including disease progression, myocardial metabolic abnormalities, and the identification of specific treatment options. For all these reasons, metabolomics is used as an invaluable tool for better understanding the pathogenesis and management of cardiovascular diseases. (Kordalewska & Markuszewski, 2015; McGarrah et al., 2018)

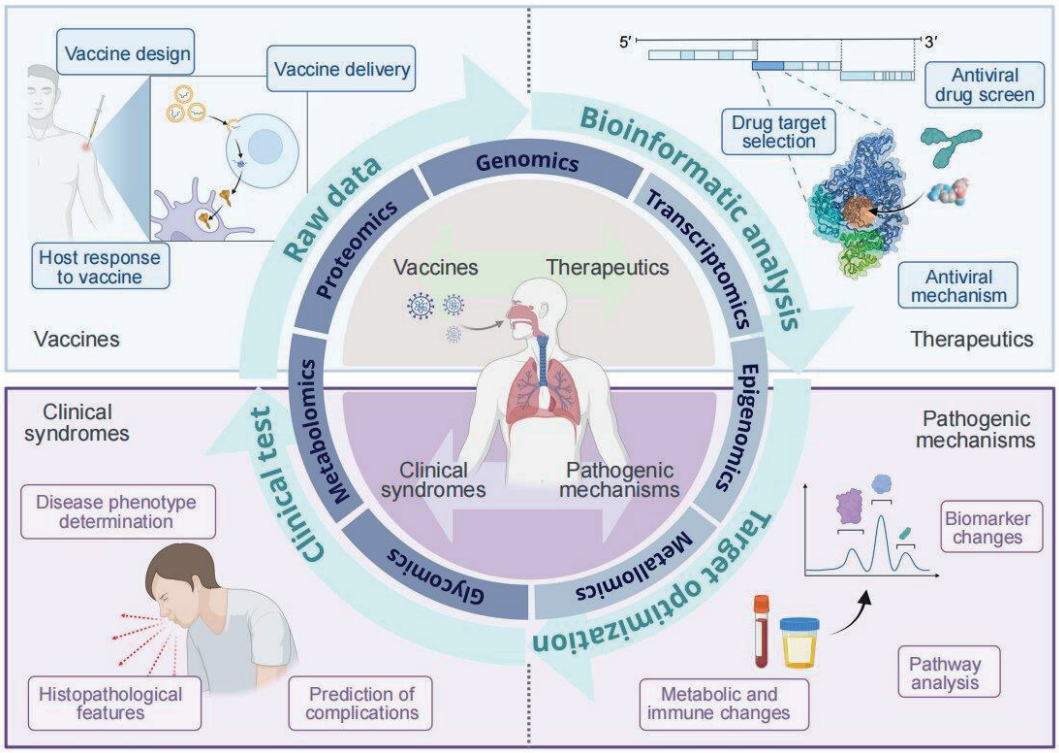

Figure 1. Stages of the metabolomics study

2. Example metabolomics studies in cardiovascular diseases

2.1. Metabolomics in Atherosclerosis

Teul and colleagues investigated the relationship between atherosclerosis and plasma, whole blood, urine, and serum metabolites using GC-MS and 1H NMR. In their metabolomics analysis of plasma samples from patients with atherosclerosis, they reported statistically significant changes in the concentrations of at least 24 metabolites, including l-Glycine, l-Alanine, l-Serine, l-Threonine, l-Tryptophan, l-Histidine, l-Proline, l-Glutamine, l-Valine, and l-Isoleucine. This metabolomics study revealed that changes in amino acid concentrations, in particular, are associated with atherosclerosis and insulin resistance. (Teul et al., 2009)

In a metabolomics study conducted by Xi Chan, Lian Lio, and colleagues, plasma samples obtained from individuals diagnosed with atherosclerosis were analyzed using GC-MS. This analysis revealed that various fatty acids, including palmitate, stearate, and 1-monolinoleoylglycerol, are associated with metabolic changes associated with atherosclerosis. Palmitate, in particular, was confirmed to play a significant role in the atherosclerotic process by affecting apoptosis and inflammatory signaling pathways. The findings suggest that impaired fatty acid metabolism directly contributes to the development of atherosclerosis and that palmitate metabolism could be considered a potential phenotypic biomarker in the clinical diagnosis of the disease. (Chen et al., 2010)

In a study on the metabolic phenotyping of atherosclerotic plaques, P. A. Vorkas and colleagues investigated the relationship between free cholesterol and ceramide metabolism in atherogenesis. Metabolomic analyses were performed using LC-MS/MS. Significant changes were detected in various metabolites belonging to cholesterol, purine, pyrimidine, and

ceramide metabolism, which are among the fundamental biochemical pathways associated with atherosclerosis. Changes were detected in the levels of phosphatidylethanolamine-ceramides, a molecule not previously linked to atherogenesis, suggesting that these molecules may serve as a bridge between two distinct pathways known to play a role in atherosclerosis. Furthermore, phosphatidylethanolamine-ceramides, which exhibit a high statistical correlation with cholesterol, have attracted attention as a novel biomarker candidate. All these results provide important scientific insights into the development of new pharmaceutical targets, particularly those aimed at reducing lipid load within plaque, and support potential approaches that may contribute to addressing unmet clinical needs. (Vorkas et al., 2015)

2.2. Metabolomics in heart failure

Heart failure causes numerous changes in energy metabolism. A study conducted by Martino Deidda, Cristina Piras, and colleagues investigated whether changes in metabolites paralleled heart failure. Biological samples obtained from three different groups were analyzed using NMR-based metabolomics methods, and three distinct metabolic clusters were identified. The primary metabolites found to be effective in this classification were 2-hydroxybutyrate, glycine, methylmalonate, and myo-inositol. Cardiac metabolic derangements associated with heart failure are thought to arise from a decrease in adequate ATP synthesis capacity coupled with increased energy requirements. (Deidda et al., 2015)

Charlotte Andersson, Chunyu Liu, and colleagues investigated the risk of heart failure in the community by analyzing circulating metabolite profiles. Participants' metabolic profiles were analyzed using mass spectrometry and liquid chromatography. Various amino acids, amines, nucleotides, organic acids, lipids, and intermediate metabolites were measured. These metabolites were monitored using echocardiographic variables to predict heart failure risk. High-throughput mass spectroscopy metabolomics studies identified a number of metabolites associated with adverse cardiac remodeling, and the onset of heart failure was identified more than 10 years earlier. (Andersson et al., 2020)

In this study investigating the diagnostic value of metabolomics in heart failure, Mei-Ling Cheng, Chao-Hung Wang, and others used mass spectrometry to profile plasma metabolites. A group of metabolites, including histidine, phenylalanine, spermidine, and phosphatidylcholine, has diagnostic value. Significant improvements were observed in this group of metabolites during recovery. Metabolomics provides powerful diagnostic data for predicting metabolic disorders associated with heart failure. Metabolite profiles provide better values than conventional biomarkers. (Cheng et al., 2015)

2.3. Metabolomics in hypertension

John O. Onuh and Michel Aliani's metabolomic profiling study on hypertension and blood pressure regulation used LC-MS and GC-MS analytical techniques to understand the pathophysiology of hypertension and investigate the relationship between circulating plasma metabolites and changes in blood pressure. Plasma metabolites and blood pressure were regularly measured over a 5-year period, and correlations were established. Changes in blood pressure were found to be related to ceramide, triacylglycerol, total glycerolipids, oleic acid, and cholesterol. Additionally, significant increases in VLDL and LDL lipid levels, as well as lactic acid and acetone levels, were found in individuals diagnosed with hypertension compared to healthy controls. This metabolomics study demonstrated that changes in metabolite profiles can be considered an effective diagnostic approach for identifying hypertensive individuals. Furthermore, it is believed that it could significantly contribute to biomarker development efforts to identify potential complications associated with hypertension and other cardiovascular diseases. (Onuh & Aliani, 2020)

A comprehensive metabolomic analysis of hypertension and dyslipidemia by Chaofu Ke and colleagues used GC-MS and ultra-performance liquid chromatography followed by mass spectrometry (UPLC-MS)-based techniques. Based on the analysis criteria, several metabolites were identified as potential biomarker candidates. These metabolites, which are involved in fat metabolism, glycerophospholipid metabolism, and alanine, aspartate, and glutamate metabolism, were found to play important roles in vascular remodeling processes and the formation of oxidized LDL. This study identified distinct pathways and biomarkers for hypertension and dyslipidemia. It demonstrated that dyslipidemia and hypertension share both common and distinct metabolites. This study demonstrated that metabolomics can uncover new pathological mechanisms and offer potential targets for intervention. (Ke et al., 2018)

In a study conducted by Y. Zhao, J. Peng, and their colleagues, a comprehensive metabolomic analysis was performed using high-throughput liquid and gas chromatography-based mass spectrometry techniques. This study revealed that in patients with pulmonary arterial hypertension (PAH), glycolysis in the lung tissue is impaired, the tricarboxylic acid cycle (TCA) is significantly increased, and significant changes occur in the oxidation pathways of fatty acid metabolism. The data indicate that PAH possesses unique metabolic mechanisms that support ATP production to meet the increased energy requirements during the severe pulmonary vascular remodeling process. These identified metabolites are thought to be potential biomarkers for the diagnosis of PAH. Furthermore, detailed elucidation of PAH-specific metabolomic changes will enable the development of new therapeutic strategies targeting the metabolic pathways involved in disease progression and offer new insights into the pathogenesis of PAH. (Zhao et al., 2014)

2.4. Metabolomics in myocardial infarction

To identify metabolomic markers for the pathogenesis of myocardial infarction (MI), Mingdan Zhu, Yangi Han et al analyzed plasma samples obtained from 30 patients with MI and 30 healthy individuals. Ultra-performance liquid chromatography-electrospray ionization quadruple time-of-flight mass spectrometry (UPLC-ESI-QTOF-MS) analysis revealed significant changes in plasma levels of 10 metabolites in MI patients: linoleic acid, arachidonic acid, C16-sphingosine, phosphatidylserine, lyso-PC (C18:2), lyso-PC (18:1), lyso-PC (C16:0), linoleamidoglycerophosphate choline, N-(2-methoxy ethyl) arachidonic amine, and N-methyl arachidonic amine. These differences have been reported to indicate disruptions in energy metabolism, phospholipid turnover, and fatty acid metabolism in MI cases. Research findings suggest that these metabolites may be important biomarker candidates in studies addressing the diagnosis, treatment, and prevention of MI. (Zhu et al., 2018)

Sara E. Ali, Mohamed A. Farag, and colleagues used GC-MS and 1H-NMR techniques to evaluate the serum metabolite profiles of ST-elevation myocardial infarction (STEMI), unstable angina (UA), and healthy individuals using a comparative metabolomics approach. Multivariate statistical analyses revealed a distinct metabolic signature that reliably distinguished STEMI patients from both the UA group and healthy controls. This metabolic signature was represented by a biomarker panel consisting of 19 different metabolites detected in the STEMI group. The findings showed that glucose, glycerol, urea, lactic acid, β-hydroxybutyric acid, and S levels were higher in the serum of STEMI patients compared to the control group. Therefore, these metabolites are considered promising biomarker candidates for STEMI. Among these metabolites, hydrogen sulfide (H_2S) has been reported to stand out as a notable biomarker. It was emphasized that by revealing the biochemical relationships between metabolites identified in STEMI patients and the most significant

metabolic pathway changes, scientific contributions could be made to the development of early diagnosis and treatment approaches to the disease. (Ali et al., 2016)

Xingking Wang, Dian Wang, Jiayan Wu, and others attempted to determine the metabolic characteristics of early MI in their study on rats. Metabolic profiles of infarcted and non-infarcted myocardium collected from 34 pairs of rats were compared using GC-MS-based tissue metabolomics to characterize the metabolic characteristics of MI. To support the metabolomic findings obtained in myocardial tissue, serum samples taken before and after the development of MI were also analyzed. The results revealed that the metabolic profile of infarcted myocardium was significantly altered compared to non-infarcted myocardium. The analysis identified significant differences in the levels of twenty-two metabolites between the two tissues. These metabolic changes are thought to reflect processes such as energy deficiency, acidosis, oxidative stress, ionic imbalance, and cardiac cell damage that occur after MI. Furthermore, because glutamine, glutamate, and lactate exhibited verifiable differences in serum levels, they were identified as potential biomarker candidates that could be effective in the diagnosis of MI. (Wang et al., 2017)

2.5. Metabolomics in atrial fibrillation

A study conducted by Manual Mayr, Shamil Yusuf, and other colleagues investigated how metabolic disturbances in patients with permanent atrial fibrillation affect arrhythmias. Heart tissue from patients with permanent AF was examined using high-resolution proton nuclear magnetic resonance (NMR) spectroscopy. An increase in beta-hydroxybutyrate, a key substrate for ketone body metabolism, as well as ketogenic amino acids and glycine, was revealed. This study demonstrated the maladaptive regulation of energy metabolites, highlighting the potential role of ketone bodies in AF patients. (Mayr et al., 2008)

2.6. Metabolomics in pulmonary embolism

Renata Bujak, Ana Garcia-Alvarez, and others analyzed plasma from pigs before and after PE induction using LC-QTOF-MS (positive and negative ionization) and GC-Q-MS to obtain metabolic fingerprints to gain insights into pulmonary embolism (PE) and pulmonary hypertension (PH). Metabolic fingerprints were identified, primarily related to glycolysis-derived metabolites, ketone bodies, and TCA cycle intermediates, which are involved in energy imbalance under hypoxic conditions, as well as a group of lipids that may play a role in signal transduction to cells, such as sphingolipids and lysophospholipids. The findings obtained in this report indicate that the combined use of LC-MS and GC-MS based metabolomics approaches may provide an effective and powerful method for a more accurate understanding and diagnostic evaluation of the pathophysiological mechanisms associated with acute pulmonary embolism (PE). (Bujak et al., 2014)

O. A. Zeleznik, E. M. Poale et al. attempted to distinguish low-risk PE from high-risk PE by stratifying PE patients according to risk stratification using metabolomics in a nested case-control study. Plasma samples were collected from 92 patients diagnosed with acute PE within 24 hours of PE diagnosis. Metabolomic analyses were performed using UPLC-MS. In an analysis comparing 46 low-risk patients with 46 intermediate/high-risk PE cases, significant differences were identified in a total of 50 metabolites. Significant changes were noted in metabolites related to purine metabolism (xanthine/inosine derivatives), fatty acid metabolism (acylcarnitines), and the tricarboxylic acid (TCA) cycle. Furthermore, alterations in pathways related to energy production, amino acid metabolism, and nucleotide metabolism were also identified in the intermediate/high-risk PE patients. Furthermore, a comparison of 28 intermediate-risk patients with 18 high-risk PE patients revealed differences in 41 metabolites. These differing metabolites included compounds associated with hemoglobin and porphyrin metabolism, as well as fatty acid derivatives such as acylcholines. This study

demonstrates that a high-throughput metabolomics approach can provide important insights into the pathophysiology of pulmonary embolism. Specifically, changes in circulating metabolite levels reflect a significant impairment of energy metabolism in the intermediate/high-risk group. The findings demonstrate that metabolites play critical roles in the development and progression of PE, while also emphasizing that metabolomic analyses can be considered a potential diagnostic tool in PE risk stratification. (Zeleznik et al., 2018)

2.7. Metabolomics in cerebral infarction

Jee Young Jung, Ho-Sub Lie, and their research team conducted a metabolomics study to evaluate metabolic changes in plasma and urine samples obtained from patients with cerebral infarction. These samples from patients with small vessel occlusion stroke were analyzed using 1H-NMR spectroscopy, and multivariate statistical analyses revealed significant metabolic differences between the patient group and healthy individuals. Increased levels of pyruvate, formate, glycolate, and lactate were found in the plasma of stroke patients, while methanol and glutamine levels were decreased. Urine analyses revealed decreased levels of glycine, citrate, and hippurate. These metabolic differences are thought to be related to processes such as increased anaerobic glycolysis, impaired folate metabolism, and hyperhomocysteinemia. The findings demonstrate the significant potential of metabolomics approaches in the early diagnosis of cerebral infarction and in elucidating the pathogenesis of stroke. (Jung et al., 2011)

2.8. Metabolomics in myocardial ischemia

Compound Danshen Tablets (CDT), a herbal preparation, is a traditional treatment option widely used in China for the treatment of MI. This study, conducted by Yonghai Lv, Xinru Liu, and their team, aimed to identify new biomarkers of MI and to elucidate the therapeutic effects of CDT using a metabolomic approach. In this study, rats with an MI model were treated with CDT and Western drugs such as isosorbide dinitrate, captopril, verapamil, propranolol, and trimetazine. Plasma extracts were then analyzed using UPLC-Q-TOF-MS. The findings demonstrated that CDT treatment restored the levels of inosine, allantoin, xanthine, and hypoxanthine, which are involved in purine metabolism, to levels similar to the control group, and that this effect played a protective role against MI. (Lv et al., 2010)

In a study conducted by Marc S. Sabatine, Emerson Liu, and colleagues, blood samples taken from patients and controls before and after an exercise stress test were analyzed using liquid chromatography and mass spectrometry. The results showed that lactic acid and several other metabolites associated with skeletal muscle AMP catabolism increased after exercise in both the patient and controls. However, many metabolites, which increased or decreased only in the patient group and remained stable in the control group, were found to be significantly dysregulated. Furthermore, six metabolites belonging to the TCA cycle, particularly citric acid, were among the 23 metabolites most significantly altered in patients, demonstrating that these changes significantly differentiated patients from controls. This study demonstrates that the metabolomics approach offers an innovative method for the assessment of acute myocardial ischemia and contributes to the identification of new disease-specific biomarkers. (Sabatine et al., 2005)

3. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

In recent years, omics technology has gained significant interest in the scientific community. Metabolomics approaches are frequently used in scientific applications. Metabolomics analyses require a combination of sensitive analytical techniques and advanced chemometric tools.

The goal of metabolomics applications is to identify the underlying causes of the development of different diseases, investigate their pathogenesis and molecular processes, optimize treatment, and identify specific diagnostic biomarkers to facilitate disease understanding and explanation. The application of metabolomics approaches is particularly important for diseases that develop asymptomatically or lack specific diagnostic biomarkers.

A key challenge in metabolomics analyses is that parameters such as sex, age, diet, medication, and other environmental factors influence metabolite levels. To draw meaningful conclusions, changes in metabolite composition should be solely due to the onset of disease. The untargeted metabolomics approach reduces the impact of these parameters on metabolome profiles by applying them to a large sample population. This provides reliable information about the metabolome profiles of organisms. However, targeted quantitative analysis of only selected metabolites can confirm disease markers. Parallel validation of these compounds in large-scale populations can provide quantification, diagnostic, or prognostic information.

In summary, the application of metabolomics in biomedical research can accelerate progress in medical science. Studies have demonstrated the benefits of metabolomics in cardiovascular diseases, and interest in this field has increased. The simultaneous application of specific analytical techniques allows the use of multiple sample types (blood, urine, saliva, breath, tissue) to reflect the full range of metabolites present in a biological system.

In this study, where we investigate metabolomics approaches related to cardiovascular diseases, our aim is to emphasize the importance of metabolomics, given the diversity and complexity of cardiovascular diseases. We aim to demonstrate that changes in metabolome profiles can be used in the diagnosis, treatment, or prevention of diseases by examining biological materials using analytical methods to elucidate the pathogenesis of cardiovascular diseases, and to emphasize its importance. The general results of metabolomics studies conducted on cardiovascular diseases within the scope of this study are summarized below.

