Medical Diagnosis and Treatment Methods in Basic Medical Sciences

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Editor
Prof. Dr. Sıddık Keskin

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PREFACE

Basic medical sciences are the entire of studies that aim to diagnose and treatments of diseases in order to maintain healthy life or to protect health and to treat disorders. Malpractices in health are still an important problem for many societies even for developed societies. The reflection of this problem on patients, relatives of patient and doctors is very serious financially and morally. It is obvious that this problem can be reduced substantially by applying the proper diagnosis and treatment methods in Medical Sciences. Today, Evidence-Based Medicine (EBM) aims to minimize the problems arising from malpractices in Health Sciences and directly affecting the patients, doctors and institution. Therefore, diagnosis and treatment methods in medical sciences are very important in the process of treating the patient and eliminating the diseases. In order to apply the proper treatment method, the proper diagnosis of the disease is basic prerequisite. When considering the role of diagnosis and treatment for clinicians, well-designed, accurate and reliable diagnostic and treatment methods are needed. Thus, many studies have been carried out on the diagnosis and treatment methods as well as efficacy and reliability of these methods used in Basic Medical Sciences. In this framework, the aim of this book is to gather and to present the studies to clinician for easy access and evaluation medical diagnosis and treatment methods. Thus, our book has 9 chapters. The first and second chapters present conceptual overview of biomedical and biochemical histopathology, respectively. The third chapter is about the review of cancer treatment and the fourth chapter is about the coagulation analysis in diagnosis and treatment. The 5^{th} chapter is about the prevalence of Palmaris longus. The 6^{th} chapter is focused on Treatment of erectile dysfunction. The 7^{th} chapter describes possible effect of pre-eclampsia and eclampsia on pregnancy, and examination of relationship between smoking and pregnancy is presented in the 8^{th} chapter. Finally, Chapter 9 addresses the effect of lavender on wound healing as alternative medicine.

We tried to prepare this book as carefully as possible and by minimizing mistakes as time and possibilities allowed. However, I apologize to you readers for the mistakes. I would like to thank for all my teammates who contributed to the preparation of this book. We hope that this book will be useful to our readers and researchers who want to make new studies.

Prof. Dr. Sıddık KESKİN

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CHAPTER I

A BIOMEDICAL TREASURE: NANOFIBERS IN WOUND HEALING

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INTRODUCTION

The skin that covers the body from the outside, protects against external-environmental factors and has the largest surface; It is also very prone to damage. The most important function of healthy skin is to provide homostasis. It acts as a barrier for exogenous substances, chemicals, moisture loss and pathogens. It is vitally important to quickly and effectively recycle any damage that may occur in the skin.

Wound is the deterioration of normal anatomical structure and functional integrity. Skin; It can be damaged by the effect of many harmful stimuli such as trauma, surgical applications, heat, cold, radiation, infection and neoplasia. In the event of injury, a complex healing and regeneration process occurs in the skin. The healing process firstly aims to restore homeostasis and within the framework of this process; It covers the steps of blood coagulation, inflammation, epithelium regeneration, granulation tissue and tissue reconstruction.

Normal wound healing takes place through the regular and sequential work of many cellular and biochemical functions. Under normal circumstances, these steps result in repairing the damage to the injured skin area. However, as a result of damage repair, a permanent scar formation occurs in the healing area. Scar tissue, unlike normal tissue; It is a texture that does not contain any leather additions and has lost its appearance - elasticity. While wound healing continues rapidly and regularly in healthy individuals, it is delayed in cases such as age, infection, diabetes, use of systemic steroids and cancer treatment. In these cases, chronic and incomplete healing ulcers may occur by disrupting the healing process (1, 2, 3). The aim of topical and systemic applications used to prevent this is

to provide ideal scar formation by affecting factors such as inflammatory cells, thrombocytes, mediators and extracellular matrix (4). In this context, different treatment methods are tried, new treatment searches are continued to accelerate the healing process, prevent excessive inflammation and infections, and to prevent any symptoms by monitoring the healing process (5).

Open wounds mean an open door for infection. Therefore, the wound surface should not be left open and should be covered with suitable materials. Wound dressings were originally fabricated from natural materials such as plant fibers and animal fats to simply cover the wounds. This has evolved to the present day whereby artificial materials can be fabricated by various advanced technologies to create multifunctional wound dressings. The two essential requirements of suitable modern wound dressings include rapid hemostasis property and good antibacterial property. The purpose of a wound dressing is to achieve rapid hemostasis function and it should also possess good antibacterial property to prevent infections from surrounding bacteria.

Electrospinning has attracted much interest for its versatility to fabricate nanofibrous membranes for a wound dressing which can create a moist environment around the wound area to promote healing (6).

ELECTROSPINNING

Historically, cells were grown and studied as monolayers on tissue culture plates. In recent years, advances in biomaterial synthesis and microfabrication have made it possible to pattern cells into complex, three-dimensional structures by using appropriate scaffolds as the templates (7, 8).

Electrospinning is a remarkably simple, robust, and versatile technique capable of generating fibers with diameters down to the nanoscale (7, 9). A non-woven mat of electrospun nanofibers possesses high porosity and spatial interconnectivity well-suited for nutrient and waste transport and cell communication (7, 10). A scaffold based on electrospun nanofibers also has a large specific surface area for loading of bioactive molecules to facilitate efficient and selective cellular responses (9).

Electrospinning was first being introduced in early 1930s for fabrication of nanofibers as filter materials and textile yarns. Since 1990s, after Reneker et al. demonstrated the feasibility to produce electrospun nanofibers from many polymers, the number of publications about electrospinning has grown exponentially, especially for recent ten years (11, 12, 13). Electrospinning received much attention for biomedical applications mainly due to the growing interest in nano-technologies and

the unique material properties. Electrospinning is an inexpensive and simple method to create nanoscale polymer fibers with diameter range from 3–5000 nm (11, 14). Nanofibers are suitable to mimic biological environment because they are in the same scale as biological molecules. In fact, nanomaterials like particles, fibrous morphologies or other complex forms, have shown improved interactionswith cells, for example, selective endocytosis, adhesion and orientation (11, 15, 16).

A useful wound healing patch should have the following properties (a) antibacterial, (b) able to promote rapid hemostasis and (c) having good biocompatibility to promote cell growth (6). Nanofibers were usually fabricated by incorporating antibacterial materials in the polymers. To date, five methods of incorporating active agents in the fibers; including the active agent blending with polymer solution before electrospinning, fabricating core/shell structure through coaxial electrospinning, encapsulating the active agent before mixing them with electrospinning solution, posttreatment of the fiber after electrospinning to convert a precursor to its active form or attaching the fiber surface with the active compound (6).

The electrospinning process utilizes high electrostatic forces for nanofiber generation; generally from a polymeric solution or melt. The main assembly of the electrospinning unit comprises of electrical supply (for the generation of high electrical voltage), a syringe (contains solution/ melt/suspension to be electrospun), a metallic needle (as a capillary), and a grounded conductive collector (rotatable drum or static plate type) all enclosed within a chamber (17, 18). During the spinning process, high voltage (5–15 kV) is applied between a needle capillary end and a collector. The polymer solution is electrically charged. At the needle tip, the polymer solution deforms from a spherical pendant droplet to a conical shape, known as “Taylor cone”. As the electric field is stronger than the surface tension of the polymer solution, the jet is ejected from the cone surface. As the jet travels, the solvent evaporates in the air, together with the stretching and acceleration of the polymer jet, leading to the extremely thin polymer fibers deposition on the collector (11, 19). Electrical bending instability occurs when the distance from the tip to the collector is sufficiently long; in the case of a short distance, the jet is typically straight. Under the action of the electric field, polymer jets experience bending instability primarily due to mutual repulsion of the excess electric charges carried by electrospun jets. The electrospinning process and the formation of polymer fibers are affected by many parameters. Spinnability, fiber diameters, fiber uniformity, fiber alignment, defects control (e.g. beads, junctions, and pores), and other properties are tunable by changing these parameters, (a) substrate-related parameters (polymer concentration,

viscosity, molecular weight, surface tension); and (b) apparatus-related parameters (flow rate and electric field) (11).

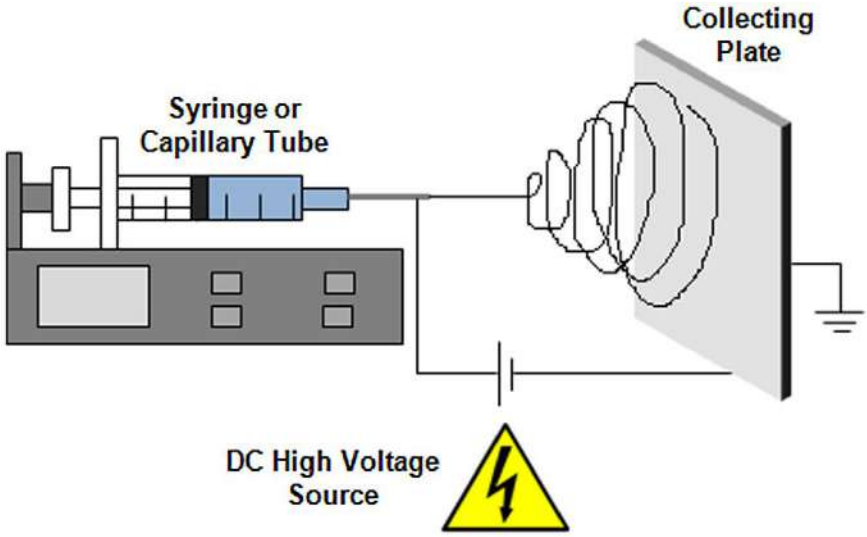

Figure 1: Schematic representation of a typical electrospinning apparatus. (Felgueiras, H. P., & Amorim, M. T. P. (2017). Functionalization of electrospun polymeric wound dressings with antimicrobial peptides. *Colloids and Surfaces B: Biointerfaces*, *156*, 133-148.)

Electrospinning has been applied to more than 100 different types of polymers. While preparing a product using the electrospin method, it is generally desired to obtain a high effect result by adding an active ingredient to a polymer solution. The most used polymer solutions can be listed as; PAA (poly(acrylic acid)), PVA (poly(vinyl alcohol)), PLA (poly(lactic asid)), PAN(polyacrylonitrile), PCL (poly(ε-caprolactone)), PVP (poly(vinyl pyrrolidone)), PEG (polyethylene glycol), PEO (poly(ethylene oxide)), PLLA (poly(L-lactide)), and PLGA (Poly (lactic-co-glycolic) acid) for biomedical production (20). For the purpose of the experimental study to be done on polymer solutions; Antibacterial agents, herbal extracts, various antioxidant agents are added to these polymer based solutions.

Figure 2: Diagrammatic illustration to high light the practical applications of E-spun NFs. (Thenmozhi, S., Dharmaraj, N., Kadirvelu, K., & Kim, H. Y. (2017). Electrospun nanofibers: New generation materials for advanced applications. *Materials Science and Engineering: B*, *217*, 36-48.)

NANOFIBERS (NFs) on WOUND HEALING

Many tissues in the human body do not have sufficient regenerative capacity, so damage to these tissues is irreversible. In addition to poor healing ability, injuries to tissues such as nerves, tendons, cartilage, and myocardium also cause severe pain and disability. At the end of the healing process that is completed after the injuries, especially the formation of scar tissue on the skin; It is a difficult situation to overcome both in terms of reducing the quality of life and cosmetics. Even with surgery, the return of function is often limited, and the healing response is scar mediated rather than regenerative (7, 21). Patients with organ trauma, disease, or congenital abnormalities should rely on organ transplantation to regain function. Despite its tremendous clinical success, this approach grapples with post-surgical immune reactions and severe limitations in the number of donors available, leaving thousands of patients on waiting lists. These limitations of organ transplantation have pushed researchers to find solutions in different areas for decades.

WOUND DRESSER PROPORTIES of NFs

When the advantages and disadvantages of wound dressings are evaluated; A good dressing must have some distinctive features. Wound

dressings prepared by electrospin method; It has many prominent features that will provide advantages in the wound healing process. NFs based wound dresses can promote hemostasis without resorting to a haemostatic agent, water adsorption is 8–92 times superior on electrospun dressings compared to typical film dressings. The arrangement of the nanofibers results in small pores that not only allow cell respiration, oxygen exchange, and to control the moisture at the wounded site preventing dehydration, but, because of its small size, can also effectively protect the wound from infection preventing microorganisms from penetrating. These wound dresses also comfortable, functional because unlike other commercially available dressings that use multi-layer technology, which tends to be more unpredictable and less stable, with electrospinning it is possible to blend various functional materials simultaneously to produce an all-in-one wound dressing. Use of electrospinning to produce biomimetic nanofibrous structures with good cell conductivity can enhance blood and tissue compatibility, which facilitates wound healing and tissue regeneration and provide scar-free healing (22, 23, 24).

ANTIBACTERIAL PROPORTIES of NFs

Microbes on the human skin are divided into three general categories: pathogens, potential pathogens, and harmless symbiotic organisms (25, 26). Bacteria are one of these cutaneous microbes. Normal bacteria that are commonly found on the skin include *Staphylococcus epidermidis*, corynebacterium, propionibacterium, occasionally *Staphylococcus aureus*, and peptostreptococcus species (27). Some of the aforementioned cutaneous bacteria, which are known as opportunistic pathogens, can become pathogenic when the human immune system is weakened, or when there are concurrent infections and illnesses such as cancer. They can infect the human body and cause many diseases (25, 28).

When the human skin barrier is removed or damaged, chances of more serious infections and issues increase (25). Usually, the polymers used for fabricating antibacterial nanofibers possess inherent abilities such as non-toxicity, goodmechanical properties, biocompatibility and biodegradability including PCL, PLA, PLGA, PVA and chitosan. The common antibacterialmaterials such as antibiotics, triclosan, chlorhexidine, quaternary ammonium compounds (QACs), biguanides, silver nanoparticles, and metal oxide nanoparticles have been reported to be used in fabricating antibacterial nanofibers (6).

Sohrabi A. et al used ampicillin incorporated PMMA (polymethyl methacrylate)–nylon6 core/shell fibers were fabricated utilizing the coaxial electrospinning technique for investigate these materials antibacterial activity in gram-positive *Listeria innocua*. They confirmed their fibers

formation of the core/shell structure and the smooth surface morphology with TEM and SEM analysis. They designed drug delivery system for all the concentrations of the encapsulated drug indicates a three stages drug release over a period of 31 days with a sustained manner and suppressed burst release, which occurred only for 6 h. Antibacterial investigations showed a gradual decrease in the OD (concentration of the bacteria) with an increase in the concentration of the encapsulated drug from 1% to 20%. A higher amount of drug in the fibers led to enhanced drug release after 18 h incubation which resulted in a higher degree of growth inhibition (29).

Khampieng T. et al, used electrospinning technique and post-spinning sorption method DOXY-h loaded-poly(acrylic acid) (PAA) nanofiber mats (PAA/DOXY-h nanofiber mats) at various doses: PAA/DOXY-h125, PAA/DOXY-h250, PAA/DOXY-h500, and PAA/DOXY-h1000. The morphology, drug content, release characteristics, and antibacterial activities of the PAA/DOXY-h nanofiber mats were investigated with SEM, UV–vis spectrophotometry, and disc diffusion methodology. They tested these products in gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus agalactiae*, and gram-negative bacteria, *Pseudomonas aeruginosa*. They found that; these particular nanofiber mats demonstrated antibacterial properties against gram-positive bacteria; *S. aureus and S. agalactiae*, therewithal appeared to be more effective and concentration-dependent against gram-negative bacteria; *P. aeruginosa* (30).

Kim SS. et al, producted Polyacrylonitrile (PAN)–chitosan double-face films and nanofibers for investigating their antibacterial activity. They dissolved PAN and a chitosan salt in dimethyl sulfoxide, and then thin-layered on a glass plate or electrospun followed by coagulation in sodium hydroxide solution. The morphology of the PAN–chitosan double-face films and nanofibers was analyzed by SEM. The antibacterial efficacy was measured by a swatch test with bacterial suspensions. They concluded that; the PAN–chitosan nanofibers produced a 5-log reduction (Log reduction is a mathematical term that is used to express the relative number of living microbes that are eliminated by disinfection) against *Escherichia coli*, *Staphylococcus aureus*, and *Micrococcus luteus* (31).

Sarhan WA and Azzazy HME, produced chitosan and honey were co-spun with polyvinyl alcohol (PVA) allowing the fabrication of nanofibers with high honey concentrations up to 40% and high chitosan concentrations up to 5.5% of the total weight of the fibers using biocompatible solvents (1% acetic acid). The fabricated nanofibers were further chemically crosslinked, by exposure to glutaraldehyde vapor, and physically crosslinked by heating and freezing/thawing. The new HP–chitosan nanofibers showed pronounced antibacterial activity against *Staphylococcus aureus* but weak antibacterial activity against *Escherichia*

coli. The developed HP–chitosan nanofibers revealed no cytotoxicity effects on cultured fibroblasts. And they concluded that, biocompatible, antimicrobial crosslinked honey/polyvinyl alcohol/chitosan nanofibers were developed which hold potential as an effective wound dressing (31).

CONCLUSION

Despite their potential for continuous exploration and evolu-tion, antimicrobial dressings are already one of most importantdiscoveries of our century in biomedical research and their thera-peutic abilities are well recognized (22). Electrospinning is a simple and cost effective but fascinating fiber forming technology with less time consumption under optimized conditions (32). Electrospinning technique, allows the fabrication of fibers with high-surface area due to their diameters being scalable down to a few nanometers. Electrospin based wound dressers can be surface functionalized to tune the physical and chemical properties of the fiber surface while the fiber structure, morphology, and spatialdistribution can be controlled to achieve specific mechanical properties. In addition, electrospinning allows the combination of different synthetic and natural polymers to be used to make nanofibers. The possibility of large-scale production combined with simplicity andversatility makes the electrospinning process very attractive for a broad variety of applications (33). Hundreds of polymer options and hundreds of healing agents that can be added to these polymer solutions show that this biomedical research field is in need of much more research.

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CHAPTER II

BIOCHEMICAL AND HISTOPATHOLOGICAL PARAMETERS USED IN EXPERIMENTAL OVARIAN ISCHEMIA / REPERFUSION MODELS IN RECENT YEARS

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INTRODUCTION

Ovarian torsion, which affects females of all ages, is a gynecological emergency (1). Adnexal torsion is uncommon, occurring most often during reproductive years. Torsion of the normal adnexa, which is rare, is more common among children than adults. Typically, one ovary is involved, but sometimes tuba uterina is also involved. Adnexal torsion causes sudden, severe pelvic pain, and sometimes nausea and vomiting. Adnexal torsion is defined as at least one full turn around the midline of the adnexa, ovary or, more rarely, the tuba uterina alone, including the infundibulopelvic and tubo-ovarian ligaments (2). The infundibulopelvic ligaments suspend the movable ovary, allowing the ovary to position laterally or posteriorly to the uterus (1). Adnexal torsion usually indicates an ovarian abnormality. Risk factors for adnexal torsion include; pregnancy, induction of ovulation for IVF, ovarian enlargement, particularly by benign tumors. Benign tumors are more likely to cause torsion than malignant ones. Because of adnexal tissue is not fixed, a big leading point, such as tumorous growth, can induce twisting (1). Adnexal torsions, which are the fifth most common cause in patients operated on due to gynecological emergencies, constitute 2.7% of gynecological emergencies. Although it is seen in all age groups, its incidence is higher in women of reproductive age. Adnexal torsion (AT) in children is rare, approximately 15% of cases occur during infancy and childhood (3). In a large series by Guthrie et al (4), the estimated incidence of AT among females 1 to 20 years old was estimated to be 4.9 of 100,000 (3, 5).Although the exact etiology is unknown, an ovarian mass is detected in the majority of cases. Torsion is very rare in normal sized ovaries. Its incidence is increasing in pathologies that enlarge the size of the ovary, such as polycystic ovary and benign cystic teratoma (6).

Ischemia due to ovarian torsion has been the subject of research in recent years. Ischemia occurs when some of them cannot get enough blood and oxygen through a reduction or interruption in blood flow. The gold standard to treat ovary torsion is surgery, and this is also the only way to confirm the torsion by using the techniques like laparoscopy and laparotomy (1). In cases of ovarian torsion, blood flow is provided to the region again with surgical intervention and thus detorsion is performed.

EXPERIMENTAL DESIGN

Animals

Many researchers, who have set up experimental research in recent years, have focused on supporting the ovarian damage caused by torsion not only with surgical intervention but also with systemic therapeutic agent applications. When Wistar albino or Spraque Dawley rats were evaluated in terms of feeding, housing, cost, response to surgical intervention; They are among the most suitable laboratory animals to these kind of experiments. While creating the experimental groups, young adult / adult, healthy, non-pregnant, 200 – 290 g weighing female rats were generally used.

Procedure of experimental ischemia/reperfusion model

Rats are starved by cutting their access to food, 24 hours before the operation. Surgical procedures in the rats were performed under anesthesia. Different anesthesia applying protocols and amounts of anesthetic agents used in ovarian ischemia / reperfusion studies conducted in recent years are listed in Table 1. The skin around the incision was shaved and disinfected. After the rats were placed in the dorsal recumbent position, under sterile conditions; A laparotomy was performed by making a 2-cm (7, 8, 9, 10, 11, 12, 13, 14, 15) or 2,5-cm (16, 17, 18, 19, 20, 21) longitudinal incision in the midline area of the lower abdomen and the uterine horns and adnexa were located. The abdomen opened and then gently separated the intestines. After incision in the right / left or bilateral fossa paralumbalis area, adnexa were torsioned 360^0 clockwise (13, 18, 22) or 720^0 counter clockwise (23) and fixed on abdominal wall to stay for during the predetermined time as ischemic. Then incision was sutured; re-laparotomy was performed and adnexa were detorsioned; reperfusion was allowed for additional predetermined time after operation sites were sutured, at the end of experimental time detorsion ovaries were harvested, and blood samples were collected. Rats were sacrificed after collecting samples. Ischemia / reperfusion times that vary according to studies are shown in the Table 2. The therapeutic agents to be applied in the experiments are usually administered intraperitoneally or by gavage half an hour before the detorsion application (with exceptions). Comparison of

ischemia / reperfusion procedures of experimental studies and agents used for treatment is presented in the Table 2.

BIOCHEMICAL PARAMETERS

Ischemic damage, which is caused by a drop in adnexal circulation, is one possible cause of adnexal injury resulting from torsion. Levels of lactic acid, hypoxanthine, and lipid peroxide in the tissues increase because of the hypoxia that is caused by this decreased blood flow (5). Removing the cause of ischemia and restoring the tissue blood supply (reperfusion/detosion) are significant procedures, unavoidable in cases of ischemia. During reperfusion, however, the return of oxygenated blood starts a reaction that can cause even more damage to the tissue. Reperfusion injury starts with the formation of reactive oxygen species, triggering a series of events (24).

Among all possible pathological mechanisms of ischemia-reperfusion injury, free radical damage (mainly oxidative/nitrosative stress injury) has been found to play a key role in the process. Free radicals lead to protein dysfunction, DNA damage, and lipid peroxidation, resulting in cell death. Free radicals are divided into two main groups: reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS play key roles in many pathological processes during ischemia reperfusion. Currently, free radicals' toxicity in ischemia-reperfusion injury is being intensively studied (25). Lipidoxidation results in the production of toxic substances, includingmalondialdehyde (MDA). MDA, a marker of tissue injury, is a secondary product of oxidative damage formed during lipidperoxidation (26, 27). MDA disrupts ionic transport and enzymaticactivity, severely modifying cell membrane permeability and flu-idity to cause discontinuities and breaks that separate cells and organelle contents (7). For this reason, MDA has been chosen as the basic biochemical parameter among many studies we have examined in this review.

The cells are protected against ROS damage by various ways such as scavenging enzyme systems including Catalase which converts hydrogen peroxide into hydrogen oxide (water) and superoxide dismutase (SOD). SOD catalyzes the partitioning of the superoxide (O2_) radical into either ordinary molecular oxygen (O2) or hydrogen peroxide (H2O2). Superoxide is produced as a by-product of oxygen metabolism. It needs to be regulated; otherwise it causes many types of cell damage. Lipid peroxidation which plays a role in decreased binding of hypothalamic hormones to their receptors causes a decline in antioxidant systems (28).

The concentrations of the metabolic intermediates of ROS are kept under strict control by a complex defense system that includes enzymes such as superoxide dismutase (SOD), catalase (CAT), and

glutathione peroxidase (GSH-Px) (29, 30). Excess ROS and their toxic products cause DNA damage, lipid peroxidation in the cellular and mitochondrial membranes, and cellular damage (8).

Reactive oxygen species, including hydrogen peroxide, hydroxyl radicals, superoxide anions, and the formation of NO and peroxynitrite are enhanced during reperfusion of the ischemic tissue. These free radicals cause further cellular damage through the peroxidation of lipids in mitochondrial and cell membranes (31). NO acts in a variety of tissues to regulate a diverse range of physiological processes, but excess of NO can also be toxic (22).

The reasons for IR-induced ovarian injury include release of cytokines and free radicals, thrombocyte and neutrophil activation, apoptosis, and nitric oxide (17). The reactive oxygen species (ROS) are produced during the ischemia or reperfusion period and release activated neutrophils such as superoxide, hydrogen peroxide, and hydroxyl radical. Independent evaluation on these markers would be unpractical and also insufficient for a wide-ranging assessment. The total antioxidant status (TAS) was employed to assess the general antioxidative status (32). As well, total oxidant status (TOS) is obtained to ascertain the overall oxidation status (33). Represented as the ratio of TOS to TAS, the oxidative stress index (OSI) is regarded as a more precise index of oxidative stress on the tissue. A number of anti-inflammatory and antioxidant agents were used to prevent I/R injury (17). Biochemical parameters frequently preferred in ovarian ischemia / reperfusion studies in recent years are listed in the Table 3.

HISTOPATHOLOGICAL CHANGES

For histopathological examinations, tissues are collected immediately after the lethal dose of anesthesia administered at the end of the experimental procedure. In small-sized organs such as the ovary, at least half of the organ should be taken into the fixation solution for histopathological examination. Half of the right ovary of each rat was put separately into a formaldehyde media. Tissues were detected in the 10% buffered formalin, after which a routine tissue follow-up was performed and they were placed in paraffin blocks. Slides of 4–5 μm thickness were cut from the parafin blocks using a microtome and deparaffinized. The samples were dyed with hematoxylin–eosin stain and examined under a light microscope (11).

Basic histopathological changes noted in ovarian ischemia / reperfusion studies; cherry like appearance across the tissue (5, 8, 18), congestion / dilatation of the vessels, diffuse hemorrhage in the ovarian tissue, leukocyte infiltration, edema, necrosis, and follicular degeneration.

A healthy evaluation of these histopathological changes is only possible with a semiquantitative scoring. A semiquantitative histological evaluation scoring system was used to determine histopathological changes. These parameterswere evaluated after scoring each individual criterion between 0 and 3. The criteria that were used to evaluate for ovarian injury were follicular cell degeneration, vascular congestion, hemorrhage, and infiltration by inflammatory cells. Each specimen was scored using a scale ranging from 0 to 3 (0: none; 1: mild; 2: moderate; 3: severe) for each criterion (35).

When the other strategy is examined, it is seen that the scoring is done as follows. Five microscopy fields were used to determine the presence or severity of tissue damage. The scoring system used for histopathologic evaluation of the ovarian tissues was the same as in Sagsöz et al (6). According to this system, congestion (vascular dilation), hemorrhage, and interstitial edema were scored from 0 to 3 according to their severity: 0 represented no pathologic findings; and 1, 2, and 3 represented pathologic findings of less than 25%, 25% to 75%, and more than 75% of the ovarian section, respectively. The total tissue damage scores were calculated by adding the scores for each parameter, and the total tissue damage scores were calculated between 0 and 9 for each ovary. Examination and scoring of the ovarian sections were performed in a blinded fashion by the same pathologist (8, 37). A healthy evaluation of these histopathological changes is only possible with a semiquantitative scoring. In the experimental studies reviewed in this review, the use of scoring system for histopathological changes is about 37%. Apart from studies using hematoxylin eosin staining, there are also studies evaluating immunohistochemical parameters, but they are in a minority (13, 18, 38, 39).

DISCUSSION

Ischaemia-Reperfusion injury (IRI) is defined as the paradoxical exacerbation of cellular dysfunction and death, following restoration of blood flow to previously ischaemic tissues. Reestablishment of blood flow is essential to salvage ischaemic tissues. However reperfusion itself paradoxically causes further damage, threatening function and viability of the organ. IRI occurs in a wide range of organs including the heart, lung, kidney, gut, skeletal muscle and brain and may involve not only the ischaemic organ itself but may also induce systemic damage to distant organs (34). Medical therapies such as anti-inflammatory and antioxidant free radical scavengers directed toward the mediators responsible for ischemic damage may be used to prevent tissue damage and irreversible changes (5). This was observed through a reduction in oxidative stress, an increase in antioxidant enzymes, and less histopathologic damage. These results agree with earlier experimental models showing that oxidative

stress plays an important role in ovarian torsion (5) and the results of many experimental studies we gathered from this study show that antioxidants used for therapeutic purposes can reduce ovarian tissue damage. We hope that our study will be a helpful resource for the design of future ovarian ischemia / reperfusion studies.

Table 1: Anesthetics used for surgical procedure in ovaryan torsion studies.

STUDY	ANESTHETICS USED for THE SURGICAL PROCEDURE
Cemgil Arıkan D. et al (2010), Bozkurt S. et al (2012)	60 mg / kg Ketamine hydrochloride *via IP*
Aslan MK. et al (2012)	80 mg / kg Ketamine hydrochloride *via IP*
Çadıcı E. et al (2010), Kurt A. et al (2011), Sengül O. et al (2013), Bayır Y. et al (2016), Unlübilgin E. et al (2017) , Turkler C. et al (2018)	25 mg / kg Sodium thiopental *via IP*
Akdemir A. et al (2014), Aslan M. et al (2017), Ilgen O. et al (2020)	50 mg / kg Ketamine hydrochloride + 7 mg / kg Xylazine hydrochloride combination *via IP*
Ergun Y. et al (2010), Sak ME. et al (2013), Eser A. et al (2015), Incebıyık A. et al (2015), Melekoğlu R. et al (2018)	50 mg / kg Ketamine hydrochloride + 10 mg / kg Xylazine hydrochloride combination *via IM*
Çakır Güngör AN. et al (2014)	75 mg / kg Ketamine hydrochloride + 10 mg / kg Xylazine hydrochloride combination *via IP*
Yurtçu E. et al (2015)	40 mg / kg Ketamine hydrochloride + 10 mg / kg Xylazine hydrochloride combination *via IP*
Topdağı Yılmaz EP. et al (2020)	45 mg / kg Ketamine hydrochloride + 5 mg / kg Xylazine hydrochloride combination *via IP*
Behroozi-Lak T. et al (2017)	90 mg / kg Ketamine hydrochloride + 5 mg / kg Xylazine hydrochloride combination *via IP*

Table 2: Experimental procedures and therapeutic agents used in ovarian ischemia / reperfusion studies in recent years.

STUDY	*I/R PROCEDURE*	*THERAPEUTIC AGENTS*
Çadıcı E. et al (2010)	3 hours İ / 3 hours R	Atorvastatin 10 mg / kg *via Gavage*
Ergun Y. et al (2010)	3 hours İ / 3 hours R	Erythropoetin 1200 IU / kg - Dimethyl sulfoxide 1.5 mg / kg *via IP*
Cemgil Arıkan D. et al (2010)	3 hours İ / 12 hours R - 3 hours İ / 24 hours R	Tadalafil (TDF) 5 mg / kg *via IP*
Kurt A. et al (2011)	3 hour İ / 2 hour R	Famotidine 20 mg / kg *via Gavage*
Bozkurt S. et al (2012)	4 hour İ / 6 hour R and 4 hour İ / 12 hour R	Selenium 0.2 mg / kg *via IP*
Aslan MK. et al (2012)	2 hours İ / 2 hours R	Ozone 0.5 mg / kg *via IP*
Sak ME. et al (2013)	3 hours İ / 3 hours R	Curcumin 100 mg / kg *via IP*
Sengül O. et al (2013)	3 hours İ / 3 hours R	Bosentan 30-60 mg / kg *via Gavage*
Akdemir A. et al (2014)	3 hours İ / 3 hours R	Oxytocin 80 IU / kg *via IP*
Çakır Güngör AN. et al (2014)	3 hours İ / 3 hours R	Hesperedin (dissolved in DMSO) 50 mg / kg *via IP*
Yurtçu E. et al (2015)	2 hours İ / 2 hours R	Vardenafil 1 and 2 mg / kg *via IP*
Eser A. et al (2015)	2 hours İ / 2 hours R - 4 hours İ / 4 hours R	Curcumin 200 mg / kg *via IP*
Incebıyık A. et al (2015)	2 hours İ / 2 hours R	Sildenafil 0.7 and 1.4 mg / kg *via IP*
Bayır Y. et al (2016)	3 hours İ / 3 hours R	Aliskiren 50 and 100 mg / kg *via Gavage*
Aslan M. et al (2017)	2 hours İ / 2 hours R	Oxytocin 0.5 µg / kg - Kisspeptin 0.5 µg / kg *via IP*
Behroozi-Lak T. et al (2017)	3 hours İ / 3 hours R	Nimodipine 1 mg / kg *via IP*
Unlübilgin E. et al (2017)	2 hours İ / 2 hours R	Benidipine 2 mg / kg and 4 mg / kg *via IP*
Melekoğlu R. et al (2018)	2 hours İ / 2 hours R	Chrysin 50 mg / kg / day - Glycyrrhetinic acid *via Gavage*
Turkler C. et al (2018)	2 hours İ / 2 hours R	Lutein 1 mg / kg *via IP*
Naykı C. et al (2018)	2 hours İ / 2 hours R	Rutin 50 mg / kg *via IP*
Güleç Başer B. et al (2018)	3 hours İ / 3 hours R	Progesterone 8 mg / kg *via IP*
Gezer S. et al (2020)	3 hours İ / 3 hours R	Letrozole 1 mg / kg *via Gavage*
Topdağı Yılmaz EP. et al (2020)	3 hours İ / 3 hours R	Lycopene 100 and 200 mg / kg *via Gavage*
Ilgen O. et al (2020)	3 hours İ / 3 hours R	Methylene blue 15 mg / kg *via IP*