Palmitate, stearate, and 1-monolinoleoylglycerol have been shown to be potential biomarkers in atherosclerosis. Furthermore, a significant association between this disease and phosphatidylethanolamine-ceramide levels has been demonstrated, based on the relationship between free cholesterol and ceramide metabolism.

2-hydroxybutyrate, glycine, methylmalonate, and myo-inositol were found to be the metabolites most affected in heart failure. Furthermore, metabolite groups including histidine, phenylalanine, spermidine, and phosphotidylcholine were found to have diagnostic value in heart failure and to show significant changes during recovery periods.

It has been emphasized that lipid components (VLDL and LDL), lactic acid, and acetone show a significant increase in patients with hypertension compared to healthy individuals.

Significant differences were observed in the plasma levels of 10 metabolites in patients with myocardial infarction: phosphatidylserine, C16-sphingosine, N-methyl arachidonic amine, N-(2-methoxy ethyl) arachidonic amine, linoleamidoglycerophosphate choline, lyso-PC(C18:2), lyso-PC(C16:0), lyso-PC(18:1), arachidonic acid, and linoleic acid. It is anticipated that these metabolites may be used in the diagnosis and treatment of myocardial infarction. Hydrogen sulfide (H2S) has been shown to be a specific metabolite in ST-elevation myocardial infarction (STEMI).

The increase in ketone metabolites beta-hydroxybutyrate, ketogenic amino acids and glycine in atrial fibrillation patients revealed the importance of ketone bodies in atrial fibrillation.

Studies on pulmonary embolism have identified significant differences in the fatty acid metabolism (acylcholines), hemoglobin metabolism, and porphyrin metabolism-related metabolites of patients with intermediate/high-risk PE (pulmonary embolism).

Studies on cerebral infarction have demonstrated increased lactate, pyruvate, glycolate, and formate excretion, decreased glutamine and methanol excretion, and decreased urinary citrate, hippurate, and glycine levels.

As can be seen, the metabolomics approach has contributed important information to the literature regarding the diagnosis, development, and treatment of cardiovascular diseases. The application of metabolomics to various diseases and treatment approaches will enable the development of effective treatment and diagnostic methods in the fields of medicine and pharmacy.

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CHAPTER 4

COVID-19 AND PREGNANCY: DISEASE COURSE, PATHOPHYSIOLOGY, OBSTETRIC OUTCOMES, AND MANAGEMENT STRATEGIES

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INTRODUCTION

COVID-19 is a novel coronavirus infection that first emerged in Wuhan, China, in December 2019 and rapidly evolved into a global pandemic. The causative agent, SARS-CoV-2, has affected millions of people worldwide due to its high transmissibility, broad host cell tropism, and capacity to cause severe respiratory disease (Zhou et al., 2020). The effects of SARS-CoV-2 on the immune system, particularly cytokine storm development, endothelial damage, and coagulopathy, represent key pathophysiological mechanisms contributing to disease severity (Su et al., 2016).

Pregnancy is a unique physiological state characterized by immune modulation, increased metabolic demands on the respiratory and circulatory systems, and pronounced hormonal changes. Consequently, pregnant women may be more susceptible to viral infections, and COVID-19 infection may lead to more severe clinical outcomes in this population (Allotey et al., 2020). The higher complication rates observed in pregnant women during previous coronavirus outbreaks, such as SARS-CoV and MERS-CoV, have underscored the importance of investigating the effects of SARS-CoV-2 on pregnancy (Zimmermann & Curtis, 2020).

Studies examining the impact of COVID-19 on pregnancy indicate that the disease poses various risks to maternal and fetal health. These risks include preterm birth, fetal distress, premature rupture of membranes, preeclampsia-like syndromes, and low birth weight (Knight et al., 2020). In a study by Chen et al. (2020), pneumonia was detected in a significant proportion of pregnant women diagnosed with COVID-19, and most deliveries were performed via cesarean section.

Physiological immunosuppression during pregnancy, reduced lung capacity, increased oxygen consumption, and heightened cardiovascular workload may contribute to a more severe disease course of COVID-19 in pregnant women (Akkuzu & Gökbulut, 2020). Research has demonstrated that pregnant women with COVID-19 have higher rates of intensive care unit (ICU) admission and mechanical ventilation compared to non-pregnant women (Allotey et al., 2020).

From a fetal perspective, while the likelihood of vertical transmission of SARS-CoV-2 appears to be low, it cannot be entirely excluded. Current evidence suggests that fetal effects are more strongly associated with maternal hypoxia, inflammation, and impaired placental perfusion rather than direct viral transmission (Norman et al., 2021). Studies conducted in Türkiye have also reported significantly increased rates of preterm birth among pregnant women with COVID-19 and highlighted the indirect impact of maternal stress on neonatal outcomes (Durankuş & Aksu, 2020).

In addition to biological effects, the COVID-19 pandemic has exerted profound psychological and social impacts on pregnancy. Social isolation, weakened support systems, and difficulties in accessing healthcare services have contributed to increased rates of anxiety and depression among pregnant women (Ertan et al., 2021). These findings emphasize the need to address pregnancy care during pandemics not only from a medical perspective but also from a psychosocial standpoint.

This section aims to comprehensively examine the effects of COVID-19 on pregnancy, childbirth, and the postpartum period; maternal and fetal complications; findings from national and international literature; psychosocial outcomes; and changes in health policies, based on current scientific evidence. By systematically presenting existing data, this review seeks to inform strategies for improving maternal healthcare services in future pandemics.

2. Maternal Effects of COVID-19 in Pregnancy

The clinical effects of COVID-19 in pregnant women differ from those in non-pregnant women due to pregnancy-specific physiological changes. Immune modulation during pregnancy—particularly the suppression of cellular immune responses and alterations in inflammatory processes—increases susceptibility to viral infections and may contribute to a more severe disease course (Allotey et al., 2020). Within this context of physiological vulnerability, SARS-CoV-2 infection has been associated with increased maternal morbidity and a higher need for intensive care (Akkuzu & Gökbulut, 2020).

2.1. Respiratory and Systemic Clinical Manifestations

The most common symptoms of COVID-19 in pregnant women include fever, cough, dyspnea, and fatigue. Clinical studies have reported radiological findings consistent with viral pneumonia in a substantial proportion of infected pregnant women, which may result in more severe clinical presentations due to increased oxygen demand and reduced lung capacity during pregnancy (Chen et al., 2020). Knight et al. (2020) reported higher rates of severe respiratory distress among pregnant women with COVID-19 compared to non-pregnant women.

In addition, increased diaphragmatic pressure, reduced respiratory reserve, and enhanced capillary permeability in pregnancy further elevate the risk of SARS-CoV-2–related hypoxemia (Zimmermann & Curtis, 2020).

2.2. Intensive Care and Mechanical Ventilation Requirements

One of the key indicators of disease severity in pregnant women with COVID-19 is the increased need for ICU admission. A large-scale meta-analysis by Allotey et al. (2020) demonstrated significantly higher ICU admission rates among pregnant women diagnosed with COVID-19. The same study also reported an increased requirement for mechanical ventilation compared to non-pregnant infected women.

Akkuzu and Gökbulut (2020) emphasized that increased cardiovascular workload and altered immune responses during pregnancy inherently elevate the risk of severe disease.

2.3. Association with Pregnancy Complications

The relationship between COVID-19 and pregnancy complications has been explored in various studies, revealing associations particularly with preterm birth, preeclampsia-like syndromes, and premature rupture of membranes. Knight et al. (2020) reported that the increased inflammatory burden associated with COVID-19 elevates the risk of early onset of labor.

In the study by Chen et al. (2020), a significant proportion of pregnant women diagnosed with COVID-19 experienced preterm delivery, and cesarean section was frequently preferred. This approach is thought to be influenced by both maternal hypoxia and concerns regarding fetal distress.

2.4. Placental Effects and Endothelial Dysfunction

The literature has documented associations between COVID-19 and placental inflammation, thrombosis, and vascular malperfusion. SARS-CoV-2 may induce inflammatory responses in trophoblast cells via ACE2 receptors, potentially resulting in placental dysfunction (Su et al., 2016).

Norman et al. (2021) reported that placental perfusion abnormalities observed in pregnant women with COVID-19 may indirectly affect fetal development.

2.5. Psychological and Psychosocial Effects

The stressors associated with the pandemic—including isolation, economic uncertainty, and challenges in accessing healthcare—have been shown to significantly increase anxiety and depression levels among pregnant women. A study conducted in Türkiye reported markedly elevated anxiety levels among pregnant women during the COVID-19 pandemic (Durankuş & Aksu, 2020).

Ertan et al. (2020) found that pandemic-related conditions negatively affected the mental health of pregnant women and increased psychological stress, which may extend into the postpartum period.

3. Fetal and Neonatal Effects of COVID-19

Evaluating the effects of COVID-19 on the fetus and neonate is critical for understanding the interaction between the virus and pregnancy physiology. The systemic inflammatory response, impaired oxygenation, and reduced placental perfusion associated with SARS-CoV-2 infection can

directly influence fetal well-being (Knight et al., 2020). Current evidence suggests that the severity of maternal disease is a key determinant of fetal outcomes (Akkuzu & Gökbulut, 2020).

3.1. Risk of Vertical Transmission

The potential for transplacental transmission of SARS-CoV-2 has been a central research question throughout the pandemic. Most studies report a low risk of vertical transmission (Zimmermann & Curtis, 2020). However, a limited number of cases demonstrating viral RNA in placental tissue indicate that vertical transmission cannot be entirely excluded (Norman et al., 2021). In the study by Chen et al. (2020), the majority of neonates born to mothers with COVID-19 tested negative by PCR, supporting the rarity of intrauterine transmission.

3.2. Risk of Preterm Birth

One of the most frequently reported fetal outcomes associated with COVID-19 is an increased rate of preterm birth. Knight et al. (2020) observed a significantly higher likelihood of preterm delivery among pregnant women diagnosed with COVID-19. Many of these preterm births were iatrogenic, prompted by maternal respiratory compromise or fetal distress (Zimmermann & Curtis, 2020). Studies conducted in Türkiye similarly reported increased rates of preterm birth, largely associated with maternal hypoxia and inflammatory burden (Durankuş & Aksu, 2020).

3.3. Intrauterine Growth and Placental Function

SARS-CoV-2 infection may disrupt placental vascular structures, impairing maternal–fetal blood flow and potentially increasing the risk of fetal growth restriction (Su et al., 2016). Norman et al. (2021) documented placental malperfusion findings in pregnant women with COVID-19, suggesting possible adverse effects on fetal development. Although rates of fetal growth restriction are not consistent across all studies, increased risk has been emphasized in cases of severe maternal disease (Akkuzu & Gökbulut, 2020).

3.4. Fetal Distress and Pregnancy Outcomes

Maternal respiratory distress and systemic inflammation related to COVID-19 may compromise fetal oxygenation and increase the risk of fetal distress. Chen et al. (2020) reported cesarean delivery indications due to fetal distress in a subset of pregnant women with COVID-19. These findings suggest that fetal effects are primarily secondary to maternal clinical status rather than direct viral mechanisms.

3.5. Neonatal Outcomes

Most neonates born to mothers with COVID-19 are healthy, and the incidence of severe neonatal complications is low. Zimmermann and Curtis (2020) reported that serious COVID-19, related clinical manifestations in the neonatal period are rare. Chen et al. (2020) similarly demonstrated negative PCR results and generally favorable postnatal courses among neonates.

Complications related to preterm birth, such as low birth weight, respiratory distress, and short-term neonatal intensive care unit admission, have been reported as secondary outcomes associated with COVID-19 (Knight et al., 2020).

3.6. Breastfeeding and Postnatal Transmission

The World Health Organization (WHO) and multiple scientific studies have reported no evidence of SARS-CoV-2 transmission via breast milk and recommend the continuation of breastfeeding. Zimmermann and Curtis (2020) emphasized that viral particles have not been detected in breast milk and that its immunological protective benefits remain essential.

4. Psychosocial Effects of COVID-19 During Pregnancy

The COVID-19 pandemic has not only constituted a biological health crisis but has also profoundly affected the psychosocial well-being of pregnant women, who are already in a physiologically and emotionally vulnerable period. Social isolation, uncertainty, limited access to healthcare, and heightened fear of infection have significantly increased psychological stress levels among pregnant women (Durankuş & Aksu, 2020).

4.1. Increased Levels of Anxiety and Depression

Elevated anxiety levels among pregnant women during the pandemic have been well documented, including in studies conducted in Türkiye. Durankuş and Aksu (2020) reported high levels of anxiety and depressive symptoms among pregnant women during the early stages of the pandemic, posing risks to both maternal and fetal health. Ertan et al. (2021) demonstrated that the psychological burden experienced by pregnant women during the pandemic was significantly higher than in previous years and persisted into the postpartum period.

These findings highlight increased psychological vulnerability during pregnancy and underscore the importance of mental health monitoring during pandemics.

4.2. Weakening of Social Support Mechanisms

Social isolation policies implemented during the pandemic reduced access to family and social support, becoming a major source of stress—particularly for women experiencing pregnancy for the first time. Zimmermann and Curtis (2020) reported that reduced social support negatively affected the pregnancy experience and increased maternal stress levels. This reduction in support also influenced healthcare-seeking behaviors, with many pregnant women avoiding hospital visits due to fear of infection, leading to disruptions in prenatal care (Knight et al., 2020).

4.3. Difficulties in Accessing Healthcare Services

The prioritization of COVID-19 patients within healthcare systems resulted in the postponement or reduction of routine prenatal care in many countries. This situation led to both insufficient medical follow-up and increased anxiety among pregnant women. Knight et al. (2020) noted that healthcare disruptions during the pandemic undermined pregnant women's sense of safety. Akkuzu and Gökbulut (2020) observed that pregnant women tended to delay prenatal visits, particularly in later gestational weeks, adversely affecting the monitoring of pregnancy-related complications.

4.4. Prenatal and Postnatal Anxiety

The uncertainty associated with the pandemic intensified anxiety related to childbirth. Pregnant women reported increased stress due to concerns about infection risk during delivery, limited communication with healthcare staff, restrictions on birth companions, and postnatal isolation practices. Zimmermann and Curtis (2020) emphasized that the psychosocial burden imposed by the pandemic may also increase the risk of postnatal depression.

4.5. Sleep Disturbances and Quality of Life in Pregnant Women During the Pandemic

The stress and social isolation caused by the pandemic have negatively affected not only mental health but also overall quality of life. Durankuş and Aksu (2020) demonstrated that sleep quality deteriorated and life satisfaction decreased among pregnant women during the pandemic period. These findings indicate that psychosocial stressors are closely linked to physiological processes and may indirectly influence maternal and fetal health outcomes.

4.6. Increased Misinformation and Media-Related Anxiety Among Pregnant Women

The uncontrolled flow of information related to COVID-19 has emerged as another factor contributing to increased anxiety levels among pregnant women. Knight et al. (2020) reported that

inaccurate and exaggerated media coverage heightened risk perception among pregnant women. Similarly, Ertan et al. (2021) emphasized that misinformation circulating on social media platforms significantly increased anxiety levels, highlighting the growing importance of accessing accurate and verified information from healthcare professionals.

5. COVID-19 and Mode of Delivery and Management Approaches in Pregnancy

COVID-19 infection during pregnancy has necessitated the reassessment of critical clinical decisions affecting both maternal health and fetal outcomes. In particular, the determination of delivery mode, infection control strategies, and intrapartum hospital protocols have been revised in accordance with updated international guidelines. Recommendations regarding the mode of delivery during the pandemic have been redefined based on maternal clinical status, respiratory function, gestational age, fetal well-being, and the severity of COVID-19 infection (WHO, 2021).

5.1. Determination of Mode of Delivery in COVID-19–Positive Pregnant Women

International guidelines do not recommend altering the mode of delivery solely based on COVID-19 positivity. WHO (2021) states that COVID-19 infection alone does not constitute an indication for cesarean delivery and that the mode of delivery should be determined by obstetric indications. Knight et al. (2020) reported that cesarean section rates among COVID-19–positive pregnant women were unnecessarily high during the early stages of the pandemic; however, subsequent studies demonstrated that COVID-19 infection does not contraindicate vaginal delivery. In addition, Ertan et al. (2021) emphasized that emergency cesarean delivery may be required to stabilize maternal health in cases of severe respiratory distress, whereas vaginal delivery can be safely preferred in clinically stable patients.

5.2. Safety of Vaginal Delivery

Most studies investigating the vertical transmission potential of COVID-19 have reported that vaginal delivery does not increase the risk of fetal infection. Zimmermann and Curtis (2020) noted that although exposure to respiratory secretions may occur during vaginal delivery, the placental barrier plays a protective role against SARS-CoV-2, making vertical transmission extremely rare. Clinical observations from Türkiye also demonstrated that the risk of maternal–fetal transmission during vaginal delivery is remarkably low and that labor can be safely managed with appropriate isolation measures (Akkuzu & Gökbulut, 2020). Therefore, vaginal delivery is considered a feasible option both clinically and from an infection control perspective.

5.3. Indications for Cesarean Delivery

Cesarean delivery is recommended in COVID-19–positive pregnant women only under specific conditions. According to the WHO (2020) guidelines, cesarean section should be considered in the following situations:

- Acute maternal respiratory distress
- Evidence of fetal distress
- Obstetric indications (e.g., placenta previa, breech presentation)
- Requirement for maternal intensive care support

Knight et al. (2020) reported that rapid termination of pregnancy in cases of rapidly deteriorating maternal oxygenation may improve maternal prognosis. Accordingly, cesarean delivery should be regarded as a medical necessity only when maternal or fetal life is at risk in COVID-19–positive pregnancies.

5.4. Infection Control Measures During Labor and Delivery

Infection control has become a critical component of obstetric care during the pandemic. According to WHO (2021) guidelines, the following measures have been standardized for the delivery of pregnant women with suspected or confirmed COVID-19:

- Conducting deliveries in negative-pressure rooms
- Use of N95 masks, gloves, eye protection, and face shields by healthcare personnel
- Limiting the number of accompanying persons
- Regulating postpartum mother–infant contact under controlled conditions

Durankuş and Aksu (2020) noted that isolation measures applied to COVID-19–positive pregnant women affected maternal quality of life and birth experience; however, these measures were deemed essential for infection control.

5.5. Postpartum Management and Mother–Infant Contact

Infection control remains essential during the postpartum period. Zimmermann and Curtis (2020) reported that SARS-CoV-2 infection in term neonates is generally mild, but postpartum contact should be carefully managed with appropriate precautions.

The WHO (2020) recommends the following practices:

- Skin-to-skin contact is permissible as long as the mother wears a mask.
- Mother–infant separation is recommended only in cases of severe maternal illness.
- Breastfeeding is safe with respect to COVID-19 transmission.

Ertan et al. (2021) further emphasized that the viral load of SARS-CoV-2 in breast milk is extremely low and that breastfeeding should be encouraged due to its benefits for both maternal and neonatal health.