Table 3: Parameters used for biochemical evaluation in ovarian ischemia / reperfusion studies in recent years. *(MDA (Malondialdehyde), SOD (Superoxide dismutase), GSH/tGSH (Total glutathione), MPO (Myeloperoxidase), LPO (Lactoperoxidase), CAT (Catalase), GPx (Glutathione peroxidase), NO (Nitric oxide), TAS (Total antioxidant status), TOS (Total oxidant status), OSI (Oxidative stability index)).*

STUDY	I/R PROCEDURE	BIOCHEMICAL PARAMETERS
Çadıcı E. et al (2010)	3 hours İ / 3 hours R	*SOD, MPO, GSH, LPO*
Ergun Y. et al (2010)	3 hours İ / 3 hours R	*MDA, NO*
Çemgil Arıkan D. et al (2010)	3 hours İ / 12 hours R - 3 hours İ / 24 hours R	*MDA, NO, SOD, CAT*
Kurt A. et al (2011)	3 hour İ / 2 hour R	*tGSH, SOD*
Bozkurt S. et al (2012)	4 hour İ / 6 hour R and 4 hour İ / 12 hour R	*MDA, SOD, NO, CAT, GPx*
Aslan MK. et al (2012)	2 hours İ / 2 hours R	*MDA, NO, T-SH*
Sak ME. et al (2013)	3 hours İ / 3 hours R	*TOS, TAS, OSİ*
Sengül O. et al (2013)	3 hours İ / 3 hours R	*MDA, SOD, GSH, plus (IL-1B, IL-6, TNF-A)*
Akdemir A. et al (2014)	3 hours İ / 3 hours R	*MDA*
Çakır Güngör AN. et al (2014)	3 hours İ / 3 hours R	*N/A*
Yurtçu E. et al (2015)	2 hours İ / 2 hours R	*TAS, TOS, OSİ*
Eser A. et al (2015)	2 hours İ / 2 hours R - 4 hours İ / 4 hours R	*TAS, TOS, NOS, XO*
İncebıyık A. et al (2015)	2 hours İ / 2 hours R	*TAS, TOS, OSİ*
Bayır Y. et al (2016)	3 hours İ / 3 hours R	*MDA, SOD, GSH, plus (IL-1B, IL-6, TNF-A, iNOS, Renin, Angiotensin-2)*
Aslan M. et al (2017)	2 hours İ / 2 hours R	*MDA, SOD, GSH*
Behroozi-Lak T. et al (2017)	3 hours İ / 3 hours R	*MDA, SOD, tGSH, NOS, MPO, plus (DNA damage, GPO, GSHRD, GST)*
Unlübilgin E. et al (2017)	2 hours İ / 2 hours R	*MDA, tGSH, plus (TNF-A, IL-1B)*
Melekoğlu R. et al (2018)	2 hours İ / 2 hours R	*MDA, SOD, GSH, CAT, GPx*
Turkler C. et al (2018)	2 hours İ / 2 hours R	*MDA, tGSH*
Naylı C. et al (2018)	2 hours İ / 2 hours R	*MDA, tGSH, plus (TNF-A, IL-1B)*
Güleç Başer B. et al (2018)	3 hours İ / 3 hours R	*MDA, TAS, TOS, OSİ*
Gezer S. et al (2020)	3 hours İ / 3 hours R	*N/A*
Topdağı Yılmaz EP. et al (2020)	3 hours İ / 3 hours R	*MDA, SOD, GSH, MPO*
Ilgen O. et al (2020)	3 hours İ / 3 hours R	*MDA, Total protein*

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CHAPTER III

CLINICAL DEVELOPMENT OF HIF-1α INHIBITORS FOR CANCER THERAPY

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INTRODUCTION

Hypoxia inducible factors (HIFs) are transcription factors that play a central role in adaptation of cells to hypoxia. HIFs are stabilized in the hypoxic environment leading to transcription of many target genes that induce angiogenesis to re-establish oxygen homeostasis and regulate cellular energetic metabolism to adapt to hypoxic conditions [1]. Cancer cells utilize these mechanisms in their advantage to survive despite unfavorable conditions, gain replicative immortality and stem cell characteristics, escape from apoptotic stimuli and immunosurveillance, metastasize to different areas and resist anti-cancer therapy. As a result, hypoxia or increased activity of HIFs bring more malignant characteristics to tumors and worsen patient prognosis [2].

Among the HIF isoforms HIF-1 come forward with its prominent involvement in tumor progression and chemoresistance. HIF-1 is a heterodimer of HIF-1α and HIF-1β subunits. HIF-1α is overexpressed in several tumors and its expression is associated with poor overall survival [3-5]. HIF-1α expression was found to be more abundant at the invasive margins of tumor specimens [6-8]. High expression of HIF-1α is also associated with high a risk of metastasis in cancer [9-11]. Therefore, targeting HIF-1α is of prime importance to increase therapeutic success in cancer chemotherapy.

REGULATION OF HIF-1α ACTIVITY

In the presence of oxygen HIF-1α is hydroxylated by prolyl hydroxylases (PHDs). Then hydroxylated HIF-1α is ubiquitinated and degraded by ubiquitin proteasome system with a reaction dependent on tumor suppressor protein von Hippel–Lindau (pVHL). On the other hand, in hypoxic conditions HIF-1α cannot be hydroxylated by PHDs and becomes stable. Then, it translocates to the nucleus and couples with HIF-1β to form HIF-1. After that, HIF-1 binds p300/CBP co-activator forming the HIF transcription complex which binds to hypoxia response elements

and induces the transcription of target genes such as vascular endothelial growth factor (VEGF), glucose transporter-1, epithelial-mesenchymal transition genes and matrix metalloproteinases [12-14].

The expression of HIF-1α is also regulated by several other mechanisms besides hypoxia in cancer cells. Reactive oxygen species generated by various stimuli stabilize HIF-1α and increases HIF-1 activity. PI3K/Akt/mTOR and RAS/MAPK pathways which are oncogenic pathways overactivated in several cancers are positive regulators of HIF-1α expression. Mutations in tumor suppressor genes phosphatase and tensin homologue (PTEN), VHL and p53 are also associated with increased HIF-1α expression in cancer cells [14,15].

HIF-1α INHIBITORS IN CLINICAL TRIALS

In accordance with the importance of HIF-1α in tumor progression and chemoresistance, last 20 years have witnessed the testing of several direct or indirect inhibitors of HIF-1α in clinical trials for cancer. Some of these agents such as camptothecins, rapamycin analogs, and anthracyclines, which exhibit antitumor action by mechanisms that does not directly involve HIF-1α inhibition, have already been approved and widely used for the treatment of several cancers. However, efforts to develop new agents that directly inhibit HIF-1α is still growing [16,17]. In this chapter, we present the major HIF-1α inhibitors that were translated to clinical studies so far with registered clinical trials in ClinicalTrials.gov (https://www.clinicaltrials.gov/). Relevant clinical trial identifiers are given for each of the studies in the text. According to their mechanism of action, anti-HIF-1α agents were classified into five in this chapter, namely HIF-1α antisense oligonucleotides, drugs that inhibit HIF-1α translation, drugs that destabilize HIF-1α, inhibitors of HIF-1/DNA binding, and drugs that inhibitor HIF-1α at multiple levels**.**

1. HIF-1α Antisense Oligonucleotides

Efforts to translate antisense oligonucleotides into the treatment of cancer patients is growing for the last decade [18]. EZN-2968 is a HIF-1α antisense oligonucleotide developed by Enzon Pharmaceuticals. A phase I trial has been conducted between 2007 and 2011 for determination of the safety, pharmacokinetic profile, and anticancer activity of EZN-2968 in patients with advanced solid tumors and lymphoma (NCT00466583). First results suggested well-tolerability in cancer patients who have undergone previous treatments [19]. In 2010, a second phase I study for EZN-2968 was commenced under the sponsorship of National Health Institute to determine the safety profile and efficacy in advanced solid tumors with liver metastasis (NCT01120288). A reduction in HIF-1α expression was observed in a very limited number of patients enrolled into the study and the trial was ended early [20]. Later in 2016, a phase Ib study was started to

test a synthetic locked nucleic acid form of EZN-2968 (RO7070179) in hepatocellular carcinoma (HCC) (NCT02564614), since dose limiting toxicity in the previous studies was hepatotoxicity due to accumulation in liver. Study results suggested that HCC patients may benefit from RO7070179 and, decrease in HIF-1α mRNA during the first cycle may have predictive value for therapeutic effect [21].

2. Drugs That Inhibit HIF-1α Translation

2a. Camptothecins

Camptothecins which act primarily by inhibition of topoisomerase I enzyme, is an important group of chemotherapeutics used in the treatment of cancer. Camptothecin and its derivatives like topotecan has been shown to down-regulate HIF-1α through regulation of the HIF-1α-targeting miRNAs [22]. After the confirmation of its anti-HIF1α activity together with the anti-angiogenetic and anti-tumorigenic effects in xenograft models, topotecan was evaluated in a phase I study for its effectiveness in suppressing HIF-1α expression in advanced solid tumors refractory to treatment (NCT00117013). Although a median reduction of 7.5% percent was detected in HIF-1α expression and a correlation was observed between this reduction and radiological outcomes, the results were not statistically significant because of low patient accrual [23].

Besides their use as single agents, inclusion of campothecins in combination chemotherapy where an anti-VEGF agent is involved may prevent the increase in HIF-1α due to inhibition of angiogenesis. With this notion, combination of camptothecins with anti-angiogenetic agents have been tested in several clinical trials. Combination of irinotecan with anti-VEGF monoclonal antibody bevacuzimab was tested for its efficacy in pediatric patients with recurrent, progressive, or refractory glioma, medulloblastoma, ependymoma, or low-grade glioma in a phase II trial started in 2006 (NCT00381797). In another phase II trial started in 2007, efficacy of topotecan in combination with cisplatin and bevacuzimab was tested in patients with recurrent or persistent cervical cancer (NCT00548418). However, the toxicity profile of this combination was disappointing [24].

EZN-2208, a pegylated form of SN38, which is the active metabolite of irinotecan, was combined with bevacuzimab in a phase I trial to test its safety, efficacy and anti-HIF-1α activity in solid tumors refractory to treatment (NCT01251926, no posted results). CRLX101, which is a nanoparticle formulation of camptothecin, was tested for its safety and efficacy profile in combination with bevacizumab in patients with ovarian, tubal, and peritoneal cancers resistant to platinium-based therapy (NCT01652079) [25]. The trial was completed in 2018 with no results posted yet. Although the emphasis was not on inhibition of HIF-1α, the efficacy

of anti-VEGF aflibercept in combination with the FOLFIRI (folinic acid, fluorouracil, and irinotecan) regimen was investigated in metastatic colorectal cancer (NCT02129257). The regimen was found to be tolerable. However, 6 months of first-line treatment with FOLFIRI + aflibercept was followed by aflibercept without accompanying anti-HIF1α treatment and a substantial increase in efficacy has not been detected [26].

2b. PI3K/AKT/mTOR Pathway Inhibitors

PI3K/AKT/mTOR pathway is an important intracellular signaling pathways that induces cell proliferation, migration, and tumor progression. Several anticancer drugs that inhibit this pathway at distinct molecular levels have been used or being tested in chemotherapy of several cancers. Rapamycin (sirolimus) is the first agent discovered among these, which gives its name to the mTOR protein, the mammalian target of rapamycin [27]. Rapamycin analogs (rapalogs), temsirolimus and everolimus are first approved for the treatment of advanced renal carcinoma and have been tested in preclinical and clinical studies for the treatment of several other cancers [28]. Besides their primary action on inhibition of cell proliferation and migration, rapalogs also downregulate HIF-1α since PI3K/AKT/mTOR pathway is a positive regulator of HIF-1α expression [14,17]. Due to this action, rapalogs have been started to be tested clinically in combination with other chemotherapeutics and anti-angiogenic agents.

A phase I study started in 2010 to determine maximum tolerated dose of everolimus (RAD001), in combination with capecitabine and oxaliplatin (XELOX) regimen in patients with advanced gastric cancer (NCT01049620). The same year, another clinical trial started to determine safety of everolimus and its efficacy in combination with FOLFOX and bevacizumab in the first-line treatment of colorectal cancer (NCT01047293). The combination exhibited a tolerable profile and fair efficacy [29]. Combination of everolimus with sorafenib, which is an anti-tumorigenic and anti-angiogenic agent due to its multi-kinase inhibitor action, was tested in a Phase I-II study for advanced solid tumors. However, the study was suspended early due to toxicity (NCT01226056). Combination of rapamycin and irinotecan has also been tested in refractory solid tumors in children for the synergistic effect in inhibition of angiogenesis and suppression of HIF-1α (NCT01282697, no results posted).

Perifosine is a multitarget tyrosine kinase inhibitor that has AKT inhibitor activity. It has been extensively tested as a single agent or combination in several different cancers in tens of different clinical trials (e.g., NCT00398879, NCT01048580, NCT00398814, NCT00060437, NCT00873457, NCT02238496 and NCT02238496). Due to AKT inhibitory action, perifosine downregulates HIF-1α [16]. However, this action

was not the main emphasis in the clinical trials of perifosine conducted so far. Despite the promising results in preclinical studies, its clinical effectiveness in cancer is not fully established yet.

2c. Digoxin

Since preclinical studies suggested that digoxin blocks HIF-1α [30], its efficacy in blocking HIF-1α in breast cancer and Kaposi's Sarcoma has been tested in two different clinical trials. The former one aimed to investigate whether two weeks of digoxin intake starting from the first biopsy to the time of surgery decreases HIF-1α expression in newly diagnosed breast cancer (NCT01763931). However the trial stopped to recruit patients early since accrual goals could not be met [31]. HIF-1α has been shown to induce lytic life cycle of Human Herpes Virus 8 [32]. Regarding the efficacy of digoxin in downregulation of HIF-1α, digoxin may have a potential in the treatment of HHV-8 related Kaposi Sarcoma. A multi-centre phase II study was conducted to test this hypothesis (NCT02212639). The study was expected to be completed in 2019. However, the results are not published yet. In addition to these two trials that put the emphasis on anti-HIF-1α action, there are more than 20 different clinical trials that test digoxin alone or in combination with chemotherapy in several different cancers as a hypoxia modulator agent or p-glycoprotein inhibitor (e.g., NCT04141995, NCT02106845). A Phase II study in prostate cancer patients exhibited promising results suggesting that digoxin can prolong prostate specific antigen doubling time and may be effective in androgen-dependent prostate cancer [33] (NCT01162135). New clinical trials are recruiting patients or will start accrual to evaluate digoxin in solid tumors and pancreatic cancer (NCT03889795, NCT04141995).

2d. 2-Methoxyestradiol

2-methoxyestradiol (2ME2) is an estradiol metabolite which inhibits microtubule activity and acts as an antitumor agent. 2ME2 inhibits the translation of HIF-1α mRNA. Due to this action, 2ME2 was started to be evaluated as an agent to inhibit tumor blood flow in two different phase I trials in 2001 and 2002 (NCT00030095, NCT00028821). Since 2ME2 exhibit inhibitory action on cell proliferation, migration, and angiogenesis [17], a nanocrystal colloidal dispersion formulation of 2ME2, Panzem was developed which has higher bioavailability. Panzem entered several phase II trials, starting from 2006 (NCT00394810, NCT00306618, NCT00481455, and NCT00444314). However, the results were disappointing in terms of anti-tumor activity and tolerability [34,35]. Later a new analogue of 2ME2, ENMD-1198 was developed. ENMD-1198 exhibited potent anti-angiogenic activity in cell models [36]. However, to the

best of our knowledge, there is no registered clinical trial for ENMD-1198 in clinicaltrials.gov yet.

3. Drugs That Destabilize HIF-1α

3a. Geldanamycin and Its Derivatives

Geldanamycin and its derivatives are ansamycin antibiotics with anti-cancer activity. They inhibit heat shock protein 90 (HSP90), leading to degradation of its targets which regulate the activity of several intracellular pathways [37]. Geldanamycin and its derivative 17AAG (17-allylamino 17-demethoxygeldanamycin) or tanespimycin have been tested in clinical trials for the treatment of several cancers since the beginning of 2000's (e.g., NCT00003969, NCT00058253, NCT00093405, NCT00119236, NCT00004065, NCT00019708, NCT00093821, NCT00087386, and NCT00319930). Since HSP90 takes part in the stabilization of HIF-1α, this makes it an important target to induce destabilization and degradation of HIF-1α. With the emphasis on this mechanism, efficacy of 17AAG was evaluated in VHL disease and renal tumors in a phase II trial started in 2004 (NCT00088374). The study could not achieve accrual goals. However, the evidence that point out to the potential of geldanamycin and its derivatives in cancer therapy is growing[38,39].

3b. Histone Deacetylase Inhibitors

Histone deacetylases (HDAC) play important roles in epigenetic regulation of genes associated with cancer. HDAC inhibitors were approved for the treatment of several cancers such as lymphoma and multiple myeloma. They show antitumor effect by inducing cell cycle arrest, apoptosis, and anti-angiogenic response [40]. HDAC is also important for the acetylation of HIF-1α/p300 complex and therefore HDAC inhibitors lead to destabilization of HIF-1α [41]. Among the HDAC inhibitors romidepsin come forward with this action and there are more than 20 different active clinical trials that assess romidepsin in the treatment of lymphoma and several other cancers (e.g., NCT02512172, NCT04257448, NCT01947140, NCT01755975, and NCT01638533).