6. COVID-19 and Pregnancy-Related Complications

When combined with pregnancy-related physiological changes, COVID-19 infection may increase the risk of maternal and fetal complications. Immune modulation during pregnancy, reduced pulmonary reserve due to diaphragmatic elevation, and increased cardiovascular workload are among the biological factors that heighten susceptibility to infection (Knight et al., 2020). Similarly, increased systemic inflammation and heightened cytokine responses further raise the likelihood of pregnancy-related complications (Zimmermann & Curtis, 2020).

6.1. Preeclampsia and Hypertensive Disorders

Associations have been reported between the vascular endothelial effects of COVID-19 and preeclampsia-like clinical presentations. Zimmermann and Curtis (2020) suggested that SARS-CoV-2–induced endothelial dysfunction may exacerbate hypertensive disorders of pregnancy. Knight et al. (2020) also reported a modest increase in the incidence of preeclampsia among pregnant women with COVID-19, potentially linked to heightened maternal inflammatory responses.

6.2. Gestational Diabetes and Metabolic Complications

Restrictions on physical activity and increased stress levels during the pandemic have been reported to influence rates of gestational diabetes. Akkuzu and Gökbulut (2020) emphasized that lifestyle changes under pandemic conditions may disrupt metabolic processes in pregnant women, thereby increasing the risk of gestational diabetes. Durankuş and Aksu (2020) similarly reported that elevated stress levels can adversely affect glycemic control.

6.3. Placental Malperfusion and Perinatal Risks

Evidence suggests that COVID-19 may indirectly threaten fetal development by impairing placental circulation. Zimmermann and Curtis (2020) reported that placental inflammation and malperfusion findings may be associated with COVID-19 infection. Knight et al. (2020) highlighted that placental hypoperfusion may negatively affect fetal well-being, necessitating close maternal–fetal surveillance.

6.4. Thromboembolic Events

The effects of COVID-19 on the coagulation system may increase the risk of thromboembolism in pregnant women. Zimmermann and Curtis (2020) stated that SARS-CoV-2 infection can induce a hypercoagulable state, further elevating the already increased risk of thrombosis during pregnancy. Knight et al. (2020) emphasized the importance of prophylactic anticoagulation in pregnant women with severe COVID-19.

6.5. Respiratory Failure and Intensive Care Requirement

Pregnant women are reported to be more susceptible to respiratory complications of COVID-19 due to reduced pulmonary reserve, diaphragmatic elevation, and increased oxygen consumption. Knight et al. (2020) reported that COVID-19–positive pregnant women required intensive care at higher rates compared to the general population. This increased vulnerability is particularly evident in the third trimester, when changes in thoracic mechanics become more pronounced.

6.6. Preterm Birth and Premature Rupture of Membranes

One of the most frequently reported complications of COVID-19 during pregnancy is preterm birth. Zimmermann and Curtis (2020) noted that preterm delivery rates increased due to both spontaneous mechanisms and maternal–fetal indications. Knight et al. (2020) further demonstrated that iatrogenic preterm birth rates were higher among pregnant women with severe COVID-19.

6.7. Maternal Mortality and Morbidity

One of the most severe consequences of COVID-19 during pregnancy is an increased risk of maternal mortality. Knight et al. (2020) reported higher mortality rates among pregnant women with severe COVID-19 compared to the general population. Zimmermann and Curtis (2020) emphasized that immunological alterations during pregnancy may contribute to a more severe course of infection.

7. COVID-19 Vaccination During Pregnancy

With the emergence of the COVID-19 pandemic, vaccination in pregnant women has become a critical public health strategy for protecting both maternal and fetal health. The increased risks of morbidity, mortality, and pregnancy-related complications associated with SARS-CoV-2 infection have further underscored the importance of vaccination. Knight et al. (2020) highlighted the potentially severe course of COVID-19 in pregnancy and the increased need for intensive care, emphasizing the vital protective role of vaccination.

7.1. Safety of COVID-19 Vaccines During Pregnancy

Observational studies evaluating the safety of COVID-19 vaccines administered during pregnancy indicate that these vaccines do not pose additional risks to the fetus. Zimmermann and Curtis (2020) reported favorable safety profiles for inactivated and mRNA-based COVID-19 vaccines during pregnancy, with no observed increase in the risk of fetal malformations. Furthermore, multiple reports have demonstrated that mRNA vaccines do not cross the placenta and do not exert direct effects on fetal tissues (Akkuzu & Gökbulut, 2020).

7.2. Vaccine Effectiveness and Maternal Antibody Response

The immune response elicited by COVID-19 vaccines in pregnant women has also been evaluated. Zimmermann and Curtis (2020) emphasized that mRNA vaccines induce strong neutralizing antibody

responses in maternal serum. Knight et al. (2020) reported that vaccine-induced maternal antibodies can cross the placenta, thereby providing passive immunity to the newborn during the early postnatal period. These findings suggest that vaccination during pregnancy enhances not only maternal protection but also neonatal immune defense.

7.3. Role of Vaccination in Reducing COVID-19–Related Maternal Complications

Administration of COVID-19 vaccines during pregnancy has been shown to reduce hospitalization rates and the risk of severe disease. Knight et al. (2020) reported significantly lower rates of intensive care admission and severe respiratory failure among vaccinated pregnant women. Zimmermann and Curtis (2020) further suggested that maternal vaccination may indirectly reduce rates of preterm birth, fetal distress, and cesarean delivery by mitigating disease severity.

7.4. Vaccine Hesitancy and Psychosocial Factors

Several studies have reported widespread vaccine hesitancy among pregnant women during the pandemic. Durankuş and Aksu (2020) noted that uncertainty and misinformation increased anxiety levels among pregnant women, negatively influencing vaccine acceptance. Ertan et al. (2021) similarly demonstrated that misinformation on social media platforms heightened perceived risk and influenced vaccination decisions. These findings highlight the critical importance of providing accurate, evidence-based information to improve vaccine uptake during pregnancy.

7.5. Recommended Timing of Vaccination

According to international guidelines, COVID-19 vaccines may be administered during any trimester of pregnancy. Zimmermann and Curtis (2020) reported evidence supporting the safety of vaccination during the first trimester, while noting that the most robust immune responses are typically achieved during the second and third trimesters. Knight et al. (2020) emphasized that vaccination should not be delayed in pregnant women with high-risk conditions such as obesity, hypertension, and diabetes.

8. Treatment Approaches for COVID-19 During Pregnancy

The management of COVID-19 during pregnancy requires a specialized clinical approach due to the necessity of safeguarding both maternal and fetal health. Knight et al. (2020) reported that COVID-19 can follow a severe course in pregnant women, with increased requirements for respiratory support and intensive care admission, underscoring the importance of prompt treatment planning. Zimmermann and Curtis (2020) further noted that physiological immune alterations during pregnancy may influence disease progression, making the maintenance of maternal–fetal balance critical in therapeutic decision-making.

8.1. Supportive Care

Supportive therapies constitute the cornerstone of COVID-19 treatment during pregnancy. Knight et al. (2020) emphasized maintaining oxygen saturation levels above 94% to optimize perinatal outcomes. Zimmermann and Curtis (2020) cautioned that fluid therapy should be administered judiciously, as excessive fluid loading may increase the risk of pulmonary edema. Durankuş and Aksu (2020) reported elevated psychological stress levels among pregnant women with COVID-19, which may affect treatment adherence. Therefore, psychosocial support should be considered an integral component of management.

8.2. Antiviral Treatment Strategies

Zimmermann and Curtis (2020) recommended that antiviral therapy during pregnancy be guided by a careful risk–benefit assessment, noting that agents such as remdesivir have limited but reassuring safety data in pregnant populations. Knight et al. (2020) reported that antiviral treatments may improve maternal outcomes in cases of severe COVID-19.

8.3. Corticosteroid Use

Corticosteroid use during COVID-19 treatment in pregnancy serves two distinct purposes: improving maternal respiratory function and promoting fetal lung maturation. Zimmermann and Curtis (2020) reported that dexamethasone may improve respiratory outcomes in severe COVID-19 cases. Knight et al. (2020) further demonstrated that betamethasone administration in pregnant women at risk of preterm delivery reduced neonatal respiratory distress.

8.4. Anticoagulant Therapy

COVID-19 is known to increase the risk of thromboembolic events. Knight et al. (2020) reported that the combination of pregnancy-related hypercoagulability and COVID-19–induced prothrombotic tendencies poses a significant risk. Consequently, low-molecular-weight heparin is considered a key prophylactic treatment option to mitigate thrombotic risk associated with both pregnancy and COVID-19.

8.5. Psychiatric Support During Pregnancy and COVID-19

Durankuş and Aksu (2020) reported increased levels of depression and anxiety among pregnant women during the pandemic, which may adversely affect treatment adherence. Ertan et al. (2021) also noted that social isolation and perceived uncertainty heightened psychological burden in pregnant women. Accordingly, Zimmermann and Curtis (2020) emphasized that psychosocial support should be a critical component of treatment planning.

8.6. Impact of Treatment Approaches on Obstetric Outcomes

Knight et al. (2020) reported that timely initiation of COVID-19 treatment reduced the incidence of preterm birth and maternal complications. Zimmermann and Curtis (2020) further noted that reductions in metabolic and immunological stress were associated with improved perinatal outcomes.

9. Effects of COVID-19 on Pregnancy and Fetal Development

The effects of COVID-19 on pregnancy and fetal development have been extensively investigated since the onset of the pandemic. Knight et al. (2020) reported that SARS-CoV-2 infection increases the risk of severe disease in pregnant women, which may adversely affect perinatal outcomes. Zimmermann and Curtis (2020) emphasized that immunological changes during pregnancy may disrupt maternal–fetal balance and indirectly affect fetal development.

9.1. COVID-19 and Placental Function

The placenta is a central organ in fetal health due to its protective and metabolic roles during pregnancy. Zimmermann and Curtis (2020) reported that placental inflammation, hypoperfusion, and vascular abnormalities were more frequently observed in pregnant women with COVID-19. Knight et al. (2020) further demonstrated that COVID-19 may impair placental circulation, reducing fetal oxygenation and increasing the risk of fetal distress and preterm birth. Ertan et al. (2021) suggested that heightened maternal stress during the COVID-19 pandemic may negatively affect placental function, as elevated stress levels can reduce uteroplacental blood flow through hormonal dysregulation.

9.2. Fetal Development and Birth Outcomes

Knight et al. (2020) reported increased rates of preterm birth among pregnant women with COVID-19, attributable to both direct effects of infection and maternal complications. Zimmermann and Curtis (2020) suggested that COVID-19 may be associated with intrauterine growth restriction and impaired fetal nutrition. Durankuş and Aksu (2020) emphasized that increased psychological stress among pregnant women during the pandemic may indirectly impair fetal development, as stress hormones can elevate the risk of fetal growth restriction. Ertan et al. (2021) further reported that social

isolation and uncertainty during the pandemic increased anxiety levels among pregnant women, imposing additional burdens on both maternal and fetal health.

9.3. Risk of Fetal Infection (Vertical Transmission)

Zimmermann and Curtis (2020) reported that the likelihood of SARS-CoV-2 crossing the placental barrier is extremely low, and vertical transmission remains rare. Knight et al. (2020) similarly reported that the majority of neonates born to mothers diagnosed with COVID-19 tested negative for SARS-CoV-2. These findings suggest that most adverse fetal outcomes associated with COVID-19 arise not from vertical transmission but from indirect mechanisms such as maternal hypoxemia, inflammation, and stress.

9.4. Effects on Fetal and Neonatal Immunity

Zimmermann and Curtis (2020) suggested that maternal immune activation during COVID-19 infection may influence fetal immune system development, with excessive maternal cytokine responses potentially altering fetal immune maturation. Knight et al. (2020) further reported that maternal infection may increase the risk of neonatal respiratory distress, low Apgar scores, and the need for neonatal intensive care during the perinatal period.

9.5. Effects of COVID-19 on Fetal Outcomes Through Maternal Mental Health

One of the indirect effects of COVID-19 during pregnancy is the increased psychological burden experienced by pregnant women. Durankuş and Aksu (2020) emphasized that the pandemic has increased levels of depression and anxiety among pregnant women, which may pose additional risks to fetal development. Ertan et al. (2021) reported that heightened anxiety may elevate prenatal stress hormones, thereby adversely affecting fetal developmental processes. Zimmermann and Curtis (2020) stated that maternal stress can influence the fetus through both immunological and neuroendocrine pathways, underscoring the importance of psychological monitoring as an integral component of prenatal care

10. Psychological Effects of COVID-19 During Pregnancy and Their Management

The COVID-19 pandemic has markedly increased levels of psychological stress, anxiety, and depression during pregnancy, with documented effects on both maternal and fetal health. Durankuş and Aksu (2020) reported that the pandemic heightened perceptions of uncertainty among pregnant women, increasing psychological burden and rendering them more vulnerable. Ertan et al. (2021) indicated that social isolation and the persistent threat posed by COVID-19 led to substantial anxiety in pregnant women. Zimmermann and Curtis (2020) further reported that elevated stress hormone levels in pregnant women exposed to COVID-19 or pandemic-related psychosocial stressors may affect maternal immune responses and fetal development. Knight et al. (2020) also suggested that psychological stress accompanying COVID-19 infection in pregnant women may influence obstetric outcomes.

10.1. Impact of the Pandemic on Anxiety and Depression Levels During Pregnancy

Durankuş and Aksu (2020) reported a significant increase in anxiety and depression rates among pregnant women during the pandemic, particularly associated with uncertainties regarding healthcare access, fear of hospital visits, and concerns about fetal health. Ertan et al. (2021) noted that the global rise in COVID-19 cases and widespread misinformation triggered heightened anxiety among pregnant women, potentially leading to recurrent cycles of fear and distress. Zimmermann and Curtis (2020) emphasized that anxiety may affect physiological processes regulating maternal immune function, thereby indirectly increasing risks for both mother and fetus.

10.2. Effects of Stress on Maternal–Fetal Health

Ertan et al. (2021) reported that increased stress during the pandemic may cause hormonal imbalances that adversely affect uteroplacental blood flow. Durankuş and Aksu (2020) similarly indicated that elevated stress levels may indirectly influence fetal development, particularly increasing the risk of growth restriction. Zimmermann and Curtis (2020) reported that maternal stress may enhance inflammatory responses through immunological activation, thereby affecting both pregnancy course and perinatal outcomes.

10.3. Pregnancy Follow-Up and Access to Healthcare Services During the Pandemic

Durankuş and Aksu (2020) reported that fear of hospital visits during the COVID-19 pandemic led many pregnant women to postpone routine prenatal appointments, thereby increasing anxiety levels.

Knight et al. (2020) emphasized that disruptions in access to healthcare services may negatively affect obstetric outcomes, particularly when high-risk pregnancies are not adequately monitored. Zimmermann and Curtis (2020) stressed that maintaining regular prenatal follow-up during pandemic conditions is critical for optimizing maternal–fetal outcomes.

10.4. Coping Strategies for Pregnancy During the COVID-19 Pandemic

Durankuş and Aksu (2020) suggested that providing psychosocial support to pregnant women during the pandemic may alleviate psychological symptoms and positively influence the pregnancy experience. Ertan et al. (2021) reported that counseling services and access to reliable information sources were effective in reducing anxiety among pregnant women. Zimmermann and Curtis (2020) further indicated that mental health support interventions may positively affect maternal immune function and overall health, thereby contributing to improved pregnancy outcomes.

10.5. Increased Psychological Burden of Social Isolation During Pregnancy

Ertan et al. (2021) reported that social isolation increased feelings of loneliness among pregnant women, thereby elevating anxiety levels. Durankuş and Aksu (2020) noted that isolation weakened social support mechanisms, reduced stress tolerance, and increased depressive symptoms in pregnant women. Knight et al. (2020) indicated that the lack of social support may complicate treatment adherence and prenatal follow-up, suggesting that isolation may indirectly affect clinical outcomes.

11. Social and Economic Effects of COVID-19 During Pregnancy

The COVID-19 pandemic has significantly affected social life, economic conditions, and overall quality of life during pregnancy. Durankuş and Aksu (2020) reported that the pandemic created heightened uncertainty, anxiety, and social isolation among pregnant women, imposing a substantial burden on daily functioning and social adaptation. Ertan et al. (2021) indicated that pandemic-related restrictions limited social interactions and weakened environmental support mechanisms, increasing psychosocial stress among pregnant women. Zimmermann and Curtis (2020) reported that COVID-19 directly affected pregnant women's access to healthcare services, employment status, and daily activities, potentially generating economic stress that negatively influenced the pregnancy process.

11.1. Weakening of Social Support Mechanisms During the Pandemic

Pregnant women are particularly sensitive to social support systems, many of which were constrained during the pandemic. Durankuş and Aksu (2020) reported that reduced social contact increased feelings of loneliness among pregnant women, adversely affecting psychological well-being. Ertan et al. (2021) noted that social isolation heightened stressors during pregnancy, with decreased contact with family and friends leading to increased anxiety and depression. Zimmermann and Curtis (2020) emphasized that the lack of social support affects both mental and physical health, underscoring the increased need for supportive environments for pregnant women during the pandemic.

11.2. Economic Hardships and Their Impact on Pregnancy

Job losses, income reduction, and economic uncertainty became widespread during the COVID-19 pandemic. Ertan et al. (2021) reported that economic difficulties intensified stress levels among pregnant women, exacerbating psychological burden. Durankuş and Aksu (2020) emphasized that financial insecurity related to COVID-19 increased pregnancy-related anxiety, highlighting the close relationship between economic conditions and both mental and physical health. Zimmermann and Curtis (2020) noted that economic pressure may limit access to healthcare services, with disruptions in routine follow-up potentially worsening perinatal outcomes.

11.3. Challenges in Accessing Healthcare Services

The pandemic caused congestion and access restrictions within healthcare systems. Knight et al. (2020) reported that pregnant women experienced difficulties accessing prenatal care services during the pandemic, complicating pregnancy management. Durankuş and Aksu (2020) noted that fear of hospital visits led some pregnant women to postpone appointments, further increasing anxiety levels. Zimmermann and Curtis (2020) emphasized that inadequate prenatal monitoring may increase the risk of complications, indicating that barriers to healthcare access can adversely affect both maternal and fetal outcomes.

11.4. Changes in Family and Home Life During the Pandemic

Ertan et al. (2021) reported that COVID-19 altered household role distributions, increased domestic stressors, and contributed to psychological burden among pregnant women. Durankuş and Aksu (2020) noted that increased time spent at home disrupted daily routines, with social isolation and reduced mobility negatively affecting mental well-being. Zimmermann and Curtis (2020) further reported that lifestyle changes during the pandemic reduced physical activity among pregnant women, potentially increasing stress and anxiety.

11.5. Socioeconomic Inequalities and the Impact of COVID-19 on Pregnancy

Knight et al. (2020) reported that pregnant women with lower socioeconomic status were at higher risk for COVID-19–related complications, attributable to disparities in healthcare access and economic conditions. Ertan et al. (2021) noted that the pandemic exacerbated psychological burden disparities across social classes, with economically disadvantaged groups experiencing higher stress levels. Zimmermann and Curtis (2020) emphasized that access to healthcare services and economic conditions are among the most fundamental determinants of pregnancy outcome

12. Reorganization of Maternity Health Services During the Pandemic

The COVID-19 pandemic necessitated a comprehensive reorganization of healthcare systems, with antenatal care being one of the most significantly affected areas. Knight et al. (2020) reported that increased patient density and heightened risk of infection in healthcare facilities directly influenced the organization of pregnancy care, underscoring the necessity for restructuring service delivery. Similarly, Zimmermann and Curtis (2020) emphasized that the increased burden on healthcare systems led to substantial changes in follow-up frequency, access modalities, and models of care delivery for pregnant women. Durankuş and Aksu (2020) noted that uncertainty and fear of hospital attendance during the pandemic altered appointment adherence among pregnant women, further highlighting the need for reorganized maternity services.

12.1. Reorganization of Antenatal Follow-up Protocols

During the pandemic, international guidelines recommended modifications to routine antenatal care protocols. Zimmermann and Curtis (2020) reported that follow-up frequency was redefined based on clinical necessity, with unnecessary in-person visits being reduced. Knight et al. (2020) emphasized that maintaining regular monitoring remained critically important for high-risk pregnancies.

Durankuş and Aksu (2020) further indicated that postponement of antenatal visits increased anxiety levels among pregnant women, necessitating careful management by healthcare systems.

12.2. Use of Telehealth Applications

The pandemic accelerated the adoption of telehealth applications in antenatal care. Ertan et al. (2021) reported that telehealth use could mitigate anxiety associated with social isolation and provide psychosocial support to pregnant women. Zimmermann and Curtis (2020) highlighted telemedicine as an effective alternative, particularly for low-risk pregnancies, enabling continuity of care while reducing infection risk. However, Knight et al. (2020) cautioned that telehealth may be insufficient for conditions requiring physical examination, advocating for hybrid care models that integrate both remote and in-person assessments.

12.3. Hospital Organization and Intrapartum Care

Special measures were implemented for maternity services during the pandemic. Knight et al. (2020) reported the establishment of isolated delivery units for COVID-19–positive pregnant women, which was essential for ensuring the safety of both mothers and healthcare professionals. Zimmermann and Curtis (2020) emphasized the mandatory use of personal protective equipment by labor ward staff and the tightening of infection control protocols. Ertan et al. (2021) noted that restrictions on partners and family members during hospital admission increased the psychological burden on pregnant women, highlighting the need for supportive interventions during childbirth.

12.4. Impact of the Pandemic on Healthcare Professionals

The COVID-19 pandemic affected not only patients but also healthcare professionals. Zimmermann and Curtis (2020) stressed that protecting healthcare workers from increased workload and infection risk was critical for the sustainability of maternity services. Ertan et al. (2021) reported that burnout among healthcare professionals could negatively impact the quality of antenatal and intrapartum care, underscoring the importance of strengthening institutional support systems.

12.5. Lasting Changes in Health Services After the Pandemic

Zimmermann and Curtis (2020) indicated that the pandemic accelerated digital integration within healthcare systems, with telemedicine likely to remain a permanent component of maternity care. Knight et al. (2020) suggested that hybrid follow-up models developed during the pandemic may continue to be used in the future, particularly within risk-based and personalized antenatal care frameworks. Ertan et al. (2021) further emphasized that providing psychosocial support through digital platforms could enhance accessibility for pregnant women and should be sustained beyond the pandemic period.

13. Long-Term Effects of the Pandemic on Maternal and Infant Health

Evidence suggests that the effects of the COVID-19 pandemic on maternal and infant health are not limited to the acute phase but may result in long-term clinical, psychological, and social consequences. Zimmermann and Curtis (2020) reported that immunological, hormonal, and psychosocial changes occurring during pregnancy in the context of COVID-19 may persist into the postpartum period. Knight et al. (2020) noted that pregnant women who experienced COVID-19 may have increased perinatal complications and greater postpartum care needs. Durankuş and Aksu (2020) highlighted that elevated stress levels during the pandemic may increase the risk of postpartum depression and anxiety. Ertan et al. (2021) further emphasized that pandemic-related disruptions to social support mechanisms could adversely affect maternal adjustment.

13.1. Long-Term Effects on Maternal Mental Health

Multiple studies have demonstrated that increased anxiety and depression during pregnancy persisted into the postpartum period during the pandemic. Durankuş and Aksu (2020) reported that women

experiencing high stress levels during pregnancy were at increased risk of postpartum depression. Ertan et al. (2021) noted that pandemic-related social isolation hindered adaptation to the maternal role and could lead to prolonged psychological maladjustment. Zimmermann and Curtis (2020) suggested that COVID-19–related immunological stress responses might persist postpartum, indirectly affecting maternal mental health.

13.2. Effects on Mother–Infant Bonding

Pandemic conditions also affected mother–infant bonding. Ertan et al. (2021) reported that lack of social support altered the maternal experience and weakened the emotional aspects of bonding. Durankuş and Aksu (2020) indicated that elevated anxiety levels could reduce mother–infant interaction, potentially compromising emotional attachment during the postpartum period. Zimmermann and Curtis (2020) emphasized that postpartum stressors may influence maternal bonding behaviors, with the pandemic introducing additional risks to this process.

13.3. Long-Term Effects on Neonatal Health and Development

The indirect effects of COVID-19 on the fetus and neonate may pose long-term developmental risks. Knight et al. (2020) reported that severe maternal COVID-19 infection during pregnancy could increase the need for neonatal intensive care and necessitate long-term health monitoring. Zimmermann and Curtis (2020) suggested that maternal immune responses may influence fetal immune system development, with potential postnatal implications. Durankuş and Aksu (2020) further reported that maternal stress may exert long-lasting effects on fetal development and neurobehavioral regulation.

13.4. Influence of Social and Economic Factors on Long-Term Outcomes

Ertan et al. (2021) emphasized that economic hardship and lack of social support during the pandemic complicated the maternal experience, potentially leading to long-term adverse outcomes for both mothers and infants. Zimmermann and Curtis (2020) noted that disruptions in access to healthcare services during the pandemic could influence long-term obstetric and neonatal outcomes. Knight et al. (2020) highlighted that socioeconomic inequalities became more pronounced during the pandemic, directly affecting the quality of maternity care and contributing to long-term disparities in maternal and infant health.

13.5. Post-Pandemic Follow-up Needs for Maternal and Infant Health

Zimmermann and Curtis (2020) stressed the importance of implementing long-term follow-up programs for both mothers and infants in the post-pandemic period, particularly with enhanced psychological and immunological monitoring. Durankuş and Aksu (2020) emphasized that early postpartum mental health screening is crucial for mitigating the psychological impact of the pandemic. Ertan et al. (2021) suggested that post-pandemic social support programs could improve maternal adjustment and strengthen long-term mother–infant relationships.

14. Current Recommendations and Clinical Approaches to COVID-19 in Pregnancy

The COVID-19 pandemic prompted updates to international guidelines and redefinition of clinical approaches in pregnancy management. Zimmermann and Curtis (2020) reported that the clinical course of COVID-19 in pregnant women is generally similar to that of the general population; however, physiological and immunological adaptations during pregnancy warrant heightened clinical vigilance. Knight et al. (2020) emphasized that pregnant women, particularly in the third trimester, may require closer monitoring due to an increased risk of severe disease in certain subgroups. Durankuş and Aksu (2020) highlighted that heightened psychological stress during the pandemic could influence perinatal clinical decision-making, underscoring the need for targeted counseling. Ertan et al. (2021) emphasized that COVID-19 management in pregnancy should extend beyond

infection control to include psychosocial support, birth planning, and postpartum follow-up within a comprehensive care framework.

14.1. Diagnosis and Clinical Assessment in Pregnancy

Diagnosis of COVID-19 in pregnant women is based on RT-PCR testing and clinical evaluation, following principles similar to those applied to the general population. Zimmermann and Curtis (2020) reported that symptoms are generally mild to moderate in pregnant women; however, manifestations such as dyspnea and fever require close monitoring. Knight et al. (2020) noted that symptoms may be more pronounced during the third trimester and emphasized that maternal oxygen saturation directly affects fetal well-being, necessitating more frequent monitoring during this period.

14.2. Therapeutic Approaches in Pregnancy

Treatment of COVID-19 during pregnancy is primarily supportive. Zimmermann and Curtis (2020) emphasized that maintaining adequate oxygen saturation is critical for both maternal and fetal survival. Knight et al. (2020) reported an increased risk of thromboembolism among pregnant women hospitalized with COVID-19, suggesting that prophylactic anticoagulation should be considered in appropriate cases. Ertan et al. (2021) underscored the importance of incorporating psychological support into clinical care and recommended regular psychosocial assessments throughout the treatment process.

14.3. Management of Labor and Delivery

In COVID-19–positive pregnant women, the mode of delivery should be determined by obstetric indications rather than infection status alone. Zimmermann and Curtis (2020) emphasized that decisions regarding elective or emergency cesarean section should be based on obstetric necessity rather than COVID-19 positivity. Knight et al. (2020) noted that in cases of severe COVID-19 with maternal respiratory compromise, the timing of delivery may need to be re-evaluated according to clinical status. Durankuş and Aksu (2020) highlighted that increased anxiety during labor may elevate the risk of complications and suggested that antenatal psychoeducation could be beneficial.

14.4. Postpartum Management and Breastfeeding

Breastfeeding is also recommended for mothers who are COVID-19 positive. Zimmermann and Curtis (2020) reported that there is no evidence supporting viral transmission through breast milk and emphasized that breastfeeding should be continued with appropriate hygiene and infection control measures. Ertan et al. (2021) noted that lack of social support during the postpartum period may increase the risk of depression, highlighting the need to strengthen family-, healthcare-, and community-based support mechanisms. Durankuş and Aksu (2020) emphasized that elevated postpartum stress levels may negatively affect the mother–infant relationship and underscored the importance of early psychological interventions during the postpartum period (Durankuş & Aksu, 2020).

14.5. Supporting Pregnant Women During the Pandemic

Access to reliable health information was of critical importance for pregnant women during the pandemic. Ertan et al. (2021) emphasized that misinformation and social isolation imposed a significant psychological burden and stressed the necessity of directing pregnant women toward trustworthy healthcare counseling. Durankuş and Aksu (2020) reported that psychological stress management programs could reduce anxiety levels among pregnant women and contribute to the development of positive attitudes toward childbirth. Knight et al. (2020) highlighted that access to maternity healthcare services should not be disrupted during pandemic conditions and identified telemedicine applications as an essential tool for maintaining continuity of care.

15. Lessons Learned and Conclusions for Future Pandemics

The COVID-19 pandemic clearly demonstrated the vulnerability of antenatal care even under normal circumstances and underscored the necessity for healthcare systems to be prepared for unforeseen crises. Zimmermann and Curtis (2020) emphasized that the impact of COVID-19 on pregnancy management extended beyond the clinical course of infection, reshaping healthcare system organization, psychosocial support structures, prenatal care models, and postpartum processes. Knight et al. (2020) reported that pregnant women, particularly those in the third trimester, may be at increased risk during pandemics, highlighting the need for pre-established pregnancy-specific protocols for crisis periods. Durankuş and Aksu (2020) noted significantly elevated stress and anxiety levels among pregnant women during the COVID-19 pandemic, identifying these psychological burdens as risk factors during both pregnancy and the postpartum period, and emphasized the importance of integrating mental health services into maternity care during future pandemics. Similarly, Ertan et al. (2021) stressed that psychological support mechanisms should be prioritized during crises and advocated for the development of alternative approaches, such as tele-psychological counseling, to mitigate the effects of social isolation.

15.1. The Need for Flexible and Resilient Maternity Care Systems

Zimmermann and Curtis (2020) reported that pandemics can lead to abrupt changes in healthcare capacity and highlighted the need for rapidly adaptable antenatal care protocols. Knight et al. (2020) emphasized that designated areas and logistical planning for pregnant patients are critical, particularly during periods of increased intensive care demand. Durankuş and Aksu (2020) noted that pandemic conditions disrupted routine antenatal visits and stressed the importance of developing effective monitoring models with minimal physical contact in future planning efforts.

15.2. The Enduring Role of Telehealth and Digital Monitoring

Telehealth applications became an essential component of maternity care during the COVID-19 pandemic. Zimmermann and Curtis (2020) reported that digital healthcare services could provide a sustainable model, particularly for low-risk pregnancies. Ertan et al. (2021) emphasized that telehealth applications could strengthen not only clinical monitoring but also psychological support processes. Knight et al. (2020) highlighted that standardizing digital monitoring systems would enable uninterrupted maternity care during future pandemic-like crises.

15.3. Long-Term Monitoring of Maternal and Infant Health

Durankuş and Aksu (2020) demonstrated that COVID-19 increased maternal stress levels, which may persist into the postpartum period, thereby necessitating long-term follow-up of both mothers and infants (Durankuş & Aksu, 2020). Ertan et al. (2021) emphasized that psychosocial effects extend beyond childbirth and affect early motherhood, underscoring the need to strengthen postpartum support programs. Zimmermann and Curtis (2020) reported that alterations in maternal immune responses during pandemics may influence fetal immune system development, increasing the importance of structured neonatal follow-up programs.

15.4. Supporting Healthcare Professionals

The workload imposed on healthcare professionals increased substantially during the pandemic. Ertan et al. (2021) reported that heightened levels of burnout and anxiety among healthcare workers could adversely affect the quality of maternity services. Zimmermann and Curtis (2020) emphasized that protecting healthcare professionals is essential for ensuring the sustainability of pregnancy care during pandemics.

15.5. Conclusions

The existing literature on the COVID-19 pandemic demonstrates that maternity care is a multidimensional process that must be organized not only medically but also psychologically and socially during crisis periods. Knight et al. (2020) emphasized the necessity of developing pregnancy care protocols in advance for potential future pandemics and highlighted the critical importance of early identification of high-risk pregnant women. Zimmermann and Curtis (2020) stressed that healthcare services should be supported by robust digital infrastructure and flexible organizational models in the event of similar crises. Durankuş and Aksu (2020) and Ertan et al. (2021) collectively highlighted that pandemics exert not only clinical but also psychological and social impacts, reinforcing the importance of holistic maternity care.

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CHAPTER 5

Lucilia sericata AND PLANT GROWTH REGULATORS: A COMPARATIVE ENDOCRINOLOGY PERSPECTIVE

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Introduction

The evolution of multicellular organisms has necessitated the development of sophisticated chemical communication systems for the coordination of cellular activities. These communication networks, termed hormonal regulation, constitute the fundamental mechanisms controlling organismal growth, development, physiological responses, and environmental adaptation. Hormones are chemical messengers that are effective even at low concentrations, capable of long-distance signaling, and induce complex gene expression changes in specific target cells (Hartenstein, 2006; Santner and Estelle, 2009).

Animals and plants have followed independent evolutionary trajectories following their divergence from a common eukaryotic ancestor approximately 1.6 billion years ago (Hedges et al., 2004). Despite this prolonged evolutionary separation, both groups have been compelled to solve similar challenges posed by multicellular life: regulation of growth, control of cellular differentiation, timing of developmental transitions, responses to environmental changes, post-injury repair, and optimization of reproductive strategies. These parallel evolutionary pressures have led to the emergence of hormonal systems that, while implemented using entirely different molecular tools, produce functionally analogous outcomes (Grollman et al., 2008; King and Carroll, 2001). This phenomenon, termed convergent evolution, suggests that a limited number of optimal strategies may exist for solving biological problems (McGhee, 2011). *Lucilia sericata* (the green bottle fly) is an insect species belonging to the family Calliphoridae of the order Diptera and is extensively studied in entomology, forensic science, ecology, and medicine. This organism possesses a complex life cycle, progressing through five distinct developmental stages: egg, three larval instars, pupa, and adult (Smith, 1986). Each developmental transition is meticulously controlled by hormonal signals, and these control mechanisms provide precise integration between the organism's internal physiological state and external environmental conditions (Nijhout, 1994).

The endogenous growth regulators of *L. sericata* consist primarily of two major hormone families: ecdysone and its derivatives, and juvenile hormones (JH). Ecdysones are steroid hormones and serve as the primary signaling molecules triggering molting and metamorphosis in insects (Riddiford, 1993; Gilbert et al., 2002). Juvenile hormones are sesquiterpenoid compounds that play regulatory roles across a broad spectrum, from maintenance of larval characteristics to adult reproductive physiology (Flatt et al., 2005). These two hormone systems coordinate all aspects of insect development through antagonistic and synergistic interactions. Additionally, neuropeptides such as insulin-like peptides (ILPs) and prothoracicotropic hormone (PTTH) assume critical roles in nutrient signaling and regulation of growth rate (Brogiolo et al., 2001; McBrayer et al., 2007). Another remarkable feature of *L. sericata* larvae is the bioactive molecules they secrete into their environment. These molecules—allantoin, antimicrobial peptides (lucifensin, defensins), proteolytic enzymes, and other factors—promote wound healing and form the foundation of the clinical practice known as maggot debridement therapy (Sherman, 2009, 2014). These secreted factors stimulate cellular proliferation, angiogenesis, immune modulation, and tissue renewal in host organism tissues, functioning essentially as "exogenous growth regulators." This dual regulatory system—both endogenous hormones controlling its own development and exogenous factors affecting its environment—renders *L. sericata* a unique model for comparative endocrinology and translational biomedical research.

Plants have evolved a growth regulatory system entirely distinct from the animal kingdom. Plant hormones, termed phytohormones, represent an extraordinarily heterogeneous group in

terms of chemical diversity. The five classical phytohormonal groups—auxins, cytokinins, gibberellins, abscisic acid (ABA), and ethylene—were discovered in the early and mid-20th century (Went and Thimann, 1937; Miller et al., 1956; Nishimura et al., 1959). In recent years, additional signaling molecules such as brassinosteroids, jasmonic acid, salicylic acid, strigolactones, karrikins, and nitric oxide have been identified as hormones or hormone-like regulators (Clouse, 2011; Wasternack and Hause, 2013; Gomez-Roldan et al., 2008). These hormones regulate virtually every aspect of plant life: seed dormancy and germination, root and shoot development, vascular tissue differentiation, tropisms (gravitropism, phototropism), flowering timing, fruit ripening, leaf senescence, stomatal movements, pathogen defense, abiotic stress responses, and even symbiotic interactions with other organisms (mycorrhiza, Rhizobium nodulation) (Santner et al., 2009; Davies, 2010). One of the most striking features of phytohormones is their operation within intensive crosstalk with one another. The effect of a hormone rarely occurs in isolation; rather, multiple hormone signals are integrated at the cellular level, and the resultant response emerges from the sum of all these signals (Nemhauser et al., 2006; Depuydt and Hardtke, 2011). For instance, auxin and cytokinin play antagonistic roles in organ formation and callus differentiation, while auxin and ethylene exhibit synergistic effects in root hair development. Similarly, ABA and gibberellin function as opposing regulators in seed dormancy and germination. These hormonal networks enable plants to fine-tune their developmental and physiological responses according to variable environmental conditions (Wolters and Jürgens, 2009; Friml, 2010). Another unique feature of plants is that hormonal signaling is accomplished through a combination of local synthesis, long-distance transport (via xylem and phloem), and cell-to-cell movement (through plasmodesmata or active transporters) (Friml and Jones, 2010; Robert and Friml, 2009). This represents a decentralized hormone production and distribution strategy, markedly different from the centralized endocrine glands of animals.