4. Inhibitors of HIF-1/DNA Binding

4a. Anthracyclines

Anthracyclines are among the most widely used and most potent chemotherapeutics in cancer therapy today. They show anti-tumor action by binding to DNA and inhibiting the progression of topoisomerase II enzyme [42]. They also inhibit binding of HIF-1 to DNA. Hence, they inhibit the expression of HIF-1α regulated genes [43,44]. Even though they are already in use in the treatment of several cancers, their anti-HIF action may

increase the use of anthracyclines further especially in chemotherapy protocols with anti-VEGF agents.

4b. Echinomycin

Echinomycin is a peptide antibiotic with DNA intercalating activity. With the aim of identifying new drugs which inhibit binding of HIF-1 to DNA, a high-throughput screening was conducted with National Cancer Institute's comprehensive small-molecule library. The screening identified echinomycin as a potent inhibitor of HIF-1/DNA binding [45]. Echinomycin has been tested in several Phase II trials for the indications of soft tissue sarcoma, endometrial carcinoma, non-small cell lung carcinoma and colorectal cancer [46-49]. However, the clinical efficacy in these trials were disappointing. Therefore, echinomycin could not progress into the clinic with the expected speed as an anti-HIF-1 agent. However, nano-liposomal formulations of echinomycin are being developed, which may offer an improved efficacy and pharmacokinetic profile for the agent [50,51].

5. Drugs That Inhibit HIF-1α at Multiple Levels

5a. Bortezomib

The ubiquitin proteasome pathway plays a central role in protein homeostasis, leading to degradation of misfolded or damaged proteins. Proteasome is an attractive target in cancer therapy since inhibition of proteosome induces cell death via endoplasmic reticulum stress, inhibition of survival pathways and stimulation of apoptosis [52]. Bortezomib is one of the most prominent proteasome inhibitors evaluated for clinical use in cancer. It was approved by FDA in the treatment of multiple myeloma in 2003 [53], and there are more than 900 different clinical trials registered that evaluate bortezomib, mostly in combination to conventional chemotherapeutics in a wide spectrum of cancers. Since proteasomal degradation of HIF-1α has substantial importance in regulation of HIF-1, bortezomib also has an anti-HIF-1 action. In the presence of bortezomib, ubiquitinated HIF-1α could not be degraded and accumulates in an inactive form. In this form HIF-1α cannot bind to p300 and induce gene expression[54]. Additionally, it was shown that bortezomib can inhibit PI3K/AKT/mTOR pathway. Moreover, bortezomib was reported to suppress nuclear translocation of HIF-1α via inhibition of MAPK pathway[55]. With these mechanisms bortezomib exhibits anti-HIF-1 action at multiple levels which makes it an attractive agent in cancer therapy.

5b. PX-478

PX-478 is a melphalan derivative that downregulates the HIF-1α mRNA and inhibits its translation. It was also reported to inhibit de-ubiquitination of HIF-1α to a smaller extent [56]. Due to inhibition of HIF-1α, PX-478 may have a potential in cancer treatment. The safety and

efficacy of PX-478 was evaluated in advanced solid tumors in a phase I trial conducted in 2007-2010 (NCT00522652). The results were promising in terms of tolerability and HIF-1α inhibition[57]. However, a registration could not be found in clinicaltrial.gov that suggest the further evaluation of PX-478 in clinical studies.

CONCLUSION

Clinical development of HIF-1α inhibitors for cancer treatment is ongoing at a great pace. Till the safety and efficacy of direct HIF-1α inhibitors is fully established, anti-cancer agents already approved in cancer such as camptothecins, rapalogs, anthracyclines and bortezomib seem to be involved more frequently in combination chemotherapy for HIF-1α inhibitory effect in addition to their primary mechanisms of anti-cancer action.

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CHAPTER IV

COAGULATION ANALYSES IN DIAGNOSIS AND TREATMENT

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INTRODUCTION

In living macroorganisms, life-long expected and unexpected bleeding and coagulation mechanisms are activated at regular intervals. Excessive coagulation can lead to life-threatening damage resulting from a blockage in the vessels, and uncontrolled bleeding can cause serious complications, especially death. To prevent bleeding, blood clotting (clotting) occurs a few seconds after the vessel injury. The primary purpose of coagulation is the vascular repair that occurs by collecting thrombocytes at the damage site. Coagulation is known as the clotting of blood or plasma. Hemostasis is the procedure that tries to stop bleeding and is the first component of the metabolism's response to vascular injury. As a result of hemostasis, a product called a hemostatic plug or hemostatic clot is formed. Thrombosis is an unexpected clot formation in a healthy vascular structure. Thrombus formation occurs as a result of thrombosis. Blood clotting can occur at an injury site (hemostasis), in a healthy vessel (thrombosis), or on a foreign surface such as a laboratory test tube. Still, hemostasis is a physiological process that can only occur in a living, bleeding organism. Hemostasis consists of primary hemostasis, in which platelets are attached and activated to a site of damage, and a fibrin network rigidly structures secondary hemostasis, in which the primary platelet plug formed. The hemostatic process represents a delicate, well-balanced chain of steps between regulatory mechanisms and control that prevent the efficient activation of local hemostatic agents and improper activation or prolongation of coagulation steps due to vascular damage. The interactions of the protein ingredients of coagulation cascade can be studied in cell-free plasma and characterized as a "cascade" of proteolytic reactions. Conversely, hemostasis's operation takes place on cell surfaces in a living organism and is dependent on regulation by various biochemical and cellular mechanisms. The competence of plasma procoagulant levels can be defined in the routine plasma clotting assays: prothrombin time (PT) and activated partial thromboplastin time (aPTT). Platelet count and

function can be determined in the clinical laboratory. Levels of individual plasma coagulation inhibitors and other regulatory proteins can also be evaluated. However, no laboratory test can provide a general evaluation of hemostasis's competency or thrombosis risk. Each laboratory test offers a small fraction of the information required, and the assessment of hemostatic function always requires a comparison of laboratory results with patients' clinical condition.

HEMOSTASIS

The hemostatic system consists of the plasma coagulation system, including blood vessels, platelets, fibrinolytic factors, and inhibitory mechanisms. When vessel damage occurs, three local agencies are activated to control bleeding: (1) vessel wall contraction, (2) platelet adhesion and aggregation (platelet plug formation), and (3) clot to form fibrin. All these mechanisms need to work, and defects in one or more of them result in abnormal bleeding. Primary hemostasis is used for rapid plug formation upon damage to the vessel wall, followed by vasoconstriction, platelet adhesion, and aggregation. This stage is for a sudden and quick solution, and fibrin formation is not required. However, primary hemostasis is transient, and bleeding could begin again, except secondary hemostasis strengthens the platelet plug by forming a stable fibrin clot. Eventually, the fibrinolytic system's mechanisms ensure the fibrin clot's dissolution and the restoration of normal blood flow.

Platelets cannot adhere to the healthy, smooth endothelial surface, and coagulation is not initiated. Endothelial thromboresistance is generated by various antiplatelet and anticoagulant materials produced by the endothelial cells like; prostacyclin (prostaglandin I2, PgI2), nitrite oxide (NO), thrombomodulin, heparin-like glycosaminoglycans, tissue factor pathway inhibitor (TFPI), tissue plasminogen activator (t-PA). Endothelial cells also show procoagulant activity by synthesizing and secreting von Willebrand factor (VWF) and PAI-1. It is crucial that blood not clot inside the vascular system. In the baseline state, vascular endothelial cells maintain a nonthrombogenic interface with the circulating blood. Endothelial cells do not ordinarily reveal molecules that promote platelet adhesion or aid activation and activity of the coagulation proteins. After vascular damage, procoagulant factors and tissue factor (TF) are activated, and thrombocyte adhesion, aggregation, and local thrombin formation are achieved.

Platelets are nucleated cells that are released by the breakdown of megakaryocytes in the bone marrow. They can be found in circulation for up to nine days. The approximate number of platelets in peripheral blood is between 150,000 to 400,000 per microliter. On the outer surface of platelets are various molecules such as integrins and leucine-rich

glycoproteins. They mediate platelet adhesion and aggregation as receptors for adenosine diphosphate (ADP), arachidonic acid, and other agonists. Platelet connecting is further intervened by von Willebrand factor (vWF) bridging between collagen and the platelet receptor glycoprotein (GP) Ib. Thrombin stimulation produced as a result of coagulation reactions is required for complete platelet activation. The platelet surface receptor, GPIIb / IIIa, rapidly changes platelet formation by stimulating it with fibrinogen. This change in conformation allows platelet aggregates to be consolidated by connecting to fibrinogen ahead of fibrin conformation. Platelet activation also launches prostaglandins and thromboxanes' synthesis, which regulates platelet activation and assists vasoconstriction. Activation of platelets motivates several changes resulting in hemostasis increase via two main mechanisms:

1. Production of the hemostatic plug at the site of injury (primary hemostasis).

2. Providing of phospholipids as a procoagulant surface for plasmatic coagulation.

Early platelet plug formation can be divided into separate stages that are tightly related: platelet adhesion, structural changes, content release, and platelet aggregation. Immediately following endothelial damage, platelets bind to adhesive proteins such as collagen via specific glycoprotein surface receptors (platelet adhesion). In this process, VWF acts as a mediator that first adheres to collagen fibers and then realizes fiber strength. The platelet then binds to VWF via membrane glycoproteins (GP) Ib and IX. After adhesion, the platelets change from disc shape to spherical shape and elongate pseudopods. It accelerates plug formation by releasing ADP, serotonin, Thromboxane A2 (TxA2), beta-TG, PF4, and VWF stored in thrombocyte granules also initiates platelet aggregation. At the end of the aggregation process, tightly packed degranulated platelets form the hemostatic plug. Fibrinogen, which binds to specific glycoprotein receptors (GP IIb / IIIa), is required for platelet aggregation. Thromboxane A2 (TXA2) secreted from granules enables vasoconstriction starting with endothelin to continue. ADP stimulates platelet aggregation, while calcium activates the coagulation system starting with factor XII.

Two congenital bleeding disorders, von Willebrand disease and Bernard-Soulier syndrome, are characterized by defective adhesion that causes a lifelong tendency to bleed due to VWF and GP Ib / IX deficiency. Besides, in Glanzmann thrombasthenia, a disease characterized by the absence of GP IIb / IIIa receptors, bleeding time is significantly prolonged, platelet aggregation cannot occur, resulting in a lifelong bleeding tendency.

The transformation of inactive proenzymes activates coagulation factors into activated enzymes. Enzyme, substrate, and cofactor are

involved in all coagulation steps. These components accumulating on the phospholipid complex require calcium ions for activation, limiting the affected area's reactions. All of the coagulation factors, except factor IV (Ca + 2), are proteins, and most of them are found in the blood as inactive proenzymes (zymogen). Factors V and VIII, on the other hand, are not in the enzyme structure and are synthesized by megakaryocytes in the bone marrow. It is stored in platelet granules and released after activation. Vitamin K is required for factor II, VII, IX, X, protein C and S synthesis and function. The only coagulation factor not circulating is tissue factor (Factor III) located in the subendothelial area. The coagulation system is a chain of sequential reactions in which coagulation factors activate each other. It is known to be initiated by two classical ways, extrinsic and intrinsic. The intrinsic pathway begins with Hageman factor (factor XII) activation, and the extrinsic pathway begins with the release of tissue factor. Both paths provide stable clot formation as a result of burst reactions that activate factor X. In the coagulation cascade, many factors intertwine, trigger, and inhibit each other's work under a single system simultaneously. The interconnected structure of the system at many points also allows better control of bleeding and clotting conditions. The coagulation system is triggered by the tissue factor released due to endothelial damage or inflammation either by factor XII activated by collagen. TF then interacts with factor VIIa, activating factors IX and X. Factor Xa also produces a small amount of thrombin from prothrombin. Propagation of coagulation consists of when thrombin repeatedly activates factors XI, IX, V, and VIII. Thrombin also stimulates the aggregation and activation of platelets. Coagulation factors, which are frequently activated and multiplied in the system each time, generate too much thrombin from prothrombin. Finally, thrombin transforms fibrinogen into fibrin. Also, thrombin activates factor XIII and forms the desired stable clot with a fibrin fiber network.

The main initiating pathway of in vivo blood coagulation is the extrinsic system. The crucial component is TF, an intrinsic membrane component expressed by cells in most extravascular tissues. The terminology "extrinsic" continues to be used today in terms of education, although quite outdated. TF is not frequently extrinsic to the circulatory system, but it is also expressed by endothelial cells and leukocytes under definite pathological conditions. TF serves as a cofactor to the significant factor VII in the extrinsic pathway. TF and factor VII complex provide factor VIIa activation, whereby factor X is converted into factor Xa, the main compartment in the common path. The factor VIIa-TF complex also converts factor IX to factor IXa. Also, as levels of factor Xa increase, the factor VIIa-TF complex is subject to inhibition by the factor Xa-dependent tissue factor pathway inhibitor (TFPI). The intrinsic pathway earlier begins with the contact phase. This phase is achieved by factor XII (contact

factor), prekallikrein, and high-molecular-weight (HMW) kininogen. In vitro contact phase is launched by binding factor XII to negative surfaces of glass and kaolin. This leads to the generation of cofactors XIIa and kallikrein and the release of bradykinin from HMW kininogen. Factor XIIa then activates factor XI, which converts factor IX to factor IXa, a reaction requiring calcium presence. After factor IXa complexes with its cofactor protein factor VIIIa on a negatively charged membrane surface. This enzymatic complex, also referred to as the tenase complex, converts factor X to factor Xa. After both the extrinsic and intrinsic pathways generate factor Xa, the coagulation pathway's ensuing reactions are the same and are referred to as the common pathway. Based on the discovery that the factor VIIa-TF complex also converts factor IX to factor IXa, that is the privileged reaction, a new modified concept of coagulation was proposed in which factor VIIa-TF complex seems to be the core initiator of coagulation, but the intrinsic pathway is aforethought essential to pursue the coagulation response.

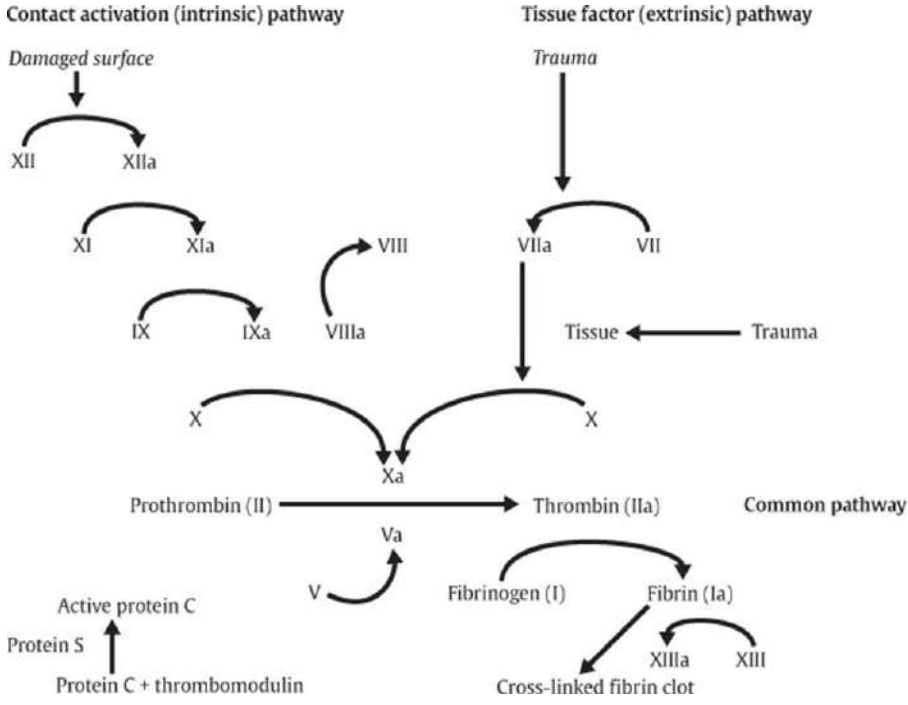

Figure 1. Coagulation pathway scheme

ENDOGENOUS ANTICOAGULANTS AND FİBRİNOLYSIS

Anticoagulant substances consist of antithrombin III (AT-III), protein C, protein S, and TFPI. Intact endothelium ensures that the formation of clots is stopped, and if endothelial repair is completed, the

fibrin is destroyed by fibrinolysis. When clot formation reaches the intact endothelium, it activates the heparin-like molecule AT-III, which runs to the endothelial surface. Antithrombin III is a potent anticoagulant substance produced in the liver and commonly found in body fluids and is a significant inhibitor of serine proteases formed during coagulation. Antithrombin III inactivates all active factors; Xa, XIa, XIIa, IXa, thrombin, and TF-VIIa complex. On the other hand, thrombin binds to thrombomodulin, which is a specific thrombin (IIa) receptor on the endothelial surface and activates protein C and protein S. Activated protein C neutralizes cofactors such as factor Va and VIIIa. Thus, the coagulation system is stopped by inactivating all active and auxiliary factors. Plasminogen is an inactive circulating protein that binds to fibrin during clot formation. Plasminogen is activated by endothelium produced tissue plasminogen activator (t-PA), urokinase-type plasminogen activator (uPA), and contact factors such as XIa, XIIa, quinine, and kallikrein and converted to plasmin. tPA is the most effective in activating fibrin-bound plasminogen. Besides, it prevents fibrinolysis from moving beyond the clot formation site. Fibrinogen is a colossal plasma protein with 340 kDa MW. It is produced from the liver, and its concentration range is 200 to 400 mg/dL. Fibrinogen is stable in plasma for about 4 to 5 days. It is a dimeric protein of highly low solubility designed with two pairs of three nonidentical polypeptide chains covalently connected by disulfide bonds. Fibrinolysis event is the breakdown of fibrin strands that hold plasmin, formed by plasminogen, and fibrin. Fibrin is broken down into fibrin breakdown products by fibrinolysis. The transformation of fibrinogen to fibrin occurs in three stages. First, thrombin splits four small peptides—the fibrinopeptides A (FPA) and fibrinopeptides B (FPB)—from the fibrinogen molecule, following the formation of a new molecule called fibrin monomer. Secondly, polymerization comprises unconsciously by noncovalent end-to-end and side-to-side relations to develop fibrin polymers. In the third step, a resistant and consistent fibrin molecule is acquired by the action of factor XIII (fibrin-stabilizing factor) and calcium ions. Fibrin degradation products (FDPs) begin to form while plasmin diminishes the thrombus. Multiple FDPs, involving fibrinopeptide B and other fibrin degradation monomers and dimers, consist. When fibrin polymers are splitted from the D fragment site, the following D-dimer fragment pictures thrombosis diffusiveness and plasmin efficiency. D-dimer assays anticipate and have prognostic value in several disease extents, bearing disseminated intravascular coagulation (DIC), pulmonary embolism, deep vein thrombosis, and cancer-associated thrombosis.