At first glance, one might consider that there are few commonalities between the hormonal system of *Lucilia sericata* and plant phytohormones. Chemical structures, biosynthetic pathways, receptor types, and even the tissues producing the hormones are entirely different. Ecdysone is a steroid while auxin is an indole derivative; juvenile hormone is a sesquiterpenoid while gibberellin is a diterpenoid; insulin-like peptides are proteinaceous while ethylene is a simple hydrocarbon gas. However, deeper analysis reveals striking functional parallels and conceptual similarities between these systems. In both systems, hormones: (1) are effective at low concentrations (nanomolar-micromolar range), (2) are detected through specific receptor proteins, (3) activate signal transduction cascades, (4) induce extensive changes in gene expression, (5) provide temporal and spatial coordination of growth and development, (6) are integrated with environmental signals, (7) exhibit antagonistic and synergistic interactions, and (8) control critical transitions in the organism's life cycle (Hartenstein, 2006; Wolters and Jürgens, 2009). Even more intriguing is that certain signal transduction components—particularly protein kinase cascades (MAPK pathways), the ubiquitin-proteasome system, second messengers (Ca^{2+}, cAMP/cGMP), and transcription factor families—are evolutionarily conserved and assume similar roles in both animals and plants (Tena et al., 2001; Smalle and Vierstra, 2004). This suggests that fundamental cellular signaling mechanisms originate from eukaryotic ancestral origins and have been adapted to different hormones in different organisms. Furthermore, functional similarities exist between the exogenous factors secreted by *L. sericata* larvae (allantoin, antimicrobial peptides) and certain aspects of plant hormones, particularly in the context of wound response and tissue regeneration. In plants, auxin and cytokinin control callus formation and organ regeneration, while jasmonic acid and salicylic acid regulate wound responses (Ikeuchi et al., 2013; Wasternack and Hause, 2013). Similarly, *L. sericata* secretions promote cellular proliferation, differentiation, and tissue repair in host

tissues (Horobin et al., 2006). These functional parallels underscore the value of examining both systems within a comparative framework.

The aim of this study is to investigate the growth regulatory systems of *Lucilia sericata* within a comprehensive and multi-level comparison with plant phytohormones. The analysis will cover the following dimensions: (1) Endogenous Growth Regulators of *Lucilia sericata*, (2) Growth Regulatory Factors Secreted by *Lucilia sericata*, (3) Growth Factors Induced in the Host Tissue, (4) Molecular Mechanisms and Signal Transduction, (5) Environmental Signals and Hormonal Responses, (6) Developmental Plasticity and Phenotypic Flexibility, (7) Evolutionary Perspective and Conservation, (8) Therapeutic Applications and Biotechnology. Each section will be supported by concrete examples from *L. sericata* and plant systems, with similarities and differences clearly highlighted and substantiated by appropriate references from the current scientific literature. This comprehensive comparative analysis will contribute to a better understanding of fundamental biological principles and demonstrate how this knowledge can be translated into translational applications. Ultimately, this study aims to elucidate the fundamental strategies of growth regulation in evolutionarily distant organisms and to evaluate how these strategies can be utilised for human health, agricultural production, and biotechnological innovations.

1. Endogenous Growth Regulators of *Lucilia sericata*

1.1.Ecdysteroids: The ‘Growth Hormones’ of Insects

Ecdysteroids are steroid hormones that control growth and development in insects. Riddiford (1993) demonstrated that ecdysteroids play a central role in insect metamorphosis and that the active form of 20-hydroxyecdysone (20E) initiates larval moulting and pupation. In *L. sericata*, this hormone is produced by the prothoracic glands and distributed throughout the body via the haemolymph (insect blood) (Nijhout, 1994). Periodic increases in ecdysone concentration trigger the transition of larvae from the first stage to the second stage and from the second stage to the third stage. During each molt period, when ecdysone levels reach a critical threshold, the larva sheds its old cuticle (exoskeleton) and synthesises a new, larger cuticle (Truman and Riddiford, 2002). This system is comparable to the function of the gibberellin (GA) hormone in plants. Giberellins, particularly GA3, regulate root elongation, flowering, and seed germination in plants (Hedden and Phillips, 2000). Both ecdysones and giberellins initiate sudden growth and developmental changes during critical developmental periods of the organism. However, at the molecular level, ecdysones directly affect gene transcription by binding to nuclear receptors (EcR/USP complex) (Koelle et al., 1991), while gibberellins eliminate growth-inhibitory effects by promoting the degradation of DELLA proteins (Sun, 2010). In both systems, the hormone signal ultimately alters the transcription of target genes, but the signal transduction pathways are evolutionarily distinct.

1.2. Juvenile Hormones (JH): The ‘Status Quo Hormone’

Juvenile hormones play a critical role in determining developmental stages in insects. Wigglesworth (1934) first described juvenile hormone as a factor that ‘preserves larval characteristics’. In *L. sericata*, JH III, produced by brain glands called corpora allata, ensures the maintenance of larval characteristics. In the presence of high JH concentrations, ecdysone triggers larval-larval moulting, whereas in the absence of JH, the same ecdysone signal initiates larval-pupal transformation (Riddiford, 2012). This is extremely important in terms of developmental plasticity; the same hormone (ecdysone) produces completely different

developmental outcomes in the presence of a different hormone (JH). This system is reminiscent of the interaction between auxin (IAA) and cytokinin in plants. Skoog and Miller (1957) demonstrated that the auxin/cytokinin ratio determines root, shoot, or callus formation in plant tissue cultures. A high auxin/low cytokinin ratio promotes root formation, while a low auxin/high cytokinin ratio supports shoot development. In both systems, the relative concentrations of the two different hormones determine developmental decisions. However, while temporal coordination is critical in the JH and ecdysone system, spatial distribution is more important in the auxin and cytokinin system (Werner et al., 2001). While hormones act systemically in insects, local hormone gradients determine organ shape in plants.

1.2.Insulin-Like Peptides (ILPs): Metabolic Growth Regulators

In recent years, it has been demonstrated that insulin-like peptides (ILPs) play important roles in growth control in insects. Brogiolo et al. (2001) reported that ILPs regulate body size, development time, and metabolism in Drosophila. Similar peptides are also found in *L. sericata*, and these molecules adjust the growth rate of the larva according to food availability. In conditions of insufficient nutrition, ILP secretion decreases, which slows growth and delays development (Colombani et al., 2005). This system integrates with the Target of Rapamycin (TOR) signalling pathway to coordinate cellular growth and division with nutritional status (Oldham et al., 2000). A similar system exists in plants. In plant cells, the TOR kinase regulates growth by sensing nutrient availability (Ren et al., 2012). However, unlike the animal insulin-like system, plants use the sucrose non-fermenting-1-related kinase (SnRK1) pathway for sugar signalling (Baena-González et al., 2007). In both systems, nutrient availability modulates growth rate, but the sensor molecules and signal transduction pathways differ. An interesting similarity is that in both systems, AMPK/SnRK1 family kinases play a central role in energy homeostasis (Hardie, 2007). This indicates an evolutionarily conserved energy sensing mechanism.

1.3.Prothoracicotropic Hormone (PTTH): Developmental Timer

PTTH is a neuropeptide produced in the insect brain that triggers ecdysone secretion. McBrayer et al. (2007) demonstrated that PTTH acts as a ‘control point’ for critical size regulation. PTTH is not secreted until the larva reaches a critical mass, preventing premature metamorphosis. This mechanism ensures that the organism does not undergo a vitally important developmental change before reaching sufficient size. A similar ‘control point’ system in plants involves florigen (FT protein) and flowering timing. Kardailsky et al. (1999) demonstrated that the FT protein produced in leaves is transported to the apex as a long-distance signal and triggers flowering under appropriate conditions. Both PTTH and florigen are produced in different parts of the organism (brain vs. leaves) and initiate important developmental transitions by acting on distant target tissues. In both cases, environmental conditions (photoperiod, temperature, nutrition) modulate the production of these signals. This is a critical mechanism for developmental plasticity and environmental adaptation (Flatt et al., 2005; Corbesier et al., 2007).

2. Growth Regulatory Factors Secreted by *Lucilia sericata*

2.1. Allantoin: A Multifunctional Growth Regulator

Allantoin (5-ureidohydantoin) is an important compound produced by *L. sericata* larvae as a result of uric acid metabolism. Sherman (2014) reported that the concentration of allantoin in

larval secretions ranged from 10 to 100 μM and that at these concentrations, it increased fibroblast proliferation by 30-50%. Allantoin promotes mitotic activity by accelerating the transition from the G1 phase to the S phase of the cell cycle. It also protects cells from oxidative stress by neutralising reactive oxygen species (ROS) (Araujo et al., 2010). It contributes to the remodelling of the extracellular matrix by regulating the activity of matrix metalloproteinases such as collagenase and elastase.

The effects of allantoin are strikingly similar to those of the cytokinin hormone in plants. Cytokinins, particularly zeatin and kinetin, promote cell division and delay ageing in plant cells (Miller et al., 1956). Both allantoin and cytokinins are related to purine metabolism; cytokinins are adenine derivatives and their biosynthesis is shared with purine metabolic pathways (Sakakibara, 2006). An interesting evolutionary link is that both molecules are closely related to nitrogen metabolism. Allantoin is a product of nitrogen waste metabolism, while cytokines play a role in nitrogen signalling (Ruffel et al., 2011). Both molecules share effects on cellular proliferation, protein synthesis, and anti-senescence (anti-ageing).

At the molecular level, the mechanism of action of allantoin has not yet been fully elucidated, but studies suggest that it activates the MAPK (mitogen-activated protein kinase) pathway (Verteramo et al., 2013). Cytokines, on the other hand, activate the two-component signal transduction system by binding to histidine kinase receptors (Hwang et al., 2002). Although their signalling mechanisms differ, both ultimately activate cell cycle genes (e.g., cyclin D-type genes) and transcription factors associated with growth.

2.2. Urea and Calcium Carbonate: pH Regulators and Growth Modulators

L. sericata larvae produce significant amounts of urea as a result of protein metabolism and secrete calcium carbonate ($CaCO_3$). Parnés and Lagan (2007) demonstrated that larval secretions raise wound pH from 5.5–6.0 to 7.0–7.5. This pH change is critical for many physiological processes. Neutral pH optimises protease activity, inhibits bacterial growth, and provides an ideal environment for fibroblast proliferation. Calcium ions (Ca^{2+}) activate intracellular signalling pathways as second messengers and potentiate the activation of growth factor receptors.

pH homeostasis and calcium signalling are similarly critical in plants. The expansion of the plant cell wall is explained by the ‘acid growth theory’ triggered by the hormone auxin. Rayle and Cleland (1992) demonstrated that auxin acidifies the cell wall by activating the H^+-ATPase pump in the plasma membrane, and that this activates expansin proteins, leading to cell wall relaxation. Therefore, in plants, low pH (4.5-5.5) promotes growth, while in animals and wound healing, neutral pH (7.0-7.5) supports optimal growth. This fundamental difference reflects the mechanical constraints of organisms with and without cell walls. Calcium signalling, however, is evolutionarily conserved in both systems. In plants, calcium binds to calmodulin and calmodulin-like proteins (CMLs) to activate various kinases and transcription factors (Bouché et al., 2005). In animal cells, similar calmodulin-dependent kinases (CaMKs) transmit growth signals. In both cases, Ca^{2+} fluctuations encode cellular responses and provide specificity (McAinsh and Pittman, 2009). The calcium carbonate secreted by *L. sericata* regulates cellular activity by providing this universal signalling molecule to the wound environment.

2.3. Antimicrobial Peptides: Immunomodulatory Growth Regulators

L. sericata larvae secrete antimicrobial peptides (AMPs) such as lucifensin and defensin. Kerridge et al. (2005) reported that lucifensin exhibits potent activity against gram-positive bacteria and is effective against MRSA. These peptides are not only antimicrobial but also possess immunomodulatory effects. At low concentrations, these peptides act as chemotactic agents, attracting macrophages and neutrophils to the wound site, but also modulate the excessive inflammatory response, thereby preventing chronic inflammation (van der Plas et al., 2008). They reduce the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while increasing anti-inflammatory IL-10 levels. Similar antimicrobial peptides are also present in plants and are called 'defensins' and 'thionins'. Broekaert et al. (1995) demonstrated that plant defensins provide protection against fungal and bacterial pathogens. An interesting point is that both animal and plant defensins exhibit similar three-dimensional structures (β-sheet rich, stabilised by disulphide bridges), which is an example of convergent evolution (Lay and Anderson, 2005). However, the amino acid sequences are quite different, indicating independent evolutionary origins.

Similar mechanisms exist in plants in terms of immunomodulatory effects. The hormone network formed by salicylic acid (SA), jasmonic acid (JA) and ethylene regulates plant immune responses (Glazebrook, 2005). SA-dependent systemic acquired resistance (SAR) and JA-dependent wound response are conceptually parallel to the balance of inflammatory and anti-inflammatory cytokines in animals. In both systems, a delicate balance must be maintained between pathogen defence and tissue repair; an excessive defence response can damage tissue, while an inadequate response leads to infection (Spoel and Dong, 2012).

2.4. Proteolytic Enzymes: Extracellular Matrix Regulators

Serine proteases and metalloproteinases secreted by *L. sericata* larvae play critical roles in the digestion of necrotic tissue and the remodelling of the extracellular matrix (ECM). Chambers et al. (2003) demonstrated that larval secretions exhibit trypsin-like, chymotrypsin-like, and collagenase activities. These enzymes create space for new tissue formation by breaking down damaged collagen and other ECM proteins. They also enhance biological activity by releasing growth factors (e.g., TGF-β, bFGF) stored in the matrix.

In plants, cell wall remodelling is also essential for growth and development. Expansins facilitate cell expansion by loosening cell wall polysaccharides (cellulose and hemicellulose) (Cosgrove, 2000). Enzymes belonging to the xyloglucan endotransglycosylase/hydrolase (XTH) family cleave and reattach xyloglucan chains, enabling cell wall remodelling (Rose et al., 2002). Pectate lyases and polygalacturonases catalyse pectin degradation. These enzymes are upregulated by auxin and increase cell wall plasticity during growth (Majda and Robert, 2018).

The common function of both larval proteases and plant cell wall enzymes is to enable growth by modifying structural barriers. *L. sericata* proteases facilitate regeneration by breaking down damaged ECM, while plant enzymes support normal growth by modifying healthy cell walls. In both cases, these enzymes work in coordination with hormonal signals (growth factors in animals, auxin in plants). In the Zinnia tracheary element differentiation system, cell wall proteases and cellulases are activated as plant cells undergo programmed cell death (Fukuda, 1996). This process resembles necrotic tissue elimination during wound healing and is an example of controlled tissue remodelling in both cases.

3. Growth Factors Induced in Host Tissue

3.1. TGF-β, VEGF and Other Growth Factors

L. sericata larval secretions increase the expression of endogenous growth factors in host tissue. Horobin et al. (2006) reported that VEGF (vascular endothelial growth factor) mRNA levels increased by 200-300% in wounds treated with larvae. VEGF promotes angiogenesis (new blood vessel formation), thereby increasing the supply of oxygen and nutrients to the wound tissue. TGF-β (transforming growth factor-beta) levels also rise, and this cytokine triggers the differentiation of fibroblasts into myofibroblasts, supporting wound contraction and collagen synthesis (Werner and Grose, 2003).

Similar growth regulatory molecules are induced during the wound response in plants. Jasmonic acid (JA) rapidly accumulates in the wound site and activates genes regulating defence and callus formation (Wasternack and Hause, 2013). Local auxin concentration also increases and coordinates cell division and differentiation. Cytokinin levels rise in callus tissue and support dedifferentiation and cell proliferation (Ikeuchi et al., 2013). The combination of these hormones is essential for the repair and regeneration of injured plant tissue.

In both systems, the wound response is triggered by damage-associated molecular patterns (DAMPs). In animals, ATP, HMGB1, and extracellular matrix fragments released from damaged cells activate Toll-like receptors (TLRs), initiating the inflammatory cascade (Bianchi, 2007). In plants, oligogalacturonides (pectin fragments from damaged cell walls) are recognised by pattern recognition receptors (PRRs) and trigger JA/ethylene synthesis (Ferrari et al., 2013). These DAMPs are upstream signals that coordinate the release of growth factors and hormones. The mechanical and enzymatic activity of *L. sericata* larvae can amplify this signalling by producing additional DAMPs.

4. Molecular Mechanisms and Signal Transduction

4.1. Receptor Systems: Nuclear Receptors vs. Membrane Receptors

Ecdysone signalling operates via a nuclear receptor mechanism. 20-hydroxyecdysone enters the cell and induces heterodimerisation of the ecdysone receptor (EcR) and ultraspiracle (USP) proteins. This complex binds to DNA and regulates the transcription of target genes (Koelle et al., 1991). Primary response genes (early genes) are activated within 4-6 hours, while secondary response genes (late genes) are expressed 10-12 hours later. This temporal gene cascade coordinates the molting process in a stepwise manner (Thummel, 1996).

In plants, auxin signalling involves both fast (membrane receptors) and slow (nuclear mechanisms) components. Auxin Binding Protein 1 (ABP1) binds auxin at the plasma membrane and within seconds leads to cell membrane hyperpolarisation and an increase in cytoplasmic Ca^{2+} (Sauer and Kleine-Vehn, 2011). Nuclear auxin signalling occurs via the Transport Inhibitor Response 1 (TIR1) receptor. When auxin binds to TIR1, it triggers the ubiquitination and degradation of Aux/IAA repressor proteins, which leads to the activation of Auxin Response Factors (ARFs) transcription factors (Dharmasiri et al., 2005). This process occurs within minutes and initiates the transcription of auxin-responsive genes.

In both systems, the speed and duration of the hormone response are determined by the receptor type and the signal transduction pathway. In ecdysone signalling, a single hormone-

receptor interaction triggers long-term developmental programmes (hours-days). In auxin signalling, both rapid responses (membrane events, seconds-minutes) and long-term transcriptional changes (minutes-hours) occur. This difference reflects the programmed and episodic nature of animal development versus the continuous and modular nature of plant growth (Wolpert, 2002).

4.2. Signal Transduction Pathways: MAPK, PI3K/Akt and TOR

Allantoin and other growth factors secreted by *L. sericata* activate numerous signal transduction pathways in host cells. The MAPK (mitogen-activated protein kinase) pathway plays a central role in cell proliferation, differentiation, and apoptosis. ERK1/2 (extracellular signal-regulated kinase) transmits growth factor signals to the nucleus and advances the cell cycle by increasing cyclin D expression (Meloche and Pouysségur, 2007). The PI3K/Akt pathway is critical for cell survival and protein synthesis; Akt activation inactivates pro-apoptotic proteins (e.g., Bad) by phosphorylating them and protects cells from death (Manning and Cantley, 2007).

Similar kinase cascades exist in plants. MAP kinase pathways regulate biotic and abiotic stress responses, hormone signalling, and developmental processes. For example, the MEKK1-MKK4/5-MPK3/6 cascade plays a role in ethylene and JA signalling (Xu and Zhang, 2015). SnRK family kinases are the plant analogues of AMPK and regulate energy homeostasis (Baena-González and Sheen, 2008). The TOR kinase is the central regulator of nutrient signalling in both animals and plants. Ren et al. (2012) demonstrated that Arabidopsis TOR controls protein synthesis, ribosome biogenesis, and cell proliferation. The existence of these evolutionarily conserved signalling pathways indicates that fundamental cellular processes are regulated by similar mechanisms across different kingdoms. However, upstream signals (hormones, growth factors) and downstream targets (specific genes, metabolic pathways) are organism-specific. *L. sericata* promotes growth by activating these universal pathways in host cells via allantoin, while plants modulate the same pathways for different developmental purposes using their own endogenous hormones.

4.3. Gene Expression and Epigenetic Regulation

Ecdysone signalling leads to extensive changes in gene expression. Microarray studies have shown that thousands of genes are differentially expressed in Drosophila during a molting cycle (White et al., 1999). These genes encode cuticular proteins, metabolic enzymes, transcription factors, and cell cycle regulators. Ecdysone-induced transcription factors (e.g., E74, E75) coordinate secondary waves of gene expression, ensuring the sequential activation of the developmental programme (Thummel, 1996). Epigenetic modifications also play an important role. Histone acetylation and methylation regulate the chromatin accessibility of ecdysone-responsive genes. The activity of HD AC (histone deacetylase) and HAT (histone acetyltransferase) enzymes is modulated by the ecdysone signal and determines gene expression profiles (Sedkov et al., 2003). DNA methylation also plays a role in developmental gene regulation, particularly in Diptera, but the role of DNA methylation in insects is more limited than in plants.

In plants, epigenetic regulation is of central importance in development and environmental adaptation. Polycomb group (PcG) proteins repress genes via histone H3 lysine 27 trimethylation (H3K27me3). For example, the flowering repressor FLOWERING LOCUS C (FLC) is permanently silenced by PcG proteins during exposure to winter cold (vernalisation)

(Sung and Amasino, 2004). This epigenetic memory allows plants to 'remember' seasonal signals. Auxin signalling also interacts with epigenetic regulators; SWI/SNF chromatin remodelling complexes are required for the activation of auxin-responsive genes (Saez et al., 2008).

In both systems, hormone signals trigger short-term transcriptional changes, while epigenetic modifications stabilise long-term developmental changes. In *L. sericata*, when a moult cycle is completed, the chromatin state is reset and prepared for the next moult. In plants, for example during the juvenile-adult phase transition, microRNA-dependent epigenetic changes establish permanent developmental states (Wu and Poethig, 2006). This reflects one of the fundamental differences between the metamorphic development of insects and the phasic development of plants: insects undergo discrete, irreversible stages, while plants exhibit modular and sometimes reversible developmental changes.

5. Environmental Signals and Hormonal Responses

5.1. Photoperiod and Thermal Signals

The development of *L. sericata* is strongly influenced by temperature and photoperiod. Niederegger et al. (2010) demonstrated that the larval development period is inversely proportional to temperature (6–8 days at 25°C, 15–20 days at 15°C). Temperature directly affects metabolic rate and modulates PTTH secretion. At low temperatures, the time to reach critical size is prolonged and metamorphosis is delayed. Photoperiod particularly controls diapause initiation; short-day conditions (< 12 hours of light) trigger pupal diapause, and in this case, ecdysone levels remain low (Saunders, 2002).

Photoperiod perception in plants is critical for flowering timing. Short-day plants (e.g., rice, soybean) flower during long nights, while long-day plants (e.g., Arabidopsis, wheat) flower under long-day conditions. This response is regulated by the molecular interaction between the CONSTANS (CO) protein and florigen (FT) (Suárez-López et al., 2001). Temperature is also important; vernalisation (prolonged exposure to cold) induces FLC repression, promoting flowering.

In both systems, environmental signals are integrated with hormonal regulation. In *L. sericata*, there is a relationship between temperature → metabolic rate → PTTH/ecdysone → moult timing. In plants, the pathway light → photoreceptors → CO/FT → flowering operates. These mechanisms enable organisms to synchronise their life cycles with environmental conditions. There are conceptual parallels between Drosophila diapause and plant seed dormancy; both are adaptations that increase survival by halting development under adverse conditions (Denlinger, 2002; Finkelstein et al., 2008).

5.2. Nutrient Signalling and Growth Coordination

Nutrient availability regulates growth rate in both *L. sericata* and plants. Colombani et al. (2005) demonstrated that amino acid sensors activate the TOR pathway in Drosophila larvae, promoting growth. Inadequate nutrition reduces insulin-like peptide secretion and slows larval growth. This mechanism prevents larvae from undergoing premature pupation under poor nutritional conditions and allows them to reach sufficient size to produce larger, more viable adults (Mirth and Riddiford, 2007).

In plants, nutrients, particularly nitrogen and phosphorus, regulate growth rate and developmental transitions. Nitrogen starvation increases the root/shoot ratio (more root growth) and delays flowering. Beyond being a nutrient source, nitrate functions as a signalling molecule and directly affects gene expression (Crawford and Forde, 2002). Nitrate transporters (e.g., NRT1.1) also act as nitrate sensors and modulate root architecture by regulating auxin transport (Krouk et al., 2010).

TOR kinase is a central hub that integrates nutrient status with cellular growth in both systems. Amino acids, glucose, and other nutrients activate TOR, which promotes protein translation, ribosome biogenesis, and lipid synthesis (Wullschleger et al., 2006). During nutrient scarcity, TOR is inactivated, and AMPK/SnRK1 kinases are activated, inducing catabolism and autophagy (Baena-González et al., 2007). This evolutionarily conserved mechanism allows organisms to coordinate growth and metabolism with nutrient availability.

6. Developmental Plasticity and Phenotypic Flexibility

6.1. Polymorphism and Alternative Developmental Pathways

In insects, environmental conditions can trigger alternative developmental pathways. For example, in some insect species, larval density affects juvenile hormone levels and determines the formation of solitary vs. gregarious phenotypes (grasshopper example) (Pener and Simpson, 2009). Although this type of polymorphism is not directly observed in *L. sericata,* nutritional quality affects pupa size and adult productivity. Poor nutritional conditions result in smaller pupae, which in turn produce smaller, less fertile adults (Tarone and Foran, 2006).

Phenotypic plasticity is much more pronounced in plants. The same genotype exhibits dramatically different morphologies under different environmental conditions. Examples include aquatic and terrestrial forms (heterophylly), shade and sun leaves, and varying stem elongation in response to different light qualities. Light quality (red/far-red ratio) alters phytohormone levels; in shade, a low R/FR ratio increases auxin and gibberellin levels, promoting stem elongation (shade avoidance syndrome) (Franklin, 2008).

Both systems exemplify the concept of the ‘norm of reaction’; the genotype determines developmental potential, while the environment shapes the realised phenotype (Schlichting and Pigliucci, 1998). Hormones play a central role in these plasticity mechanisms. Environmental modulation of JH and ecdysone levels in *L. sericata* adjusts development timing and pupa size. In plants, the dynamic balances between auxin, cytokinin, gibberellin, and abscisic acid modulate organ number, shape, and size in response to environmental inputs.

6.2. Compensatory Growth and Regeneration

Wound healing involves regenerative processes both in the natural biology of *L. sericata* and in the wounds it treats. Insect larvae possess effective repair mechanisms against external injuries. The rapid mobilisation of haemocytes (insect immune cells) to the wound site, coagulation and melanisation, and the proliferation of epidermal cells facilitate wound closure (Rajan and Perrimon, 2012). This process is modulated by JH and ecdysone; the wound can locally induce ecdysone synthesis and accelerate healing. The wound response in plants involves callus formation and organ regeneration. In injured tissue, differentiated cells undergo dedifferentiation and begin to divide (callus). This process is regulated by auxin and cytokinin; high levels of both hormones together promote callus proliferation (Ikeuchi et al., 2013). Callus

cells can then differentiate into new organs (roots, shoots) according to appropriate hormone ratios. Transcription factors such as Wound-induced dedifferentiation 1 (WIND1) coordinate this process (Iwase et al., 2011).

Factors secreted by *L. sericata* larvae support similar dedifferentiation and proliferation processes in human wound healing. Fibroblasts differentiate into myofibroblasts (TGF-β effect), keratinocytes proliferate and migrate (EGF effect), and endothelial cells form new blood vessels (VEGF effect). These cellular responses are facilitated by allantoin, antimicrobial peptides, and proteases contained in larval secretions. Conceptual similarities exist between plant callus formation and animal wound healing in terms of the dedifferentiation → proliferation → redifferentiation sequence.

7. Evolutionary Perspective and Conservation

7.1. The Evolutionary Origins of Hormone Signalling

Steroid hormone signalling is widely conserved among metazoans (multicellular animals). Ecdysone and vertebrate steroid hormones (oestrogen, testosterone, cortisol) are structurally similar and are all derived from cholesterol. The nuclear receptor superfamily is present in all bilaterian animals and represents an evolutionarily ancient signalling mechanism (Laudet, 1997). The ecdysone receptor (EcR) and vertebrate steroid receptors are derived from a common ancestor and share similar DNA-binding and ligand-binding domains.

Plant hormones, however, evolved independently. Auxin (indole-3-acetic acid), ethylene (a simple gas), and gibberellins (tetrasiklic diterpenoids) are structurally completely different from animal hormones. This reflects the independent evolutionary transition of plants and animals into multicellular organisms (King, 2004). However, interestingly, some fundamental signalling mechanisms (e.g., kinase cascades, ubiquitin-proteasome system, Ca^{2+} signalling) are conserved in both kingdoms, demonstrating their fundamental importance for eukaryotic signalling.

Brassinosteroids are plant steroid hormones and show structural similarity to animal steroid hormones, but this is convergent evolution; their receptors and signalling mechanisms are entirely different (Li and Chory, 1997). Brassinosteroids bind to membrane-localised receptor kinases (BRI1) and transmit signals via phosphorylation cascades, unlike the nuclear mechanism of animal steroid receptors (Wang et al., 2002). This is an interesting example of similar chemical structures being independently integrated into different signalling systems.

7.2. Conservation of Antimicrobial Defence Mechanisms

Antimicrobial peptides (AMPs) are evolutionarily ancient components of innate immunity. Insect defensins, human defensins, and plant defensins share similar structural motifs (β-sheet, disulphide bridges) but have low sequence homology (Bulet et al., 1999). This indicates convergent evolution; the independent evolution of optimal structures for antimicrobial activity on multiple occasions. L. sericata's lucifensin is effective against Gram-positive bacteria, and its membrane-permeabilisation mechanism is shared by many AMPs (Andersen et al., 2010).

In plants, thionins, defensins, and lipid transfer proteins (LTPs) exhibit antimicrobial activity. Some plant defensins interact with fungal β-glucan and disrupt cell wall synthesis, which is a different mechanism from that of animal AMPs (Thevissen et al., 2004). However,

in both cases, these peptides play a role not only in pathogen defence but also in developmental signalling (e.g., the phytosulfokine peptide family in plants) (Matsubayashi et al., 2006). This demonstrates that defence and developmental mechanisms are evolutionarily intertwined.

The antimicrobial peptides of *L. sericata* establish a delicate balance between pathogen control and immunomodulation in the context of wound healing. Similarly, in plants, salicylic acid activates defence responses while also limiting excessive cell death (Chandra-Shekara et al., 2004). In both systems, the 'fine-tuning' of defence responses is critical for the organism's survival; insufficient defence leads to infection, while excessive defence causes autoimmune damage.

7.3. TOR and the Evolutionary Conservation of Nutrient Signalling

The TOR (Target of Rapamycin) kinase is the central regulator of nutrient signalling in yeast, plants, insects, and mammals. This protein kinase is highly conserved evolutionarily and coordinates fundamental cellular processes (protein synthesis, ribosome biogenesis, autophagy) with nutrient availability (Wullschleger et al., 2006). In *L. sericata* larvae, TOR activity is regulated by amino acid uptake and determines growth rate. Similarly in plants, TOR responds to glucose and nitrate signals and regulates meristem activity (Ren et al., 2012).

The evolutionary conservation of TOR indicates that fundamental cellular processes are regulated by similar mechanisms across all eukaryotes. However, upstream signals (what types of nutrients are sensed) and downstream targets (which growth programmes are activated) are organism-specific. For example, in mammals, the TOR complex 1 (mTORC1) is particularly sensitive to leucine (Hara et al., 1998), whereas in plants, glucose and nitrate are the primary TOR activators (Xiong and Sheen, 2014).

An interesting point is that rapamycin (a fungal metabolite) inhibits TOR and slows growth in both animals and plants. This suggests that the evolutionary origins of TOR may predate the divergence of prokaryotes and eukaryotes. Rapamycin treatment has been shown to delay ageing and extend lifespan in various model organisms (yeast, worms, mice) (Johnson et al., 2013). This suggests that reducing growth signalling may be an evolutionarily conserved strategy for extending lifespan.

8. Therapeutic Applications and Biotechnology

8.1. Larval Therapy: Clinical Use of Natural Growth Regulators

L. sericata larval therapy is an example of the clinical application of natural growth regulatory mechanisms. Sherman (2009) reported that larval treatment provided faster debridement (average 14 days vs. 28 days) and reduced amputation rates compared to standard treatment in diabetic foot ulcers. The multifactorial effects of larval secretions—antimicrobial activity, debridement, growth factor stimulation, immunomodulation—function as combinatorial therapy in chronic wounds.

The medical applications of plant growth regulators are more limited, but some examples exist. Kinetin (a cytokinin) is used as an anti-ageing agent in skin care products; it promotes cell proliferation and collagen synthesis (McCullough and Kelly, 2006). The use of Forsyth (a synthetic cytokinin analogue) in topical treatments for hair loss has been investigated. These

examples demonstrate that plant hormones may have cross-kingdom effects in animal cells, likely via conserved cellular mechanisms (kinase activation, cell cycle regulation).

8.2. Recombinant Protein Production and Synthetic Biology

The recombinant production of allantoin and antimicrobial peptides from *L. sericata* offers advantages for scalable therapeutic production. The lucifensin gene was cloned into Escherichia coli and functional protein was produced (Altincicek et al., 2008). Recombinant lucifensin exhibits the same antimicrobial activity as the natural peptide and could potentially be formulated as a topical antibiotic. For allantoin production, the uricase enzyme can be overexpressed to optimise the conversion of uric acid to allantoin. Synthetic biology approaches can be used to create 'artificial maggot secretion'. Genetically modified bacteria or yeast can be engineered to produce a cocktail of allantoin, antimicrobial peptides, and growth factors. This preserves the therapeutic benefits while overcoming the psychological barrier to larval use. Alternatively, biopolymer hydrogels can be loaded with these factors and used as controlled-release wound dressings (Andersen et al., 2012). In plant biotechnology, the manipulation of growth-regulating genes is widely used to increase product yield and stress tolerance. Modification of the gibberellin biosynthesis pathway has enabled the development of dwarf or semi-dwarf plant lines (green revolution wheat) (Peng et al., 1999). Overexpression of auxin transport proteins can increase root systems and nutrient uptake. Similar genetic engineering strategies can be applied for *L. sericata* growth regulators; for example, modified larvae that increase allantoin production or secrete broader-spectrum antimicrobial peptides.

8.3. Biomimetic Approaches and Drug Development

L. sericata's multi-modal wound healing strategy may inspire the design of biomimetic therapeutics. The concept of developing 'multi-functional wound healing agents'—combining debridement, antimicrobial, anti-inflammatory, and regenerative properties in a single treatment agent—is gaining increasing interest. Nanoparticulate carriers (liposomes, chitosan nanoparticles) can be used for the simultaneous delivery of allantoin and antimicrobial peptides (Gomes et al., 2013). Peptidomimetics inspired by lucifensin can be designed with improved pharmacokinetic properties. Natural peptides are susceptible to proteolytic degradation; the use of D-amino acids or peptoids can increase stability. Peptide cyclisation can enhance receptor binding affinity and membrane penetration (Craik et al., 2013). These approaches can increase the clinical viability of peptide therapeutics. Analogues of plant growth regulators have also been used in drug development. Zeatin riboside (a cytokinin) has neuroprotective properties and has been investigated for the treatment of neurodegenerative diseases (Ayed et al., 2011). Brassinosteroid analogues exhibit antiviral and immunomodulatory activities (Syrov et al., 2003). This demonstrates that plant and insect growth regulators have broad therapeutic potential beyond their original biological context.

Conclusion

This comprehensive comparative analysis of growth regulatory systems in *Lucilia sericata* and plants reveals both fundamental convergence and profound divergence in hormonal control mechanisms across evolutionarily distant organisms. While the chemical structures, biosynthetic pathways, and receptor systems differ dramatically—ecdysteroids and juvenile hormones in insects versus the diverse array of phytohormones in plants—both systems share core principles of hormonal regulation: efficacy at low concentrations, specific receptor-mediated perception, signal amplification through conserved transduction cascades (MAPK, ubiquitin-proteasome system, TOR kinase, calcium signaling), and integration with environmental cues. The ecdysone-juvenile hormone antagonism controlling insect metamorphosis conceptually parallels auxin-cytokinin interactions regulating plant organ formation, while the exogenous factors secreted by *L. sericata* larvae—allantoin, antimicrobial peptides, and proteolytic enzymes—exhibit striking functional similarities to plant wound-response hormones. Allantoin's proliferative effects mirror cytokinin activity, antimicrobial peptides parallel plant defensins and jasmonic acid signaling, and proteolytic enzymes functionally resemble plant cell wall-modifying enzymes. These parallels exemplify convergent evolution, where similar biological challenges—growth control, developmental transitions, pathogen defense, tissue repair—are addressed through analogous strategies despite independent evolutionary origins.

From a translational perspective, *L. sericata* larval therapy demonstrates the successful clinical application of natural growth regulators, with multifactorial effects in chronic wound healing providing superior outcomes compared to conventional treatments. This success has catalyzed molecular characterization of larval secretions and development of biomimetic therapeutics, including recombinant antimicrobial peptides, synthetic allantoin formulations, and engineered wound-healing cocktails. Similarly, plant growth regulators are finding applications in medicine (kinetin in anti-aging, brassinosteroid analogs in immunomodulation) and agriculture (gibberellin-modified crops, auxin herbicides). Future research integrating systems biology, structural biology, epigenomics, and single-cell transcriptomics will deepen our understanding of hormonal networks in both systems, enabling rational design of novel therapeutics and agricultural innovations. The conservation of fundamental signaling modules across kingdoms suggests that insights from comparative endocrinology can inform drug discovery, synthetic biology, and biotechnology applications. This study ultimately demonstrates that examining hormonal regulation across diverse life forms—from insects to plants—not only illuminates unifying principles of biological organization but also provides a rich source of inspiration for translating nature's solutions into applications benefiting human health, sustainable agriculture, and biotechnology innovation.

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CHAPTER 6

VIRULENCE FACTORS OF STREPTOCOCCUS PYOGENES?

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Introductıon

Streptococcus pyogenes is a strictly human-adapted, Gram-positive, β-hemolytic bacterium whose ecological niche is confined to the human host. While asymptomatic colonization of the pharynx and skin is commonly observed, this organism is distinguished by its ability to initiate a remarkably broad range of clinical syndromes. These infections vary from relatively mild conditions, such as superficial skin involvement and streptococcal pharyngitis, to severe toxin-mediated disorders including streptococcal toxic shock syndrome (STSS), as well as fulminant invasive diseases such as necrotizing fasciitis involving extensive soft-tissue destruction (J. Ferretti & Köhler, 2016; Henningham, Barnett, Maamary, & Walker, 2012). The diversity of clinical manifestations is largely driven by the extensive repertoire of extracellular toxins and surface-associated proteins expressed by this pathogen (Table 1) (Reichardt, 2001).