COAGULATION TESTS

Numerous tests assess the risk of bleeding to guide clinical diagnosis and treatment approaches. Commonly used coagulation tests contain the following:

-CBC

-Bleeding time

-Platelet function tests

-Routine hemostasis tests as prothrombin time (PT), partial thromboplastin time (PTT), thrombin

time (TT), fibrinogen level, and D-dimer

- Antithrombin III levels

- Private analysis suchlike anti-Xa assay, diluted thrombin time, and tests for heparin-induced thrombocytopenia

- Thromboelastography (TEG)

-Hypercoagulability and fibrinolysis tests

Complete blood count (CBC) is one of the most common laboratory tests requested by clinicians, and this test is also required before surgery to evaluate a patient for bleeding risk. Platelet count and mean platelet volume are essential parameters in predicting bleeding complications, especially in surgical interventions. Performing CBC is for diagnosing thrombocytopenia when the platelet count is less than 150,000/ μL. Unexpected thrombocytopenia should be confirmed by peripheral blood smear. The probability of red blood cell fragments or pseudo thrombocytopenia may provide hints for further assessing the patient.

Bleeding time is defined as the time between applying a small standard incision and bleeding stops. Although it is a simple test, many factors affect bleeding time, making it difficult to standardize. Bleeding time measures platelets' interaction with the vessel wall, followed by forming the first hemostatic plug. Bleeding time will be prolonged in cases of decreased platelet count, dysfunction, or vascular wall pathology. Besides, when there is a decrease in VWF or fibrinogen, the bleeding time may be prolonged. The depth, width, and position of the skin incision may differ for each application, so its diagnostic utility as an individual test is of limited value.

Analyzers available for platelet function tests include PFA-100, Plateletworks, and VerifyNow. Also, a platelet aggregation test can be performed. These tests are used to determine platelet function and to decide whether a platelet transfusion is required. The PFA-100 system is a platelet

function analyzer that can be used to appraise platelet-related primary hemostasis. If the collagen-epinephrine (CEPI) closure time is extended but the collagen-adenosine diphosphate (CADP) closure time is standard, this is presumably due to aspirin. If both CEPI and CADP closure times are prolonged or CEPI closure time is typical and CADP closure time is abnormal, this means platelet dysfunction or von Willebrand disease. PFA-100 is senseless to von Willebrand disease type 2N, clopidogrel, ticlopidine, and storage pool disease. PFA-100 results are biased by thrombocytopenia and low hematocrit. VerifyNow is a quick, turbidimetric whole blood assay able to evaluate platelet aggregation. This assay is based on the competence of activated platelets to bind with fibrinogen. If GpIIb/IIIa (glycoprotein IIB/IIIa) competitors are present in patients' plasma, platelet aggregation lessens. Results are declared in reactive aspirin units (ARU) or clopidogrel, plavix reactive units (PRU). Plateletworks evaluates platelet function in whole blood. It compares the platelet count before and after exposure with a selected platelet agonist. In agonist exposure, functional platelets should be collected, and nonfunctional platelets should not aggregate. A hematology analyzer such as ICHOR II then measures the number of non-clumped platelets in the EDTA tube and agonist tube. With the difference, platelet functions are evaluated. The Plateletworks is useful in monitoring the platelet response in patients using antiplatelet, such as aspirin and clopidogrel. Platelet aggregometry is a widely used laboratory test to screen patients with genetic or obtained platelet function defects. Platelet aggregometry evaluates light transmission rising through platelet-rich plasma when platelets are aggregated due to an agonist's addition. Diversified agonists used for this test are; AA, collagen, ristocetin, ADP, and epinephrine. The first aggregation wave is through the activation of the GpIIb/IIIa receptor. The secondary wave is due to platelet granule release. Secondary wave deficit means a storage pool disorder by a reduced number of granules or faulty release of granule substances. The use of these agonists in advanced concentrations results in the two waves joining into one aggregation wave.

The prothrombin time (PT) is performed by adding a raw preparation of TF (usually an extract of the brain) to citrate- anticoagulated plasma, recalcification of the plasma, and assessing the clotting time. Thromboplastin and CaCl2 are generally added in a single step. Clotting time is established at 37°C using a diversity of methods, including photo-optical and electromechanical detection. The prothrombin time may be elongated because of the extrinsic coagulation pathway's deficiency, i.e., factors II, V, VII, X, and/or fibrinogen. PT is a practical adjustment of the extrinsic pathway and common pathway, and the reference range is 8.8s - 11.6 s. A circulating anticoagulant directed against these factors may also provoke the PT's prolongation. However, frequently PT is reported as the international normalized ratio (INR). The thromboplastin used may alter

from laboratory to laboratory and from country to country. However, reporting results as INR ensures results are comparable among different laboratories. The INR is calculated as dividing the patient PT by the mean normal pt and taking the result's power as the ISI (International Sensitivity Index) value. The INR has two main advantages: it lets a comparison between results acquired from several laboratories, and it allows investigators to standardize anticoagulant therapy in clinical trials and scientific publications. The normal INR range is 0.8-1.2. Reasons for prolonged PT include coumarin (warfarin), vitamin K deficiency, factor deficiency (inherited or acquired, e.g., liver disease), or factor inhibitors.

Activated partial prothrombin time (aPTT) is another practical test for the assessment of secondary homeostasis. Citrated plasma (or oxalate), surface activating agent (silica, kaolin, celite, or ellagic acid), calcium, and platelet substitute (rough phospholipid) are mixed and clotting time is determined most likely using an automated coagulation analyzer. It is a useful measure of the intrinsic pathway as well as the common pathway. It can evaluate hereditary or acquired faults of the coagulation factors XII, XI, X, IX, VIII, V, prothrombin, and fibrinogen. PTT or aPTT is named partial thromboplastin time because of the lack of tissue factor (thromboplastin) in the tests. The expected value differs from laboratory to laboratory but is generally within 25 and 39 s. Causes of extended PTT include the following;

-Heparin and other direct thrombin inhibitors (DTIs)

-Factor deficiency, factor inhibitors, or lupus anticoagulant (LA)

-von Willebrand disease

-HMWK (Fitzgerald factor) deficiency

-Pre-kallikrein (Fletcher factor) deficiency

Falsely measured prolonged PT and aPTT reasons contain high hematocrit, underfilling of the citrate tube, and EDTA contamination. Delay in transport and processing may also cause false results.

Interpretation of PT and PTT results

PT result	PTT result	Clinical Evaluation
Normal	Prolonged	Factor deficiency of the intrinsic pathway, above the common pathway (eg, factor VIII, factor IX deficiency) Heparin therapy, DTI therapy von Willebrand disease Inhibitors (eg, lupus anticoagulant, factor VIII or IX inhibitor)
Prolonged	Normal	Liver disease, vitamin K deficiency, warfarin therapy
Prolonged	Prolonged	Factor deficiency of the common pathway Heparin therapy (high dose), DTI therapy Warfarin therapy (high dose) Lupus anticoagulant (strong antibody) DIC

Figure 2. Clinical evaluation of PT and PTT results

Thrombin time (TT) is a test that the patient's plasma is mixed with thrombin, and clotting time is measured. It is an assessment of functional fibrinogen. Heparin and abnormal or low fibrinogen molecules prolonges TT. The most performed fibrinogen assay is a modified thrombin test known as the Clauss fibrinogen assay. This method contains an initial (10-fold) dilution of the plasma sample to guarantee that fibrinogen is rate-limiting for clotting and the following measurement of the clotting time by supplementing an excess of thrombin to the sample. The length of the clotting time is contrarily related to the concentration of fibrinogen. D-Dimer forms via fibrin degradation and contains two cross-linked D fragments of the fibrin protein. Elevated levels suggest increased fibrinolysis in DIC and thrombotic states. Antithrombin disables factor Xa (activated factor X) and activated factor II (thrombin). Therefore, activity is induced by heparin. Low ATIII levels may need additional heparin to obtain the desired anticoagulated state. This is called "heparin resistance." Adipose tissue may release heparin storage back into the circulation a few hours after surgery. This is mentioned as rebound heparin and could result in bleeding. Both heparin resistance and rebound heparin can be prevented if low ATIII levels are corrected before surgery. Low levels of ATIII levels may be seen in; inherited deficiency, liver disease, nephrotic syndrome, DIC, and L-Asparaginase therapy.

Coagulation tests may include different approaches other than routine analysis. PT and aPTT prolongation in individuals may be due to factor deficiency or factor inhibitors. At this point, mixing studies come to

the fore. In this test, the patient's plasma and normal plasma are mixed in equal volume, and PT, PTT, or both are evaluated at 0 and 1-2 hours. Prolonged PT / PTT is not shortening, despite factor addition indicates the presence of inhibitors. 0 and 1-2 hour PTT results mean lupus anticoagulant in the same cases. Results that extend over time reveal the presence of the antibody (typically factor VIII inhibitor). If the PT and PTT are both prolonged and the mixing study shows correction, the only inference is common pathway problems. Plasma anti-Xa testing can be used to monitor patients receiving unfractionated heparin (UFH) or low molecular weight heparin (LMWH) therapy. LMWH primarily has anti-Xa activity, and UFH shows both anti-Xa and anti-IIa activity. Coagulation-based or chromogenic methods can measure the anti-Xa activity of AT. Additionally, a patient who is bleeding but shows typical values of PT/PTT may be due to; thrombocytopenia and thrombocytopathia, factor XIII deficiency (clot is not soluble in urea), and increased primary fibrinolysis (e.g., lack of α2-antiplasmin). The Factor XIII screening test is based on mono chloric acid's ability to dissolve the fibrin clot in factor XIII deficiency. Quantitative tests depend on factor XIII transamidase properties. Although prolonged bleeding time is determinant for von Willebrand disease, it is not specific for the disease. At this point, measurements of the von Willebrand antigen amount, the functional capacity of VWF, and the function of the associated factor VIII molecule are valuable. VWF protein is measured by the ELISA method. A decrease in VWF levels is found in most patients. The average VWF antigenic level in people with blood type 0 is about 25% lower than in other blood groups. The functionality of VWF is evaluated by calculating the cofactor activity of ristocetin (standardized normal platelet suspension) in the patient plasma. In this assay, the agglutination capacity of VWF in ristocetin-added plasma is evaluated in terms of the rate or extent of platelet agglutination.

Disruption of coagulation mechanisms may cause bleeding, and impairment of control mechanisms may cause thrombosis. Control modulators include AT, protein C and immunological and functional methods can measure protein S and AT. Functional tests involving chromogenic substrates detect AT as a heparin cofactor. For protein C, tests are available evaluating the prolongation of aPTT in terms of inactivating activated protein C factors V and VIII. The determination of protein S is involved in its combination with a free protein S and C4b. Free protein S acts as the cofactor of active protein C. Protein S assay can be evaluated using protein S deficient plasma and activated protein C. In the past, various fibrin/fibrinogen degradation products were determined by different methods. Currently, only D-dimer is measured as a diagnostic parameter for DIC and other thrombotic events. Monoclonal antibodies that can detect D-dimer in plasma were synthesized. The antibody did not

react with fibrinogen, resulting in high sensitivity and specificity of the test. ELISA can measure prothrombin fragments 1 and 2 (F1 + 2), thrombin-antithrombin complex (TAT), beta-thromboglobulin, and PF 4 to assess hypercoagulability in research laboratories. However, these assays are generally not accepted for evaluating a bleeding patient or a patient with thrombotic disorders. Thromboelastography (TEG) is a method used to measure the effectiveness of blood coagulation. Although it is used more and more in emergency departments, intensive care units, and resuscitation in delivery rooms, it mainly comes to the fore in surgery and anesthesiology. TEG can also evaluate platelet function, clot strength, and fibrinolysis in addition to known coagulation tests. Thromboelastometry (TEM), previously known as rotational thromboelastography (ROTEG) or rotational thromboelastometry (ROTEM), is a different TEG modeling in which the rotating TEG is the sensor shaft instead of the container.

COAGULATION DISORDERS

Laboratory tests are essential for diagnosing coagulation disorders. However, there are no standardized panels required for accurate diagnosis, and measurements can differ significantly between laboratories. Routine laboratory tests for coagulation disorders include platelet count, prothrombin time (PT), partial thromboplastin time (aPTT), international normalized ratio (INR), and bleeding time. Platelets should have not only sufficient quantity but also be functional. Bleeding time evaluates the function of platelets. Bleeding times may increase in VWF deficiency or antithrombotic substance use (NSAID, aspirin, and valproic acid). PT shows the function of factors II, V, VII, and X, which are vitamin K-dependent factors synthesized from the liver. Drugs that reduce the synthesis of vitamin K, such as warfarin, will cause an increase in PT and INR values. INR is a ratio used to predict the percentage of active coagulation factors. For example, an INR of 2 to 3 means about 10% clotting factor activity. This activity should be at least 30% for standard coagulation. Partial thromboplastin time evaluates the von Willebrand factor's activity and factors VIII, IX, XI, and XII. Hemophilia patients have regular PT / INR, bleeding time, and thrombocyte count despite high aPTT. Low FVIII levels will cause prolonged aPTT values through the intrinsic pathway. An elevated aPTT requires investigation of factors VIII and IX. When evaluating the established factor levels, the amount of factors present will determine the disease's severity and prognosis. When prolonged aPTT values raise clinical suspicion, the focus should be on the mother's entire family history, especially the first-degree male relatives. Diagnosis of von Willebrand disease is made with increased bleeding time and decreased vWF levels in ristocetin cofactor analysis, in addition to bleeding history and family history. Since vWF levels are affected by intra-

individual variation, stress, pregnancy, exercise, and inflammatory processes, the test should be repeated three times before making a diagnosis. It can affect gender and blood type levels. Factor multimer tests may be required to diagnose the specific subtype of the disease. Also, aPTT can be slightly elevated at second to low factor VIII levels in about 50% of patients with von Willebrand disease.

Disseminated intravascular coagulation (DIC) is a clinicobiological syndrome defined by excessive clotting activation leading to the accumulation of fibrin into the vessel, organ disorders, excessive consumption of coagulation factors and platelets, and bleeding that can be fatal. DIC can be triggered by different clinical conditions (sepsis, cancer, trauma, and pregnancy complicated by eclampsia or other diseases). Elimination of the activation factor and the destruction of the trigger mechanism are the main therapeutic approaches. DIC-specific treatment strategies aim to limit the activation of blood clotting and the risk of bleeding. Fibrin formation is accompanied by plasminogen activator inhibitor 1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), and fibrinolysis activation due to comorbid diseases and clinical conditions. If fibrinolysis does not occur at the required level, fibrin accumulation will cause diffuse blockages in the microvascular system. Microvascular thrombosis, together with hemodynamic and metabolic disorders, can lead to multiple organ failure. It may have clinical consequences in organ failure, renal failure, respiratory failure, hypotension, circulatory failure, or impaired brain function. Besides, intravascular coagulation activation may contribute to macrovascular thrombus formation, leading to venous or arterial thrombus and embolization. Diagnostic criteria include:

-Presence of additional disease that may predispose to DIC,

-Clinical signs (bleeding or organ dysfunction)

-Laboratory tests (platelet count, PT, fibrinogen, and fibrin/fibrinogen degradation products [FDPs])

Rapidly evolving DIC results in severe thrombocytopenia, prolonged PT and PTT, rapidly decreasing plasma fibrinogen levels, and elevated plasma D-dimer levels.