Persistence of *S. pyogenes* within host tissues is strongly dependent on the expression of the M protein, which impairs phagocytic clearance by polymorphonuclear leukocytes in the absence of serotype-specific antibodies. Experimental evidence supporting the central role of this molecule has been obtained from M-deficient mutant strains, which, despite retaining other virulence-associated structures, exhibit markedly reduced survival in human blood. Protective immunity against group A streptococci is therefore closely related to the development of antibodies directed against the M protein. The high degree of heterogeneity among M protein variants, with more than 200 distinct serotypes described to date (e.g., M6, M12, M18, and M24), explains the frequent occurrence of repeated infections throughout an individual's lifetime (Vincent A. Fischetti, 2016).

From the 1980s onward, *S. pyogenes* has re-emerged worldwide as a major etiological agent of invasive group A streptococcal diseases, which are characterized by aggressive progression and remain associated with considerable morbidity and mortality despite timely antimicrobial treatment. The rising incidence observed over recent decades has underlined the importance of elucidating both host-related predisposition factors and bacterial determinants underlying disease severity (Walker, McArthur, McKay, & Ranson, 2005). Comprehensive global burden analyses have estimated approximately 616 million cases of streptococcal pharyngitis, 111 million cases of pyoderma, and over 517,000 deaths annually attributable to invasive GAS disease and its complications. Moreover, in 2010 alone, more than 340,000 fatalities were linked to rheumatic heart disease resulting from GAS infection, underscoring the substantial public health impact of this pathogen (Fiedler, Köller, & Kreikemeyer, 2015).

Although no individual virulence determinant has been exclusively associated with a single clinical outcome, accumulating evidence suggests that specific M protein serotypes, particularly M1 and M3, are more frequently involved in invasive infections, indicating a genotype–phenotype relationship in disease expression (Walker et al., 2005).

Table 1. Potential and established surface-associated and extracellular virulence determinants of GAS (Reichardt, 2001).

Surface-associated virulence factors	• M proteins and M-related proteins • Fibronectin-binding proteins: SfbI, F protein, and GAP dehydrogenase • Hyaluronic acid capsule • Streptolysin O • Streptolysin S
Extracellular virulence factors	• Superantigens (pyrogenic exotoxins types A, C, G, H, I, J, K, Z, and SSA) • Streptococcal pyrogenic exotoxins • Streptokinase • C5a peptidase • Apoproteinase (Opacity factor) • DNases (DNase B)

1 Virulence Factors

The understanding of bacterial pathogenicity has evolved substantially since the foundational concepts were introduced by Robert Koch nearly a century ago. In modern microbiology, virulence is generally defined as the capacity of a microorganism to cause disease within a host. However, disease pathogenesis is no longer viewed as a simple consequence of the number or potency of virulence factors expressed by a pathogen. Instead, disease outcome is increasingly recognized as the result of an intricate interplay between microbial attributes and host-associated variables, including immune competence, genetic background, and the specificity of host–pathogen interactions. Within this framework, *S. pyogenes* is regarded as a highly interactive pathogen that engages in diverse molecular and cellular processes throughout infection. Although numerous virulence-associated genes have been identified, the complete genetic architecture underlying pathogenicity remains incompletely characterized. As a result, bacterial virulence is now conceptualized as a multidimensional process shaped by both intrinsic microbial features and host-dependent regulatory dynamics (Sitkiewicz, 2018; Upton, Tagg, Wescombe, & Jenkinson, 2001).

Emerging evidence further indicates that virulence determinants are not conserved solely to facilitate infection but also because they confer ecological advantages outside the host environment. In opportunistic pathogens in particular, virulence-associated traits have been shown to enhance environmental persistence and adaptive flexibility. Consequently, virulence is no longer considered exclusively infection-related but rather part of a broader survival strategy associated with microbial fitness, niche adaptation, and phenotypic plasticity (S. P. Brown, Cornforth, & Mideo, 2012). In parallel with this view, the distinction between “opportunistic” and “obligate” pathogens has become less rigid. Microorganisms traditionally classified as obligate human pathogens, including Streptococcus pneumoniae and *S. pyogenes*, are now also recognized as organisms that exhibit facultative pathogenic behavior depending on host and environmental conditions (Brouwer, Barnett, Rivera-Hernandez, Rohde, & Walker, 2016).

A broad repertoire of virulence determinants encoded by *S. pyogenes* has been characterized and shown to influence multiple stages of GAS pathogenesis. These determinants orchestrate critical infection processes such as epithelial attachment, cellular invasion, immune interference, toxin production, and tissue injury (J. J. Ferretti, Stevens, & Fischetti, 2016). Beyond these classical virulence factors, metabolic components have also been implicated in

pathogenic progression. For example, lipoteichoic acid (LTA) has been demonstrated to facilitate bacterial adhesion to pharyngeal epithelial cells and extracellular matrix proteins, including fibronectin (Chen et al., 2020).

Several fibronectin-binding proteins, including SfbI, protein F2 (SfbII), FBB54, and FBBP, have been identified as major adhesins mediating attachment to epithelial and mucosal surfaces (Brouwer et al., 2016; Chen et al., 2020). In addition, M proteins have been reported to promote adherence to keratinocytes through interaction with the membrane-associated cofactor CD46 (Koneman, 1997). Structural group A cell wall antigens composed of L-rhamnose and N-acetyl-D-glucosamine moieties are covalently anchored to the peptidoglycan scaffold; however, their precise contribution to virulence has not yet been fully delineated (Kozińska & Sitkiewicz, 2020; J. A. Tsatsaronis et al., 2013).

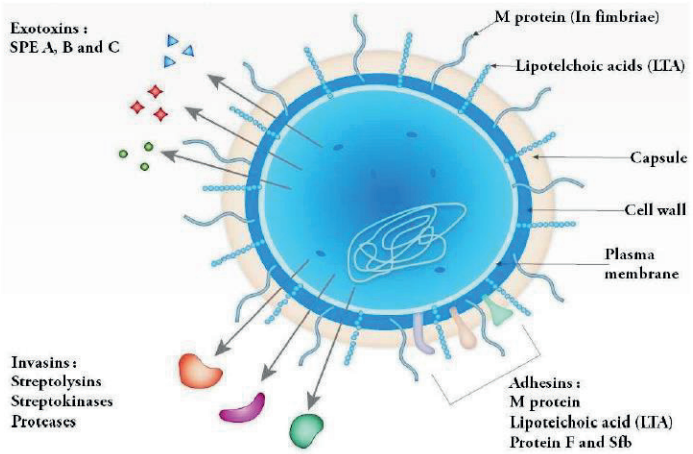

Figure 1. Schematic representation of the cell surface structures of Streptococcus pyogenes and the major virulence-associated products involved in host interaction and pathogenicity (Jasim, Hatem, & Abd Mohammed, 2021).

1.1 Capsule

Clinical isolates of group A streptococci obtained from patients with pharyngitis or invasive disease have long been characterized by their distinctive colony morphology on blood agar, where colonies typically appear large, moist, and semi-transparent (Figure 2). Upon extended incubation, a transition toward a mucoid phenotype with irregular or dull (matt) surfaces is frequently observed. Repeated laboratory passaging, however, has often been associated with loss of this phenotype and the emergence of smaller, denser, and shinier colonies, suggesting that capsule expression may be unstable under in vitro conditions (Wessels, 2016). Early experimental evidence established a functional link between colony morphology and pathogenicity. In pioneering studies conducted in the 1920s, Lancefield and Todd demonstrated that mucoid streptococcal variants displayed enhanced virulence in murine models and increased resistance to leukocyte-mediated killing in human blood (Lancefield & Todd, 1928; Todd & Lancefield, 1928). Subsequent biochemical characterization revealed that the viscous extracellular material produced by these strains consisted primarily of hyaluronic acid, a linear glycosaminoglycan composed of repeating units of N-acetylglucosamine and glucuronic acid. In contemporary investigations, hyaluronic acid production has been confirmed in many clinical GAS isolates, with synthesis occurring predominantly during

exponential growth and extracellular release becoming more prominent in stationary phase. Notably, although this polymer is synthesized by the bacterium, the hyaluronic acid capsule exhibits low immunogenicity in mammalian hosts and is inefficiently distinguished from host tissue components by the immune system, thereby facilitating immune evasion (Wang & Cleary, 2019). Capsulated phenotypes have been identified in a substantial proportion of group A β-hemolytic streptococci (GABHS). Biosynthesis of the capsule is mediated by enzymes encoded by the *has* operon (*hasA*, *hasB*, and *hasC*), which respectively encode hyaluronic acid synthase, UDP-glucose dehydrogenase, and glucose pyrophosphorylase. Differences in capsule thickness and expression level among GAS strains have been associated with regulatory variation affecting transcriptional control of these genes (Abd El-Baky et al., 2020).

Mucoid colony formation on blood agar is therefore considered a phenotypic marker of capsular expression. Regulation of capsule synthesis is largely controlled by the *CsrR/CsrS* two-component regulatory system, which modulates expression intensity in response to environmental signals. Functionally, the capsule has been shown to promote resistance to complement-dependent killing and phagocytosis. This protective effect is mediated in part through enzymatic degradation by hyaluronidase, which diminishes opsonin binding and impairs immune recognition (L. Brown, Kim, & Cho, 2016; Turner et al., 2019). Furthermore, cooperative interactions between the capsule and surface proteins, including M protein, enhance epithelial attachment via binding to the CD44 receptor expressed on host cells (Kozińska & Sitkiewicz, 2020).

Epidemiological analyses have consistently demonstrated a strong correlation between capsule production and disease severity. Mucoid GAS strains, in particular, have been linked to invasive streptococcal infections and outbreaks of acute rheumatic fever, supporting the central role of the capsule in virulence (Wessels, 2016).

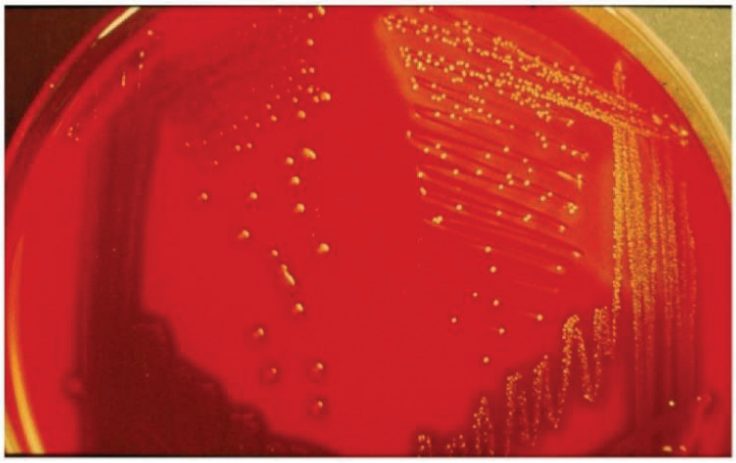

Figure 2. Blood agar plate showing typical mucoid colonies of a group A streptococcal strain (left) and non-mucoid (glossy) colonies of an acapsular mutant strain (right) (Wessels, 2016).

1.2 M Protein

The M protein represents the principal virulence-associated structure of group A β-hemolytic streptococci (GABHS) and plays a central role in immune evasion. Its antiphagocytic activity is largely mediated through interference with complement activation and inhibition of opsonization, thereby contributing to impaired neutrophil function and survival within host tissues. In addition to these effects, the M protein has been shown to exert cytotoxic activity against neutrophils, which further enhances bacterial persistence during infection. Biochemically, the M protein exhibits notable resistance to thermal stress and acidic environments, while remaining vulnerable to degradation by trypsin. Structurally, it consists of two polypeptide chains that, in the mature form, are covalently linked to the peptidoglycan layer of the streptococcal cell wall (Figure 3) (Smeesters, McMillan, & Sriprakash, 2010). At the molecular level, the protein is characterized by repetitive heptad sequence motifs capable of forming a stable α-helical coiled-coil configuration. These motifs display a conserved pattern in which hydrophobic amino acids frequently occupy the first and fourth positions of each repeat, contributing to conformational stability and functional integrity (McNamara et al., 2008).

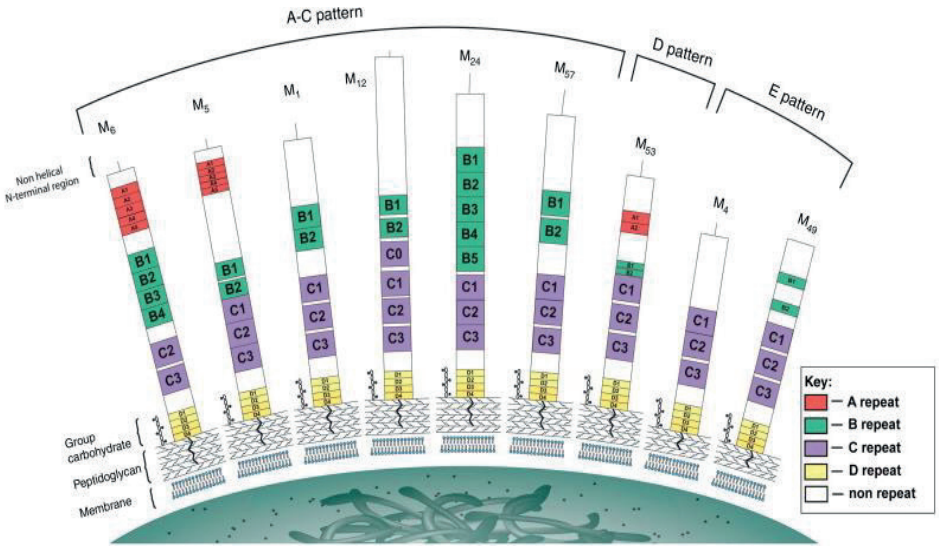

Figure 3. Representative schematics illustrating the mature forms of different M proteins. The overall organization of the mature structure of nine well-characterized M proteins is shown. The NCBI accession numbers for these proteins are as follows: M6, P08089; M5, CAM31002; M1, NP_269973; M12, YP_597455; M24, AAN04081; M57, A44643; M53, P49054; M4, CAA33269; and M49, AAA26868. All protein sequences were truncated to correspond to the mature form of the M proteins. The signal peptide and the sequence downstream of the threonine residue within the LPXTG sortase recognition motif were excluded from the alignment (Smeesters et al., 2010).

The involvement of the M protein in disease development extends beyond immune evasion and includes a central role in bacterial attachment to host tissues. Inhibition of neutrophil-mediated phagocytosis, together with facilitation of adhesion to epithelial surfaces,

underscores its importance in the pathogenic process. In addition, the M protein functions as a potent immunogen, and the generation of serotype-specific antibodies against this molecule has been shown to confer protective immunity against reinfection with homologous GAS strains. These properties collectively establish the M protein as a critical determinant of GAS virulence. The M protein is also capable of interacting with a wide range of host-derived plasma proteins. Among these interactions, the association between the M1 variant and fibrinogen has received particular attention due to its role in stimulating neutrophil activation. This interaction promotes the release of inflammatory mediators, thereby amplifying local and systemic immune responses during infection (Figure 4) (Hamada, Kawabata, & Nakagawa, 2015).

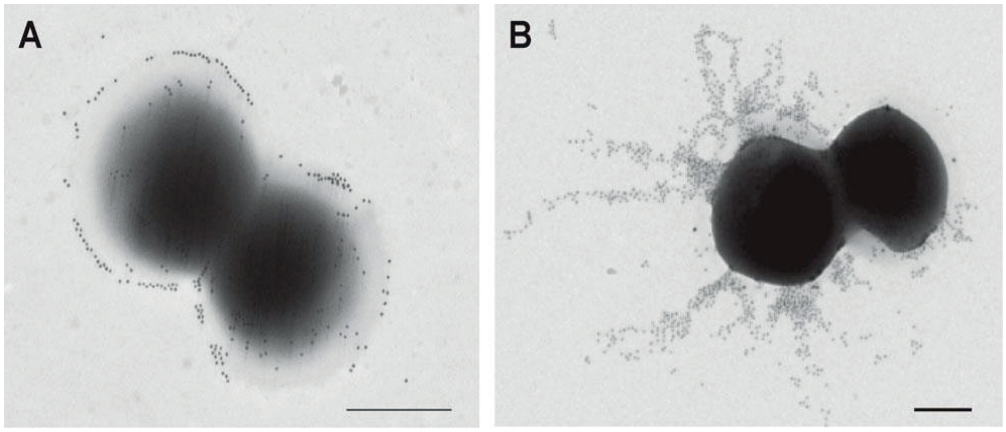

Figure 4. Immunoelectron micrographs illustrating fibrillar surface structures of GAS. M proteins (A) and pili (B) were visualized following incubation with specific primary antibodies and subsequent labeling with gold-conjugated secondary antibodies. (Scale bar: 0.5 µm.) (Hamada et al., 2015).

An additional key function of the M protein involves shielding GAS from immune elimination by restricting complement deposition on the bacterial surface (McNamara et al., 2008). Resistance to phagocytic clearance is achieved through at least two distinct but complementary mechanisms. Firstly, M protein-expressing strains recruit factor H, a negative regulator of complement activation, which limits C3b accumulation and reduces opsonization efficiency. Secondly, binding of fibrinogen by the M protein interferes with activation of the alternative complement pathway, further impairing complement-mediated bacterial clearance (Fischetti, 1989). Conversely, when neutralizing antibodies directed against the M protein or other surface structures are present, enhanced complement activation occurs. Antibody-mediated opsonization facilitates C3 deposition on the bacterial surface and promotes efficient recognition and ingestion by neutrophils (Figure 5) (Cunningham, 2000).

The streptococcal M protein is capable of imitating host muscle and connective tissue components through a mechanism referred to as molecular mimicry. On the basis of antigenic variation, more than fifty distinct M protein variants have been identified in *S. pyogenes*. Certain low-numbered types, including M1, M3, M5, M6, M14, M18, M19, and M24, are regarded as rheumatogenic, as they possess immunological epitopes that cross-react with cardiac tissue. As a consequence, these strains may initiate autoimmune responses leading to rheumatic carditis following acute pharyngeal infection, clinically manifesting as rheumatic fever. In addition to cardiac complications, post-streptococcal acute glomerulonephritis (AGN)

represents another immune-mediated sequela of infection. AGN arising after pharyngitis has been predominantly associated with M types 1, 4, 12, and 25, whereas cases linked to impetigo are typically caused by nephritogenic strains harboring higher-numbered M types such as 2, 49, 55, 57, 59, 60, and 61 (Madigan, Martinko, & Parker, 1997). Beyond structural mimicry, M proteins displayed on the bacterial surface also exhibit immunomodulatory activity. These molecules have been shown to induce polyclonal T-cell activation and promote cytokine release, thereby exerting superantigen-like effects that contribute to excessive inflammatory responses (Ghosh, 2018). Historically, M protein diversity formed the basis of serologic classification for *S. pyogenes*, and variability among serotypes has been reported both geographically and in relation to disease presentation. Over time, serological methods have largely been replaced by sequence-based typing of the *emm* gene encoding the M protein. Modern *emm* genotyping relies on nucleotide sequence variation and currently differentiates more than 250 genetic variants. Epidemiological surveillance has revealed that industrialized countries tend to be dominated by a limited number of *emm* types, whereas substantially greater genetic heterogeneity is observed in low-income regions (Mahmoud, Toth, & Stephenson, 2022). Earlier laboratory approaches, including capillary precipitin testing and agarose gel immunodiffusion, demonstrated that individual GAS isolates express a single M antigen, leading to the identification of 93 serotypes. Standardized molecular protocols have since formalized *emm* typing methodologies (Fischetti, 2016). In addition to the canonical M protein, multiple structurally related surface proteins have been identified in *S. pyogenes*. The genes encoding these proteins, including *enn*, *mrp*, *arp*, *fcrA*, and *protH*, collectively comprise what is now designated as the “emm gene superfamily” (Tsatsaronis et al., 2013).