Preanalytical requirements for routine coagulation measurements

Blue-capped tubes with a citrate concentration of 105-109 mmol / L (3.2%) are recommended for coagulation tests. Laboratory professionals should keep in mind that there may be a reference range difference depending on the citrate concentration in the same method and correct reference ranges. In ensuring the correct blood/anticoagulant ratio, care should be taken to ensure that the blood amount corresponds to the tube

filling line specified by the tube manufacturer. It should be noted that the tube filling line is determined to provide the correct blood citrate ratio (9: 1), not the volume of the citrated tube. Blood should be taken into the citrate tube first in patients who are requested coagulation tests together with other laboratory tests. Unless the manufacturer specifies a different procedure, the tubes containing anticoagulants should be mixed by inverting 3-6 times after blood is taken. Blood should be taken into the citrate tube first in patients who are requested coagulation tests together with other laboratory tests. Unless the manufacturer specifies a different procedure, the tubes containing anticoagulants should be mixed by inverting 3-6 times after blood is taken. Coagulation tests at high hematocrit values (> 55%) should be run from plasma with an adjusted citrate concentration. All coagulation measurements should be performed within the first 4 hours after the blood sample is collected. PT and D-dimer tests can be run within 24 hours. However, laboratories must evaluate the sample stability for their system. If the aPTT test is requested for standard heparin monitoring, the whole blood sample should be centrifuged within 1 hour after the sample is taken, and the plasma should be separated. To evaluate protein-S, protein-C, and activated protein C resistance, it is recommended that these treatments be discontinued two months before women receiving combined oral contraceptives and hormone replacement therapy. When sampling is required for inherited diseases such as vWF, FVIII deficiency, protein S deficiency, samples should be taken when regular menstrual cycles begin or at least two months after birth. All abnormal values, including pregnancy-associated antiphospholipid antibodies, should be confirmed by re-drawing.

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CHAPTER V

PREVALENCE OF PALMARIS LONGUS AGENESIS (PLA) AMONG TURKISH MEDICAL STUDENTS

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INTRODUCTION

The palmaris longus (Figure 1) (1) is a small, fusiform-shaped muscle located on the anterior forearm of the human upper extremity. This muscle belongs to the superficial forearm flexor group with a most common proximal attachment at the medial epicondyle of the humerus via the common forearm flexor tendon and a most common distal attachment into the connective tissue fibers of the palmar aponeurosis and the flexor retinaculum, a ligamentous structure forming the roof of the carpal tunnel and containing the median nerve and digital flexor tendons (2, 3). The palmaris longus can be morphologically quite variable but most commonly has a tendinous proximal attachment, a mid-length, spindle-shaped muscle belly, and a long and thin tendinous distal portion. The majority of fibers in the palmaris longus tendon pass superficially to the flexor retinaculum, the tendon broadens into a flattened collection of fibers, and the fibers interweave with the palmar aponeurosis (2, 3). The functional contribution of the palmaris longus appears to be minimal, but it has clinical significance due to its frequent intraoperative harvest for many surgical procedures, often involving tendon repair in the upper extremity. The thin distal tendinous portion, superficial location, and lack of functional significance make the muscle easily accessible and ideal for intraoperative harvest for tendon reconstruction and other surgical procedures. The palmaris longus muscle is commonly present but may be absent in a small percentage of the population, ranging from 2.5% to 26% of individuals, depending on the studied population (2, 3, 4). The palmaris longus tendon is located near the anatomical center of the anterior wrist, medial to the tendon of flexor carpi radialis, and lateral to the tendon of flexor carpi ulnaris (3). The presence of the palmaris longus muscle can be determined through noninvasive and standard physical examination of the volar wrist (5, 6).

MATERIALS AND METHODS

Subjects

This is a cross sectional study that involved 712 (429 male, 283 female) healthy subjects studying at Atatürk and Erzincan Binali Yıldırım University Faculty of Medicine. We excluded subjects with physically disabilities, any prior surgery (to upper or lower limbs) and any upper limb injuries. Subjects with a history of injury/disability (including those resulting from trauma, be it spine, lower or upper limb), disease or abnormality of the upper limb which would preclude examination for the presence of the PL tendon.

Tests

In our current study, the Scheaffer's test (7, 8, 9, 10, 11, 12), the Thompson's test (5, 8, 11, 13) and the Mishra's test (7, 8, 9, 11, 13), which are the classical tests frequently used in the studies, were applied to test the presence of Palmaris longus:

- ***The Schaffer's test:*** This is the Standard test for the assessment of the palmaris longus tendon. It involves opposing the thumb to the little finger and then followed by the flexion of the wrist. Each of the subjects was asked to oppose the thumb to the little finger and then flexes the wrist slightly (8,11) (Fig. 2).

- ***Thompson's test:*** The test involves the flexion of the fingers to form a fist followed by the flexion of the wrist and finally, the thumb is opposed and flexed over the fingers. The subjects were asked to make a fist and then flex the wrist and finally, the thumb is opposed and flexed over the fingers (8,11) (Fig. 3).

- ***Mishra's test:*** The test involves the abduction of the thumb against the resistance with the wrist in slightly palmar flexion. The subjects were asked to abduct the thumb against resistance with the wrist in slight palmar flexion (8,11) (Fig. 4).

Statistical analysis

IBM SPSS 22.0 package program was used for statistical analysis of the data (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Mean ± standard deviation and (minimum-maximum) value were used when summarizing age, which is a continuous variable, and number (n) and percentage (%) for categorical variables. The Chi-square test was used in the analysis of categorical variables. Phi coefficient was used to evaluate the PLA relationship between the right and left sides. For Phi coefficient <0.20 no relationship, 0.20-0.29 was considered weak, 0.30-0.49 moderate, 0.50-0.69 strong,

0.70-1.0 very strong (14). Statistical significance was accepted as p <0.05 in all tests.

RESULTS

Study was carried out with 712 medical students. There were 283 (39.7%) female and 429 (60.3%) male students. The mean age was19.5±1.4 and ranged from 18 to 26 years.

For all students PLA prevalence was found 7.6% (n=54). The prevalence of PLA for females was 6.4% (n=18 out of 283) and for males 8.4% (n=36 out of 429). Prevalence was not significantly different between males and females (p=0.316).

Out of 54 students who had PLA was examined for sides according to gender. The percentage of unilateral PLA was 50.0% (n=9 out of 18) for female sand 61.1% (n=22 out of 36) in males. Percentage of unilaterality was not statistically significant for gender (p=0.436). The distribution of agenesis according to gender was shown in (Table 1).

When the sides were examined for PLA, both for males andf emales, right and left limbs have strong positive correlation of PL agenesis (φ=0.576, p<0.001). For females correlation between agenesis and sides was (φ=0.576, p<0.001) and (φ=0.650, p<0.001) for males.

Agenesis	Gender		Total	p
	Male	Female		
No	393 (91.6%)	265 (93.6%)	658 (92.4%)	0.316
Yes	36 (8.4%)	18 (6.4%)	54(7.6%)	
Unilateral	22 (61.1%)	9 (50.0%)	31 (57.4%)	0.436
Bilateral	14 (38.9%)	9 (50.0%)	23(42.6%)	
Total	429 (60.3%)	283(39.7%)	712(100%)	

Table 1. PLA distribution according to gender

DISCUSSION

Palmaris longus (PL) is one of the forearm muscles that lie between the flexor carpi ulnaris and the flexor carpi radialis muscles. The absence of the PL has been shown to vary based on body side, gender, and ethnicity in prior studies (7, 11, 15).

Roohi et al. studied in 450 healthy subjects (equally distributed among Malaysia's 3 major ethnic groups) for the presence or absence of palmaris longus. Palmaris longus was found to be absent unilaterally in 6.4% of study subjects, and bilaterally in 2.9% of study participants. Malays have a high prevalence of palmaris longus absence at 11.3% followed closely by Indians at 10.7% whilst Chinese had a low absence rate of 6.0% (10). Godwin and Adedayo examined in 600 subjects comprising 335 males and 265 females aged 8-60years were used to assess the prevalence of agenesis of the PL in Yoruba tribe. As a result, The overall prevalence of absence both unilaterally and bilaterally in the two sexes was 6.7%. In males, unilateral absence was 5.4%. The distribution on the right and left were 2.4% and 3.0% respectively. The bilateral absence was 1.5%. In females, unilateral absence was 6.0%. The distribution on the right and left were 2.6% and 3.4% respectively. Bilaterally, it was 0.4%. In one subject unilaterally, PL was observed to have differentiated from flexor carpi radialis (13).

Sater MS et al. examined 1,043 subjects, 3–85 years old, from the Kingdom of Bahrain for the presence or absence of the palmaris longus muscle using the conventional test for the presence of this muscle. The palmaris longus muscle was absent in 36.8% of subjects. Bilateral absence (19%) was more common than unilateral absence (17.9%) with preponderance in female subjects. The muscle was absent more often on the left side than the right (P=0.003). In the right upper limbs the muscle was absent in female subjects more than male subjects (P=0.031) (16). Lahiji et al. study on Iranian people and repoted that, the prevalence of the PLA was estimated to be 22.8%; 10.2% agenesis on the right side, 5.9% on the left side, and 6.7% bilaterally PLA. The relationship between PLA and gender did not appear to be significant. Among people with PLA 43% and among people withour PLA 17% were left handed (17).

Most standard textbooks of hand surgery quote the prevalence of the absence of PL at around 15% among Caucasians. The prevalence of its agenesis has been variously reported to be from 1.5% in black people to 64% in Turkish people (17).

According to a META analysis performed by 2013, their results demonstrated that the prevalence of PLA is the lowest among East Asians and Africans, and particularly among the East and South East African population (18).

Alabbat A. et al; studied in 200 normal female students from King Faisal University – Saudi Arabia and resulted that in female subjects; the overall prevalence of absence both unilaterally and bilaterally is 40.5 %. Unilateral absence was 20.5%. The bilateral absence was 20%. The distribution on the right and left was 29% and 31.5% respectively (5).

Kikano et al, evaluated 335 Labanese patients including a total of 339 by using magnetic resonans imaging (MRI) scans. The Palmaris longus was present in 221 wrists (65.2%), and bilaterally in only one (25%) out of the 4 bilateral cases. All palmaris longus were located using the Axial T1 views. Univariate and multivariate analyses showed no correlation with side, gender, or Tesla power. The only morphological variation was a reversed PL in 2 cases (0.6%). The mean width was 4.24 ± 1.2 mm. The mean thickness was 2.75 ± 0.6 mm (19).

The PLM is located superficially, is easily accessible, and is fully developed at birth, making it one of the most frequently used donor material for tendon and joint reconstructive surgeries in all age groups. As PLM is considered an accessory muscle, its tendon is often used as a graft for tendon transfer (18, 20) and in other reconstructive surgeries (21, 22). Also, It is well known that there is a wide variation in the reported prevalence of the PL absence in different ethnic groups. The highest reported prevalence of absence of the PL tendon is seen in the Turkish population with an overall prevalence of absence of 63.9% (7, 23).

Considering its dispensability, surgeons agree that PL is the best choice for tendon grafts in tendon reconstruction, helping the function of paralyzed muscles used for repairing ptosis, treatment of facial paralysis, and urinary incontinence. Plastic surgeons also utilize the PL in lip augmentation and restoration of lip and chin defects. As its absence is immaterial to the function of the wrist, PL has the greatest variation in the human body and its most common variation is agenesis (PLA) (24). However, this muscle variation can lead to median and/or ulnar nerve compression syndromes in some rare cases (25).

A retrospective review of patient charts undergoing endoscopic carpal tunnel release was performed by Boltuch et al (26). They considered the rates of palmaris longus agenesis (PLA) and compared to a population matched data set by using Schaffers's test. Study reported that; the Palmaris longus was more prevalent in a population of patients undergoing carpal tunnel release. These findings can be used to provide further insight into the pathophysiology of carpal tunnel syndrome (26).

CONCLUSION

In conclusion, this study demonstrates absence of palmaris longus (PL) muscle in Turkish students compatible with other population studies.

ACKNOWLEDGEMENTS

The authors of this work wish to thank our mentor Prof. Dr. Samet KAPAKİN and all the Students in college of Medicine, Atatürk and Erzincan Binali Yıldırım University for their help for collecting the sample and doing the research.

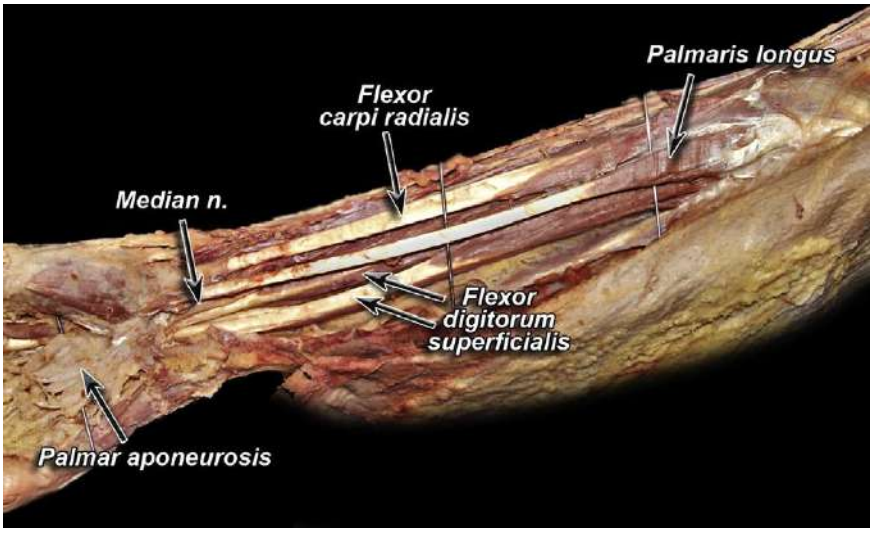

Figure 1: Dissected cadaveric forearm with present palmaris longus muscle *(Pękala, P. A., Henry, B. M., Pękala, J. R., Skinningsrud, B., Walocha, J. A., Bonczar, M., & Tomaszewski, K. A. (2017). Congenital absence of the palmaris longus muscle: A meta-analysis comparing cadaveric and functional studies. Journal of Plastic, Reconstructive & Aesthetic Surgery, 70(12), 1715-1724.)*

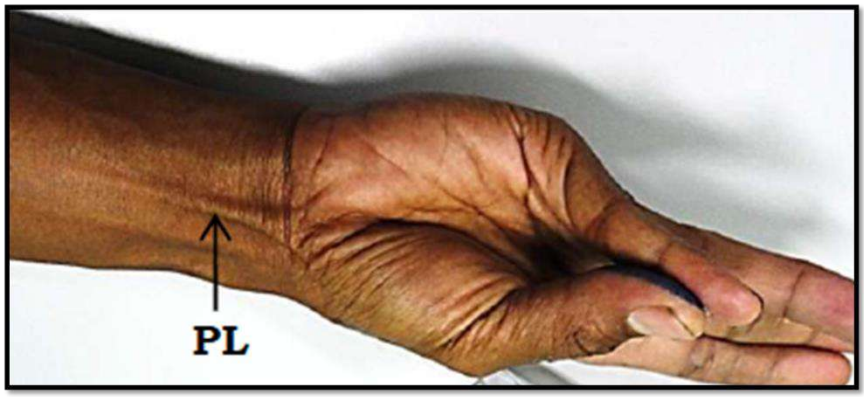

Figure 2: Schaffer test. (Thidar, A. M., Myint, T. T., Naing, D. K. S., & Mustapha, Z. A. (2013). Palmaris Longus Agenesis (PLA) among Dusun and Bajau ethnic groups of Northern Borneo. *International Journal of Collaborative Research on Internal Medicine & Public Health*, *5*(6), 0-0.)

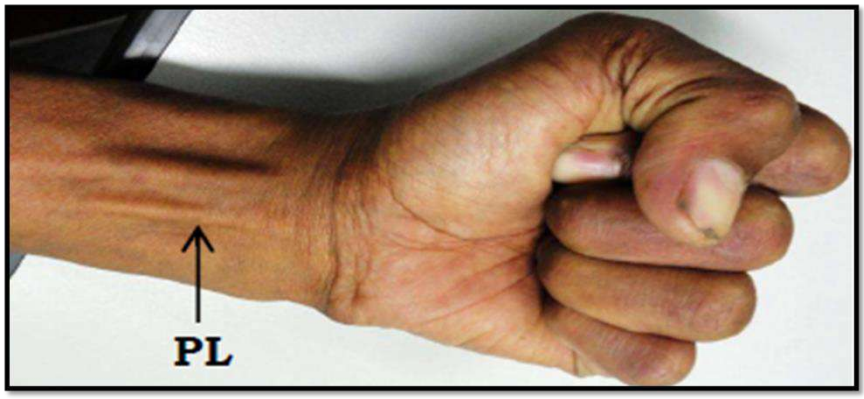

Figure 3: Thompson test. (Thidar, A. M., Myint, T. T., Naing, D. K. S., & Mustapha, Z. A. (2013). Palmaris Longus Agenesis (PLA) among Dusun and Bajau ethnic groups of Northern Borneo. *International Journal of Collaborative Research on Internal Medicine & Public Health*, *5*(6), 0-0.)

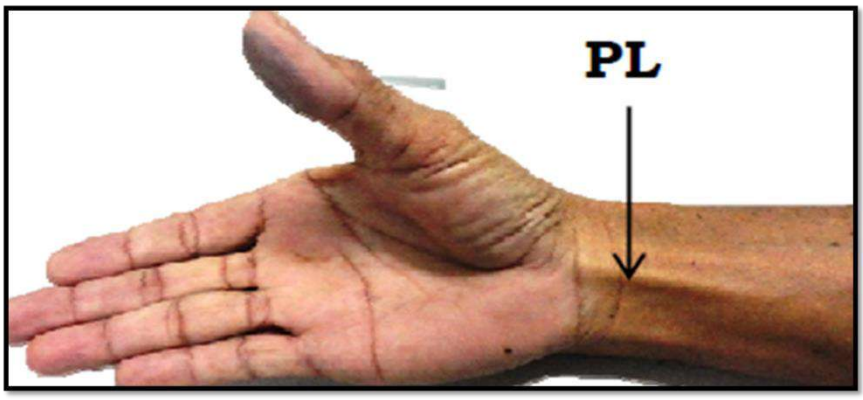

Figure 4: Mischa test. (Thidar, A. M., Myint, T. T., Naing, D. K. S., & Mustapha, Z. A. (2013). Palmaris Longus Agenesis (PLA) among Dusun and Bajau ethnic groups of Northern Borneo. *International Journal of Collaborative Research on Internal Medicine & Public Health*, *5*(6), 0-0.)

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CHAPTER VI

TARGETS FOR THE TREATMENT OF ERECTILE DYSFUNCTION

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1. INTRODUCTION

1.1. Epidemiology

Erectile dysfunction (ED) is defined as the inability to achieve or maintain a repetitive/permanent erection for any sexual activity. Worldwide; it is a condition that depends on many factors, where more than 150 million men are affected, with 39% of men around the age of 40 and 67% of men around the age of 70 [1].

The most important point in terms of the definition is that the erectile problem is not limited to one or more times, this problem must be repetitive or long-term to be diagnosed with erectile dysfunction. In addition to the age factor, the presence of some chronic diseases (heart diseases, hypertension, diabetes, and depression), smoking, alcohol use, stress, drug addiction, obesity, and some lifestyles also trigger the formation of this disorder. In fact, evidence has been shown that there is a close relationship between metabolic syndrome, cardiovascular disease and ED [2]. Erectile dysfunction can also be caused by psychogenic, organic (vascular, neurogenic, hormonal, anatomical/structural), or a combination of the organic and psychogenic state [3]. Sexual dysfunction is the most emphasized subject in psychogenic/organic differentiation. It requires a multidisciplinary approach in both its differential diagnosis and its treatment [4].

Although ED is a benign disease, this condition is associated with physical and psychosocial health and has a significant impact on the quality of life of both affected individuals and their partners and families [5]. ED is an important problem that impairs the quality of life of men with this condition all over the world.

2. NEUROPHYSIOLOGICAL AND MOLECULAR BASES OF PENIS ERECTION

Three processes that work synergistically and simultaneously are needed for normal erectile function:

1) Increased arterial blood flow through the neurogenic way,

2) Cavernosal smooth muscle relaxation,

3) The restriction of venous return flow.

ED occurs due to failure in any of these ways [6]. Penis consists of 3 anatomical compartments [7]. The corpus spongiosum supports and protects the urethra along the ventral face of the penis. On the other hand, located on the dorsal and consists of two adjacent parts the corpus cavernosum, fills with blood at the time of erection and provides a penile erection. The cavernosal compartment consists of a widespread network of vascular sinuses fed by helical arteries. When the penis is flaccid, the smooth muscle cells supporting the vascular sinuses are tonically contracted, allowing for low blood circulation. In contrast, neuromodulator release, which occurs in response to sexual stimulation and consists of cavernosal nerve terminals and endothelium, causes relaxation of cavernosal smooth muscles and consequently penile erection.

Central regulation of erectile function includes both spinal and supraspinal systems. Central supraspinal systems mainly; it is localized in the limbic system (hippocampus, medial preoptic region, nucleus accumulate), hypothalamus and its nucleus (paraventricular and ventromedial nucleus) as well as the nucleus paragigantocellularis of the reticular formation [8]. While gamma-aminobutyric acid (GABA), opioid peptides and serotonin reduce sexual responses, oxytocin, nitric oxide, excitatory amino acids, adrenocorticotropin (ACTH), and alpha-melanocyte-stimulating hormone (α-MSH) facilitates sexual responses [9].

The penis is innervated by autonomic and somatic nerves. Other factors such as cholinergic nerves, nonadrenergic/noncholinergic nerves (nitric oxide), Vaso-Intestinal Polypeptide (VIP) and Calcitonin Gene Related Peptide (CGRP) cause smooth muscle relaxation [6,10].

Apart from this, shear stress and muscarinic receptors located in the trabecular endothelium also stimulate nitric oxide production. Nitric oxide diffuses into smooth muscle cells and interacts with guanylate cyclase to increase intracellular cGMP levels. Increasing cGMP levels also causes cellular processes that result in smooth muscle relaxation and act through cGMP-dependent protein kinase and ion channels. This mechanism causes the reduction of intracellular Ca^{+2} levels and the

opening of Potassium ion channels that cause smooth muscle cell hyperpolarization [11–13].

Similarly; pathways mediated by VIP, CGRP, and Prostaglandin also cause smooth muscle cell relaxation by increasing intracellular cAMP levels [14].

A feature that is very important in all these erection mechanisms and must be remembered is the ion channels known as gap junctions and located in the cavernosal smooth muscle cell membrane. Thanks to these channels that cause tight communication, syncytial relaxation and contraction can occur, which has a very important role in erectile physiology [13].

The phosphodiesterase enzyme system is required for detumescence (reversal of erection): A series of biological events develop in the arterial blood, which is filled and stuck in the cavernous bodies, to return to the venous system and the penis to regain its flaccid state. First, the enzyme called "phosphodiesterase 5" in the cavernous structure c-GMP and possibly also enzymes called "phosphodiesterase 2,3,4" break down c-AMP into GMP and AMP, respectively. Cells in hyperpolarized states are then depolarized, calcium channels open, and muscular filaments start to contract by interacting with each other as calcium enters the cell. Blood is pumped into the congested veins, and as the amount of blood in the cavernous body decreases, the pressure on the veins stuck in the periphery decreases, and a normal venous return begins [15].

Nitric oxide (NO) is a potent peripheral smooth muscle relaxant acting through cyclic guanosine monophosphate (cGMP). NO released from nonadrenergic-noncholinergic nerve endings and endothelium is the main mediator that provides an erection. NO; It is synthesized from L-arginine by nitric oxide synthase (NOS) enzyme in many tissues in the body. NO synthase; endothelial (**eNOS**), neuronal (**nNOS**), and induced (**iNOS**), it is known that there are 3 isoforms. Mainly, eNOS and nNOS play a role in penile erection due to NO. NO is synthesized from the endothelium (eNOS) in response to shear stress, from cholinergic nerves as co-transmitter with acetylcholine, and nonadrenergic-noncholinergic (NANK) nerve endings by nNOS, and it activates intracellular guanylate cyclase by diffusing into smooth muscle cells [16]. This causes the conversion of GTP to cGMP. Thus, intracellular cGMP concentrations increase when there is NO stimulation. Increased cGMP concentrations cause cGMP-dependent protein kinase (PKG) activation and consequently smooth muscle relaxation. PKG; reduces intracellular Ca^{+2} levels by decreasing Ca^{+2} channel activity, causes smooth muscle cell

hyperpolarization by opening Ca^{+2} dependent K^{+} channels, and also causes smooth muscle relaxation by providing membrane hyperpolarization by inhibition of calcium sensitizing pathways such as the Rho-kinase pathway (Figure 1) [11,17].

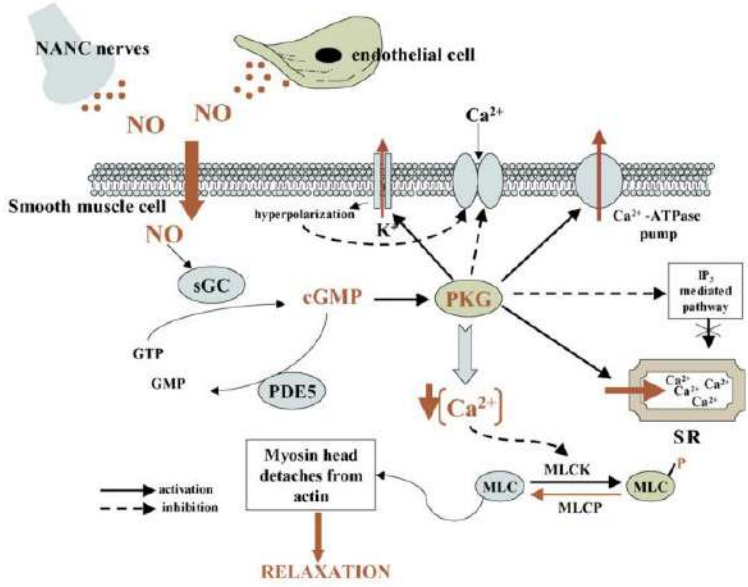

Figure 1: cGMP-mediated smooth muscle cell relaxation in corpora cavernosa[17]

NO from nNOS in nitrergic nerves is responsible for most of smooth muscle relaxation and initiation of relaxation, while NO from eNOS has been shown to contribute to the maintenance of erection [18]. It has been determined that the activity of nNOS in the penis is regulated by androgens. In castrated rats, it has been shown that the erectile response caused by cavernosal nerve stimulation and the nNOS activity in the penis decrease, and these effects can be corrected by androgen administration [18]. Molecular oxygen, along with L-arginine, is a necessary substance for NO synthesis. It has been shown that NO synthesis in the corpus cavernosum is directly regulated by oxygen concentration[19].

3. POTENTIAL GOALS FOR THE TREATMENT OF ERECTILE DYSFUNCTION

3.1. Targets Associated with NO-cGMP Pathway

3.1.1. Increasing NO Production

In the treatment of erectile dysfunction, Tetrahydrobiopterin (BH_4), a NOS cofactor, is used as a potential therapeutic agent in the

treatment of erectile dysfunction [20]. BH4 dysfunction or scarcity, which is the most important factor in eNOS dependent NO release, causes eNOS dysfunction. This leads to the release of NO-mediated oxygen radicals. Here, with the reaction of NO and free oxygen radicals, NO levels decrease and peroxynitrite is formed [20]. In vitro and in vivo studies have demonstrated that there is a significant relationship between Erectile dysfunction and ROS (Reactive Oxygen Species) production, especially in diabetic animal models [21,22]. O, which is a ROS_2^- it turns into peroxynitrite by reacting with NO. Peroxynitrite; it reacts with lipids, proteins, nucleic acids and creates structural damage. They found that basal O_2^- levels increased in the tissue segments of the aging rat model compared to the control [23]. With the increased O_2^- concentration, NO is destroyed and NO-dependent vasodilation is impaired. Superoxide dismutase enzyme (SOD) preserves NO activity by preventing the formation of peroxynitrite of ROS. It is known that arginase activity increases in ED due to age and diabetes [24]. Decreased arginase activity in the penis has been shown to improve endothelial function and restore erectile responses [24,25]. Also, pretreatment of diabetic Corpus cavernosum with arginase (ABH) inhibitors has been shown to partially inhibit the disruption of NO-mediated corporal relaxation and the enhancement of arginase activity.

An extracellular signal-regulated kinase (ERK), known to mediate cellular responses by growth factors, has recently been shown to play a role in vascular reactivity and reduce eNOS activity [26,27]. ERK inhibition has been shown to improve cavernosal relaxation in diabetic mice [28].

Another way to increase intracellular NO concentrations is by inhibiting the endogenous NOS competitive inhibitor asymmetric dimethylarginine (ADMA), the L-arginine analog [29].

Inhibition of increasing systemic levels of TNF-α, known to be positively correlated with ED, significantly improves sexual function [30].

3.1.2. Increasing the cGMP Level

3.1.2.1. PDE-5 (Phosphodiesterase-5) Inhibitors

The PDE5 enzyme has 2 subunits, catalytic, and regulatory. The catalytic domain is the target site for PDE5 inhibitors and contains the binding unit for cGMP. When the cGMP fills this area, the catalytic mechanism located right next to this connection area comes to the closest position and catalyzes the 5'-GMP by breaking the cyclic phosphate bond of the cGMP. When sildenafil or other PDE5 inhibitors also bind to the catalytic site, cGMP entry is blocked. These inhibitors, which show

affinity to the catalytic region, are competitively prevented from binding of cGMP to reach the catalytic mechanism. Thus, they inhibit cGMP degradation. However, PDE5 inhibitors cannot be destroyed by the catalytic mechanism [31].

Although the catalytic area is the direct target of PDE5 inhibitors, some features of the regulatory region affect the inhibition developed on it. This area contains allosteric cGMP binding domains and at the same time phosphorylation sites for enzyme regulation. Catalytic when cGMP is attached to the allosteric domain does not break down as in the area and enzyme function It is activated by the reaction that develops as a result of binding. In this case binding of cGMP also acts as a stimulator for binding of PDE5 inhibitor to the catalytic site. In this regard, when the cGMP level increases in smooth muscle cells after the patient intake sildenafil or other PDE5 inhibitors, this stimulates the binding of more inhibitors to the catalytic site. In this way PDE5 inhibitors stimulate their own activity. In addition to binding of cGMP to the allosteric region Phosphorylation of PDE5 by cGMP dependent protein kinase also affect PDE5 inhibition [31].

3.1.2.2. NO Donors

Currently, researchers have focused on a new class of compounds whose biological and medical significance is recognized. One of these agents is NCX 911, a derivative of sildenafil developed by NixOx, which provides NO release. NCX 911 spontaneously releases NO and increases intracellular cGMP level through PDE5 inhibition and activation of sGC in the absence of endogenous NO. NCX 911 is more potent than sildenafil both in enhancing tissue relaxation with EFS and reversing contraction in isolated human corpus cavernosum strips. While sildenafil has minimal effect on increasing tissue cGMP level in the absence of endogenous NO, it increases cGMP level dependent on NCX 911 concentration [32].

3.1.2.1. Guanilyl Cyclase (sGC) Activity

Prototype of NO-independent sGC activators, initially potent platelet aggregation YC-1 known for its inhibitory effect [33]. In experimental studies, it has been shown that YC-1 strengthens the relaxation phase created by transmural electrical field stimulation (EFS) in the rat corpus cavernosum, reverses the contraction caused by noradrenaline in the isolated rabbit corpus cavernosum, and this effect is parallel with the tissue cGMP level [34]. After that, NO-independent sGC activators were developed that were more potent than YC-1 (BAY 41-2272, BAY 41 8543, BAY 58-2667). Of these three molecules, BAY 41-2272 and BAY 41 8543 are affiliated to the heme group, and BAY 58-

2667 exerts its effects independently from the heme group. Both dependent sGC activators act synergistically with NO. Therefore, quinoxaline derivative is sensitive to inhibition by sGC inhibitors such as ODQ (1H- [1,2,4] oxadiazole [4,3, -a] quinoxaline-1). The efficacy of only BAY 41-2272 in erectile tissue has been investigated till this day, and it has been shown to induce relaxation in human and mouse corpus cavernosum samples contracted with phenylephrine. BAY 41-2272 was found to be 32 times more potent than YC-1 and 2 times more than the NO donor. Intravenous and oral administration of the compound produced weak erections in rabbits, but it was observed that the efficacy of BAY 41-2272 was potentiated when administered simultaneously with NO. [35,36].

3.1.2.3. Natriuretic Peptides

Another target that inducing penile erection by stimulating cGMP production is the particulate guanylyl cyclase (pGC). PGC activation by natriuretic peptides enables relaxation of the penile tissue and thus, erection [37].

3.1.2.4. Carbon monoxide (CO) and heme-oxygenase (HO) inducers

Limited number of studies shows that CO releasing molecules (CORMs) can be useful in the treatment of ED, and correcting the HO activity in diabetes-related ED, can improve erectile function [38,39].

3.2. Independent Goals from the NO-cGMP Pathway

3.2.1. Adenylate Cyclase (AC) -cAMP Pathway

Activation of the AC-cAMP pathway is known to be beneficial in the treatment of the ED [40]. PGE_1 (Prostaglandin E_1) or PGE_1 is using phosphodiesterase inhibitors specific to mimetic-induced AC activation, smooth muscle relaxation and penile erection can be induced to increase cAMP pathway activity and cAMP levels [40]. PGE_1 the use of Alprostadil, its analogue, is known to be useful in the treatment of ED [41].

It is thought that the vasointestinal polypeptide (VIP) and its receptors, which relax corporal tissues through the cAMP signaling pathway, may represent the therapeutic target [42,43].

3.2.1. Hydrogen sulfide (H2S) Pathway

H2S, which plays a role in the control of vascular homeostasis, is seen as a promising target for the development of new therapeutics for ED, as it not only promotes corporal dilatation but also alleviates penile fibrosis [44,45].