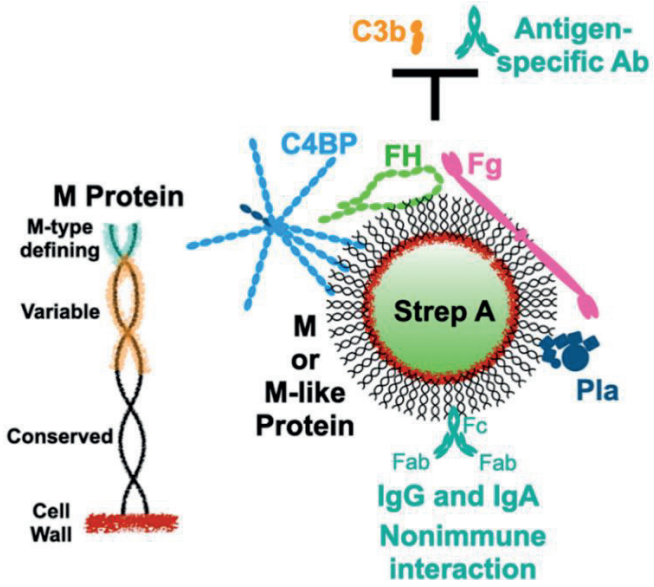

Figure 5. Surface acquisition of human proteins by *S. pyogenes.*

***Left: Schematic representation of the M protein coiled-coil structure covalently attached to the Strep A cell wall. The N-terminal 50 amino acids define the type-specific region (turquoise), while the N-terminal one-third to one-half of the molecule represents the variable region (orange).**

***Right: M and M-like proteins on the surface of Strep A were shown to recruit host molecules, including C4BP, FH, fibrinogen (Fg), and plasminogen (Pla), in order to prevent opsonin accumulation, such as C3b and antigen-specific antibodies, on the bacterial surface. In addition, M and M-like proteins have been demonstrated to interact non-immunologically with IgG and IgA via their Fc domains.**

****C4BP, C4b-binding protein; Fc, fragment crystallizable; Fg, fibrinogen; FH, factor H; IgA, immunoglobulin A; IgG, immunoglobulin G; Pla, plasminogen. (Mills & Ghosh, 2021).**

Over the past decade, substantial progress has been made toward the development of vaccines targeting GAS. Among the numerous antigens investigated, the amino-terminal domains of M proteins have emerged as the leading vaccine candidates. These regions contain type-specific epitopes capable of inducing highly bactericidal antibodies while demonstrating minimal cross-reactivity with human tissues, thereby reducing the risk of autoimmune complications (Dale, Penfound, Chiang, & Walton, 2011). Moreover, a considerable proportion of GAS strains express an M-related protein known as Mrp, which shares structural and functional similarities with the M protein. Mrp also exhibits anti-phagocytic properties and binds human IgG in a subclass-dependent hierarchy (IgG1 > IgG4 > IgG2 > IgG3), while failing to interact with IgM or IgA. This non-immune binding mechanism is thought to enhance immune escape and may partly explain the strict host specificity of GAS for humans. Accordingly, Mrp is recognized as an additional virulence determinant in GAS pathogenesis (Podbielski et al., 1996).

1.3 Serum Opacity Factor (SOF)

The serum opacity factor (SOF) is a surface-associated protein expressed by approximately 45% of *S. pyogenes* strains and is regarded as an accessory virulence determinant linked to the M protein family. Two major biological activities have been ascribed to SOF. First, it is capable of inducing serum turbidity through specific interactions with high-density lipoproteins (HDL). Second, it interferes with the development of β-hemolysis on blood agar, thereby influencing phenotypic characteristics used for laboratory identification (Zhu, Olsen, & Musser, 2017). Structurally, SOF contains two functionally distinct regions. One domain mediates the opacity reaction by altering mammalian serum lipoproteins, while a second domain facilitates binding to host fibronectin and fibrinogen, thereby contributing to bacterial adhesion and host interaction (Oehmcke, Podbielski, & Kreikemeyer, 2004). The biological relevance of SOF is further emphasized by observations that antibodies directed against this molecule inhibit serum opacification in an M type–specific fashion. Consequently, SOF profiling has been employed as an auxiliary epidemiological tool for differentiating GAS strains.

In addition to SOF, another opacity-associated factor (OF) has been identified in GAS isolates representing 29 M serotypes and has been detected predominantly among strains recovered from cutaneous infections (Zhu et al., 2017).

1.4 Streptolysin O (SLO)

Streptolysin O (SLO) and streptolysin S (SLS) are cytotoxic exotoxins expressed by the vast majority of clinical GAS isolates and are classified as members of the cholesterol-dependent cytolysin family. SLO functions as a pore-forming toxin that binds to membrane cholesterol in eukaryotic cells and self-assembles into large oligomeric structures, ultimately disrupting membrane integrity and inducing cell lysis (Palmer et al., 1998). The toxin initially associates with host membranes in a monomeric state and exhibits marked sensitivity to oxygen. As a consequence of this property, pronounced β-hemolytic activity is typically observed under anaerobic conditions or in deeper regions of blood agar cultures. In addition to GAS, SLO production has also been reported among certain group C and group G streptococcal strains (Jasim et al., 2021).

At the cellular level, SLO interacts with erythrocytes by preferentially recognizing galactose-containing moieties, followed by toxin polymerization and subsequent insertion into cholesterol-rich membrane domains. In experimental models lacking galactose substrates, SLO displays affinity for host glycans and promotes the recruitment and activation of a complementary toxin, NAD^+ glycohydrolase (SPN) (Mozola & Caparon, 2015). Beyond its cytolytic activity, SLO contributes to immune modulation by promoting neutrophil degranulation and reducing macrophage phagocytic capacity (Figure 5). In clinical practice, detection of anti-SLO antibodies, measured as anti-streptolysin O (ASO) titers, remains a valuable serological indicator of recent or prior streptococcal infection, particularly in cases of suspected streptococcal pharyngitis (Bisno, Brito, & Collins, 2003).

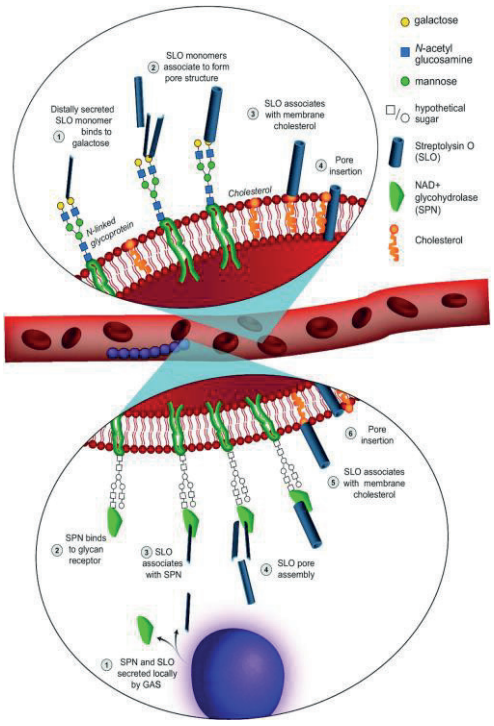

Figure 5. Proposed model for glycan recognition on erythrocyte membranes by SLO (A) and its accessory toxin SPN (B). In the absence of streptococcal NAD^+ glycohydrolase (SPN), secreted streptolysin O (SLO) polymers initially bind to a galactose residue (A) and accumulate on local or distant erythrocyte membranes. In the absence of galactose, locally secreted SPN binds to a glycan receptor and serves as a platform for SLO polymer assembly (B). In both scenarios, assembled SLO polymers subsequently interact with membrane cholesterol prior to pore insertion (Indraratna, Everest-Dass, Skropeta, & Sanderson-Smith, 2022).

1.5 Streptolysin S (SLS)

Streptolysin S (SLS) is a toxic effector molecule synthesized by *S. pyogenes* and several related *Streptococcus* species and functions as a major mediator of cellular injury (Molloy et al., 2015). Unlike secreted protein toxins, SLS is produced as a ribosomally encoded peptide and exhibits broad cytotoxic activity toward diverse host cell populations (Flaherty et al., 2015). Although the complete molecular mechanism responsible for SLS-mediated damage has not been conclusively defined, the toxin has been observed in both cell-associated and intracellular forms. Current evidence indicates that membrane phospholipids serve as primary targets of SLS, consistent with its ability to destabilize host cell membranes. The oxygen-stable nature of SLS is reflected by its hemolytic phenotype on sheep blood agar, where erythrocyte destruction occurs both at the colony surface and in the subsurface layers of the medium (Bisno et al., 2003; Jasim et al., 2021).

1.6 Streptococcal Pyrogenic Exotoxins (SPEs)

Streptococcal pyrogenic exotoxins (SPEs) represent a group of potent virulence determinants that are centrally involved in the development of the scarlatiniform rash

characteristic of scarlet fever and are key contributors to the pathophysiology of streptococcal toxic shock–like syndrome. Several immunologically distinct variants have been described, primarily classified as types A, B, and C. The genetic determinants encoding SPE-A and SPE-C (*speA* and *speC*) are typically harbored by lysogenic bacteriophages integrated into the streptococcal genome, whereas *speB* is encoded within the chromosomal DNA. Functionally, *speB* encodes a cysteine protease that exhibits broad substrate specificity. This proteolytic enzyme has been shown to degrade an array of host structural and immune proteins and to promote the production of pro-inflammatory mediators such as interleukin-1. Regulation of SpeB expression is governed by the CovR/CovS two-component signal transduction system, which modulates virulence gene expression in response to environmental cues. Disruption of the *covR/S* regulatory locus has been associated with enhanced synthesis of the hyaluronic acid capsule and increased secretion of SpyCEP, a serine protease. SpyCEP has been shown to cleave multiple host cytokines, facilitating immune evasion and promoting bacterial spread within the respiratory tract (Proft, Arcus, Handley, Baker, & Fraser, 2001).

1.7 Streptococcal Pyrogenic Exotoxin B (SpeB)

Streptococcal pyrogenic exotoxin B (SpeB) is recognized as the dominant extracellular cysteine protease produced by GAS. This enzyme exhibits broad proteolytic activity and is capable of degrading numerous host-derived proteins, including extracellular matrix components, immunoglobulins, and complement system factors. In addition, SpeB targets several streptococcal surface and secreted proteins. Through this dual activity, SpeB reshapes both host defenses and bacterial surface architecture, thereby facilitating immune evasion, tissue invasion, and dissemination. As a result of its extensive substrate range and central role in modulating host–pathogen interactions, SpeB is regarded as a pivotal virulence factor contributing to GAS pathogenicity (Chiang-Ni & Wu, 2008).

1.7.1 Streptococcal Pyrogenic Exotoxins A and B as Superantigens

Targeted degradation of host immune effector molecules, together with proteolytic processing of GAS surface structures, plays a critical role in circumventing host immune defenses. These processes facilitate bacterial penetration into deeper tissue layers and promote spread beyond the initial focus of infection. Beyond its contribution to acute disease, SpeB has also been identified as a central antigen in the development of acute post-streptococcal glomerulonephritis (APSGN), implicating this protease in immune-mediated renal pathology following GAS infection (Proft et al., 2001). In parallel, streptococcal pyrogenic exotoxins A and B (SPE-A and SPE-B) exhibit potent superantigenic activity. By directly engaging major histocompatibility complex class II (MHC-II) molecules on antigen-presenting cells and cross-linking them with T-cell receptors, these toxins bypass conventional antigen processing mechanisms and induce massive, non-specific T-cell activation. This aberrant immune stimulation leads to uncontrolled release of inflammatory cytokines from activated lymphocytes and monocytes. The resulting hyperinflammatory state triggers dysregulation of complement pathways as well as coagulation and fibrinolytic systems, which together underpin the systemic manifestations observed in streptococcal toxic shock syndrome (Abd El-Baky et al., 2020; Bidet & Bonacorsi, 2014).

1.8 C5a Peptidase

Early in vitro investigations demonstrated that virulent group A streptococci are capable of suppressing the chemotactic properties of human serum, a finding first documented by Wexler and colleagues. This inhibitory effect has since been attributed to a GAS-derived factor that contributes to pathogenicity via both immunomodulatory and enzymatic mechanisms. In particular, the complement-derived chemoattractant C5a has been identified as a primary target, and its functional inactivation markedly reduces neutrophil recruitment to sites of infection (O'Connor & Cleary, 1987; Jasim et al., 2021).

Biochemical characterization has established streptococcal C5a peptidase as a proteolytic enzyme belonging to the endopeptidase family that subsequently undergoes post-translational processing to achieve functional activity (Hamada, Kawabata, & Nakagawa, 2015).

1.9 Fibronectin-Binding Proteins (FBPs)

Fibronectin (FN) is a multifunctional glycoprotein of the extracellular matrix (ECM) synthesized by host cells and serves as a major soluble constituent of plasma and other bodily fluids. In addition to its circulating form, fibronectin is abundantly incorporated into the structural framework of the ECM. This molecule interacts with a broad range of cellular receptors and integrins expressed on host cell surfaces. A growing body of evidence indicates that numerous bacterial genera, including *Staphylococcus* and *Streptococcus*, exploit fibronectin during the initial stages of colonization, using it as a molecular bridge to establish attachment prior to cell surface proliferation and tissue invasion (Henderson, Nair, Pallas, & Williams, 2011).

Several GAS-derived fibronectin-binding proteins (FBPs) have attracted attention as potential vaccine antigens because of their ability to elicit protective humoral immune responses (Yamaguchi, Terao, & Kawabata, 2013). Antibodies targeting these proteins have been detected in sera from patients with acute glomerulonephritis and rheumatic fever, highlighting their immunogenic nature. Moreover, the *fbp54* gene, which encodes a major fibronectin-binding protein, has been detected across clinical isolates representing diverse M types. This widespread distribution among GAS lineages, coupled with strong antigenicity in humans, positions FBPs as attractive candidates for vaccine development (Yamaguchi et al., 2013). Beyond their contribution to adherence, FBPs are also involved in immune modulation by interfering with complement activation, thereby enhancing bacterial resistance to phagocytic clearance (Henderson et al., 2011).

2 Bacteriophages of *Streptococcus pyogenes*

The commemoration of Frederick Twort's discovery of bacteriophages in 2015 revitalized scientific attention on the contribution of phages to bacterial pathogenicity. The earliest indications of phage involvement in *S. pyogenes* biology were reported in the late 1920s, when erythrogenic (scarlatiniform) toxin activity was shown to be transferable from toxigenic to non-toxigenic strains using cell-free filtrates derived from infected cultures. These milestone observations provided the first evidence that toxin-encoding genetic material could be disseminated via bacteriophage-mediated mechanisms (McShan, McCullor, & Nguyen, 2019).

Subsequent investigations demonstrated that bacteriophages are widely distributed among group A streptococci and that phage carriage is frequently associated with enhanced virulence. Both lytic and lysogenic phages have since been studied in detail, leading to the identification of multiple phage-borne virulence genes, including *speA*, which encodes a streptococcal pyrogenic exotoxin. Together, these data underscore the importance of bacteriophages as major drivers of horizontal gene transfer and virulence evolution in *S. pyogenes* (McShan et al., 2019; Weeks & Ferretti, 1986).

Bacteriophage replication strategies are traditionally divided into two categories: lytic and lysogenic cycles. Lytic phages typically do not establish persistent genetic relationships with their hosts and therefore do not contribute directly to long-term phenotypic alterations. Nevertheless, they influence bacterial populations by preferentially eliminating susceptible cells and by promoting genetic exchange through transduction events. In contrast, lysogenic phages integrate into the bacterial chromosome and become stable genetic elements, where they may exert sustained effects on host fitness, adaptation, and pathogenic potential (McShan & Nguyen, 2016).

2.1 Lytic Bacteriophages of Group A Streptococci

Among the lytic bacteriophages associated with *S. pyogenes*, phage A25 has been extensively studied and remains one of the most thoroughly characterized examples. This phage was first recovered from sewage samples in Paris during the early 1950s and was subsequently shown to facilitate generalized transduction in *S. pyogenes*. It is preserved within the American Type Culture Collection under the reference designation ATCC 12204. Despite its long-standing role as a model system for bacteriophage research, a complete genomic sequence for phage A25 has yet to be established (Maxted, 1952). Beyond their involvement in virulence factor mobilization, bacteriophages also play a central role in the horizontal dissemination of antimicrobial resistance determinants. Strains carrying prophages related to phage T12 have been demonstrated to produce transducing particles following lysogenic induction, enabling the transfer of genes conferring resistance to multiple antibiotic classes, including tetracyclines, macrolides, chloramphenicol, lincomycin, and clindamycin. These findings highlight bacteriophages as critical agents in reshaping the genetic landscape of *S. pyogenes*, not only through the propagation of virulence traits but also by driving the emergence of antimicrobial resistance (Hyder & Streitfeld, 1978).

2.2 Lysogenic Bacteriophages of Group A Streptococci

Lysogenic bacteriophages are distinguished by their capacity to integrate into the bacterial chromosome through site-specific recombination, after which they are maintained as stable genetic elements and vertically transmitted during cell division (Chaussee, Liu, Stevens, & Ferretti, 1996; Kjems, 1960). Although prophages carrying toxin-encoding genes are not uniformly present in all isolates, integration has been documented at multiple chromosomal locations in S. pyogenes, thereby contributing to genome plasticity and strain-dependent variation in virulence (Chaussee et al., 1996).

Importantly, prophage-derived sequences constitute a substantial component of the accessory genome in S. pyogenes and account for a large fraction of genetic heterogeneity

among strains. A significant proportion of these genes encode products involved in immune evasion, tissue invasion, and systemic spread. Collectively, these observations support the view that prophages act as primary engines of pathogenic diversification by shaping both genomic architecture and phenotypic expression.

Conclusion

S. pyogenes is responsible for a diverse spectrum of human diseases, ranging from uncomplicated skin and upper respiratory tract infections to rapidly progressing and life-threatening conditions such as streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis. Over the past several decades, invasive GAS infections have re-emerged as a major public health challenge, with global mortality estimates exceeding 500,000 cases annually.

Notwithstanding significant progress in molecular microbiology, the mechanisms by which GAS evades host immune defenses, particularly those related to resistance against phagocytic clearance, remain only partially delineated. In order to improve prevention strategies and to better understand the heterogeneous clinical behavior of GAS infections, extensive investigation is required. Both experimental and clinical studies are needed to define regulatory networks controlling virulence factor expression and to clarify host–pathogen interactions at the molecular level.

This review aimed to present a comprehensive synthesis of current knowledge regarding the virulence attributes of S. pyogenes and to emphasize the molecular mechanisms associated with severe invasive disease. Improved insight into these processes is anticipated to inform the development of advanced diagnostic methodologies, novel therapeutic interventions, and effective vaccine strategies for the control of GAS infections.

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