3.3. Targets associated with vasoconstriction

3.3.1. RhoA/Rho-Kinase Inhibitors

Most of the time, smooth muscle cells in the erectile tissue are in a contraction and the blood flow into the cavernous sinuses is minimal. Many studies have reported that RhoA/Rho-kinase-mediated vasoconstriction may be the primary mechanism responsible for penile smooth muscles to maintain this contracted state [46,47]. RhoA/Rho-kinase actively increases intracellular calcium (Ca^{2+}) plays a role in maintaining penile flaccid state as it increases the noradrenergic tone regardless of its concentration [48]. Although there is sufficient conceptual information to support the importance of intracellular calcium in the regulation of vascular tone in the penile structure, Ca^{2+} sensitization has been recently investigated. RhoA is a small protein that binds with the GTP and is involved in many cellular events such as smooth muscle contraction, cell roof, and morphology and secretion [49]. RhoA is inactive when bound to GDP, but with heterotrimeric G protein activation, it is activated by the conversion of GDP to GTP and migrates to the cell membrane. Rho A activation process is regulated by 3 groups of proteins:

1) GDI (guanine nucleotide dissociation inhibitor) inhibits RhoA activation,

2) GEF (guanine nucleotide exchange factor) regulates the conversion of GDP to GTP,

3) GAP (GTPase associated protein) inhibits RhoA activity with intrinsic GTPase activity.

Theoretically, factors that stop RhoA activation by affecting the activity of these proteins will prevent smooth muscle contraction and allow relaxation. Activated RhoA targets Rho-kinase in penile smooth muscle. It catalyzes the phosphorylation of MLC phosphatase enzyme, which inhibits Rho-kinase enzyme activity. When MLC (myocyte light chain) phosphotase activity is weak, the MLC predominantly remains in phosphorylated form and smooth muscle contraction is continued. The interruption of this process, in which RhoA activates Rho-kinase, leads to the reversal of the flat contraction and the formation of relaxation [49]. Y-27632, a pyridine derivative, is a selective Rho-kinase inhibitor [50].

3.3.2. Angiotensin receptors

Increased Ang-II activity may contribute to the development of ED by increasing harmful effects such as vasoconstriction, proliferation, fibrosis and oxidative stress [51], in this context, angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARB; Ang-II /AT1) have been shown to have beneficial effects on erection in

recent years [52]. The view that drugs such as ACEI/ARBs such as telmisartan, ramipril, captopril and losartan should reduce these harmful effects and be seen as treatment targets for ED [53–55].

3.3.3. Endothelin and Adrenergic receptors

Downregulation of ETA receptor expression or activity of Endothelin (ET-1) synthesized by the vascular endothelium in the penis is potentially effective in alleviating ED [26]. Studies with drugs such as tamsulosin, doxazosin, and alfuzosin have shown that α-adrenergic blockade is effective in the ED treatment [56].

3.3.4. Transient receptor potential (TRP) channels and Store operated calcium entry

Intracellular Ca^{2+} is playing an important role in modulating levels of TRP channels (TRPC6) are seen as a new molecular target for ED therapy [57]. Recently, transfer of dominant negative genes for 2 proteins, named ORAI1 and STIM1 identified in cavernous smooth muscle cells has been shown to restore erectile function in diabetic rats and is seen as a new treatment target for ED [58].

3.4. Anti-inflammatory and anti-fibrotic treatment targets

This pathway is directed towards the prevention of collagen accumulation and smooth muscle cell loss in CC [59]. Upregulation of the TGF-β1/Smad pathway by inducing fibrous-muscle structural changes [60]; inhibition of Poly (adenosine diphosphate ribose) polymerase (PARP), which is important for DNA repair, by increasing NO-mediated relaxation [61]; by suppressing IL-6, a pleiotropic cytokine [62]. It has been shown to improve erectile function.

3.5. Centrally acting drug targets

Positive results have been developed in clinical trials with centrally acting drugs such as dopamine receptor agonists and melanocortin receptor agonists. Apomorphine, a centrally-acting non-opioid dopaminergic agonist, induces penile vascular and corporal smooth muscle relaxation, through the activation of the receptors D1 and D2 in the MPOA (Medial Preoptic area) and PVN (Paraventricular nucleus of hypothalamus) regions, which maintain the activation of central neurogenic pathways in erection [63].

Melanocortins such as melanocyte-stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) are involved in important homeostatic mechanisms controlled by the hypothalamus as well as sexual functions such as penile erection and sexual motivation [63]. Melanocortins act on dopaminergic neurons located in the pro-erectile

centers of the hypothalamus, causing central and spinal oxytocin release. They show their effects by binding with the MC1-R to MC5-R receptors. These receptors have been identified in the skin, gastrointestinal tract, male and female reproductive system, adrenal cortex, and brain [64,65]. Especially expressed in peripheral tissues, MC5-R provides the relationship between central and peripheral control of sexual behavior [64].

3.6. Alternative therapy goals

3.6.1 Herbs, Toxins and Polyphenols

In recent years, complementary and alternative medicine has become increasingly popular and has been used as herbal supplements for ED. These plants and the properties of active components in their content [66–68] and there are preclinical studies that reveal their mechanisms of action and significant efficacyies are promising [69–71]. The mechanism of action of some herbs has been demonstrated by its stimulatory effect on the NO-cGMP signaling pathway [71], some aphrodisiac [72] increase fertility and sexual function [73]. It has been shown to have such effects.

It shows that Tx2-6, a toxin purified from the venom of the P. nigriventer spider, induces erection, by activating the nNOS of the PnTx2-6 toxin, increasing NO production and improving vascular smooth muscle relaxation that animal venoms can be potential drugs in ED treatment [74,75].

There is some evidence that polyphenols, which have been shown to be effective in erectile dysfunction, do this by acting as antioxidants that improve the NO/cGMP pathway and stimulate angiogenesis. Resveratrol, a polyphenol found in red wine, is reported to increase cGMP in corporal smooth muscle cells, activate eNOS, thereby improving endothelial function [76]. On the other hand, Quercetin is beneficial on the corpus cavernosum with its anti-inflammatory, anti-apoptotic and antioxidant effects [77].

3.7. Regenerative medical

Regenerative medical especially in the last few decades has increased in importance, and preclinical trials have shown that growth factor therapy, gene transfer, stem cell therapy, and tissue engineering can be beneficial for the restoration of erectile function [78–81].

Although human growth hormone (GH) is not considered as the classic sex hormone, it is involved in the regulation of male reproductive functions and sexual maturation. GH has physiological significance not only in LH and FSH secretion but also in early testosterone stimulation [82]. It is assumed that the biological effect of growth hormone is through the stimulation of endothelial NO formation mediated by "insulin-like

growth factor (IGF-1)". These data indicate the importance of growth hormone in maintaining erectile capacity [83]. Beneficial effect of IGF-1 in endothelial damage caused by spinal cord injury [84] has been shown that treatment with recombinant GH creates a dose-dependent relaxation on the erectile tissue and this effect is parallel to the tissue cGMP level [83].

Gene Transplant Methods: One of the most important conditions of successful gene therapy is that the genetic material (RNA or DNA) to be transferred is transported with the appropriate vector or vehicle that will provide the most effective treatment. Some vectors used to date can be listed as: nonviral vectors (naked DNA, plasmid DNA, and liposomes), adenoviruses, adeno-associated viruses, and retroviruses. All of these vectors have specific efficacy and side effects inside the host cell. An ideal vector should be capable of providing efficient transduction and long-term gene expression with little or no side effects such as infection, immunological reaction, and host cell mutation [79].

First of all, it is necessary to consider whether gene therapy is a viable treatment method in the treatment of erectile dysfunction. Gene therapy was initially recommended as an appropriate treatment option in the treatment of cardiovascular diseases, and vascular pathologies being one of the most important causes of the etiology of ED, suggesting that the same method can be used in ED treatment [80]. Preclinical studies in recent years show that gene transfer has corrective effects on ED [79,80]. Due to the structural characteristics of the cardiovascular system, there is a drawback in the treatment of diseases in this system: gene therapy can easily reach beyond the targeted area. On the other hand, a similar drawback in ED treatment can be minimized. With a simple tourniquet to be applied to the root of the penis, the possible systemic effect that may occur after gene injection can be minimized. Another problem with gene therapy is that most of the targeted cells need to be transfected in order to achieve therapeutic efficacy. On the other hand, transfecting only a certain group of cells in the penis is sufficient to provide therapeutic effect. As is known, there is an interconnection network provided by gap junctions between corpus cavernosum smooth muscle cells. The therapeutic effect achieved after gene transplantation in a particular cell, thanks to these connections, can spread throughout the penis as if all cells were transfected [79]. Human large conductance, voltage dependent Ca^{2+} Clinical trials using naked DNA gene therapy with a plasmid (hSlo) expressing the sensitive K channel (hMaxi-K channel) represent the only gene therapy to be evaluated in phase 1 clinical trials to date [85–87]

Tissue engineering aims to reconstruct the penile tissue. Successful monocultures of cavernous endothelial and smooth muscle cells have been reported in recent years. [88].

4. OTHER GOALS

There are studies showing that physical exercise also contributes to ED treatment as a non-therapeutic approach. Corpus Cavernosum relaxation in hypertensive rats induced by NO blockade [89] and has been shown to exert anti-inflammatory effects on the hypothalamus and testicles, increase LH levels, and support the primary role of lifestyle modification in hypogonadism and erectile dysfunction [90].

5. CONCLUSION

As a result, the increasing concentration of biochemical, genetic, physiological, pharmacological and histological research on the erectile dysfunction at the molecular and tissue level will enable a better understanding of erectile function mechanism, and the formation of new treatment targets and clinical treatment protocols in the treatment of ED.

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CHAPTER VII

POSSIBLE EFFECTS OF PREEKLAMPSY AND EKLAMPSY THAT MAY OCCUR WITH MATERNAL OBESITY ON THE HEALTH OF PREGNANCY: A METAANALYSIS

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1. INTRODUCTION

It has been determined that the prevalence of overweight "obesity" has gradually increased in women in the last 20 years (88). As a result, it has been suggested that it is associated with obesity during pregnancy, gestational diabetes, hypertensive diseases, preeclampsia-eclampsia, traumboeubalism diseases, preterm labob, macrosomia, birth complications and increased cesarean delivery (89). In addition, epidemiological studies have shown that excess maternal body weight increase during pregnancy causes high birth weight newborns. (90) It has also been reported that newborns may increase the risk of being overweight and obese in childhood and adulthood (91).

According to the data of the World Health Organization (WHO), it is stated that there are over 400 million obese and 1.6 billion slightly obese individuals in the world. It was stated that in 2015, this rate could reach 2.3 billion per 700 million, respectively (92). 2016 According to the data of the adult population in the world, approximately 13% were obese, and especially if the prevalence of obesity before pregnancy with an average 0.5 point increased knowledge of literature a year i, in 2000 Turkey's National Diabetes Obesity and Hypertension Epidemiology (TURDEP) that the obesity prevalence according to study data 22.3% It has been suggested (93).

In particular, increased obesity was found to be associated with pregnancy loss and the risk of recurrent pregnancy in the first trimester (94). In overweight and obese pregnant women, in addition to the increasing number of undesirable perinatal effects, obviously obesity; It has also been determined to increase the risk of severe gestational hypertension, preeclapsia-eclipse, gestational diabetes and macrosomia in terms of the mother and fetus (95). In a study involving 1.4 million women with a large sample, it was found that the risk of preeclapse doubles with

every 5-7 kg / m2 increase in body mass index in pregnant women (96). Again, when compared with obese and normal body weight women, it was found that triglyceride, very small density lipoprotein, insulin and leptin levels were higher in obese pregnant women (97). It has been concluded that obese women, especially in the postpartum period, breastfeed less than those of normal weight (98).

It is clear that maternal obesity increases a number of serious problems such as gestational diabetes, hypertension, preeclapse and eclipse during pregnancy, as it is associated with short and long-term complications in the mother and newborn at the end of pregnancy, delivery and postpartum.

2. MATERNAL OBESITY

The alarming increase in the prevalence of obesity worldwide has explained the importance of the World Health Organization (WHO) considering obesity as one of the most serious global health problems of the 21st century (50). WHO defined obesity and obesity as abnormal or excessive fat accumulation that harm health and stated that the prevalence of obesity during pregnancy is between 1.8% and 25.3% (25).

The Center for Disease Control (The Center for Disease Control) stated that the prevalence of obesity in pregnant women mirrors obesity in women of reproductive age (42); stated that 25% of women of childbearing age are obese and 25% of them are obese especially in the USA and maternal obesity is a major risk factor for maternal and perinatal mortality (40).

It was found that the mother who was overweight and obese during pregnancy and at birth was at significant risk in terms of antenatal, intapartum, postpartum and neonatal complications (25). Among the prenatal complications; It has also been determined that recurrent miscarriages, congenital anomalies cause pregnancy-induced hypertension, preeclampsia, gestational diabetes mellitus (GDM) and venous thromboembolism (29). The risk of induction and cesarean section increases in obese and obese mothers and it has been stated that the babies of these mothers are mostly macrosomic and stay in the hospital for a longer time (1).

It has been emphasized that obesity is a strong risk factor for gestational diabetes (35) and is effective in the development of diabetes, cardiovascular disease and hypertension in the following years (30).

The Royal Collage of Obstetricios and Gynecologist (2004) emphasizes that those with prenatal and postnatal BMI over 30 or body weight over 90 kg carry a significant risk for postpartum troboembolic

events (30) Stated that between 2003-2005, body mass index (BMI) was above 25 in 65% of maternal deaths due to thromboembolism (40).

Madan et al. (2010) (28) found that 23% of women were obese and 5% were extremely obese; He stated that as the age and parity of women increased, their BMI increased. Pre-pregnancy diabetes, gestational diabetes, chronic hypertension and preeclampsia, dysfunctional birth, use of induction, premature rupture of membranes (PROM) and cesarean delivery were found to be increased in extremely obese patients. In this study, premature birth was found in 8% of normal weight women, 8% of obese women, 9% of obese women, and 10% of extremely obese women; It has been stated that it may be associated with the increased risk of preterm birth in obese patients. Chorioamnionitis, low birth weight baby (LBW) and placental detachment were observed more frequently as a result of PROM and preterm delivery in obese pregnant women (29).

In a systematic analysis of 13 scientific studies, it was found that increasing BMI is an independent risk factor for preeclampsia; It has been reported that obese mothers are at twice the risk of preeclampsia than mothers of normal weight. Gestational hypertension is more common in morbidly obese women who did not have hypertension before; Therefore, maternal obesity is not only associated with a high risk of preeclampsia,but has also been associated with gestational hypertension (40).

In a randomized controlled study with 1661 women in order to evaluate peripartum and neonatal outcomes in obese and obese mothers; It was found that 27% of women were obese, 16% were obese, there was an artificial initiation of labor and an increase in cesarean rate, and more antibiotics were used for wound infection in the postpartum period. Again in this study, it was found that the risk of developing gestational diabetes was two and four times higher in obese and obese than normal weight women, respectively; It has been reported that babies born to obese and fat mothers are larger and macrosomic (1).

Pregnancy outcomes were examined in obese women who gave birth in a large university hospital in Europe; Singleton pregnancies with birth weight> 500 g and women whose BMI was calculated in the first trimester (n = 5824) were included in the study; It was determined that 0.6% of women were morbidly obese. While the mean age of morbidly obese women was 31, 46.1% of women with normal BMI were found to be primigravida. While 35.8% of morbidly obese women have hypertension or preeclampsia caused by pregnancy, these problems were detected in 9.8% of women in the normal BMI group. The frequency of fetal macrosomia (birth weight> 4-5 kg) is 1.7% in the normal BMI group and 6.3% in the obese group (13).

2.1. EFFECT OF MATERNAL OBESITY ON HYPERTENSIVE DISEASES

It has been suggested that hypertensive disorders are the most common medical problems complicating pregnancy in developing and developed countries, and cause significant morbidity and mortality in mother, fetus and newborn, and hypertensive disorders are seen in approximately 10% of pregnancies worldwide (11). Previously, 30 mmHg systolic blood pressure (SBP) increase or 15 mmHg diastolic blood pressure (DBP) increase were included in the definition of hypertension (HT) in pregnant women, but it was no longer defined since it was found that it had no effect on prognosis (6). However, these pregnant women were followed up more closely in terms of the possibility of developing hypertension (HT). High blood pressure is a symptom and has been the result of different pathogenesis. Accordingly, its effect on the mother and fetus was also different. In addition, hypertension during pregnancy has varied from mild high blood pressure to severe high blood pressure causing multiorgan dysfunction.

HT can be present before pregnancy as well as detected during pregnancy for the first time. In some, it becomes evident only in the intrapartum or postpartum period. Classification is extremely important in terms of prognosis, management and determination of maternal fetal risks. The National High Blood Pressure Education Program (NHBPEP) made the following classification in 2000 (34):

1. Preeclampsia-eclampsia
2. Chronic hypertension
3. Preeclampsia combined with chronic hypertension
4. Gestational hypertension

Preeclampsia-Eclampsia: It was defined as de novo hypertension accompanied by proteinuria, which emerged after 20 weeks of gestation. Hypertension blood pressure was found to be above 140/90 mmHg in at least two measurements. Proteinuria is defined as 300 mg protein leakage in 24-hour urine. Since edema, which was included in the old definition, is nonspecific and is seen in most normotensive pregnant women, it has not been included in the new definition. It has been said that preeclampsia is not severe if there is no target organ damage. The definition of severe preeclampsia is defined as excessive elevation in blood pressure or target organ damage.

Chronic Hypertension: Known to be present before pregnancy. It was detected before or before the 20th week of pregnancy. In the first half of the pregnancy, despite the increase in intravascular and extravascular volume as well as the increase in cardiac output and

intrvascular volume, the mean blood pressure decreased as the total peripheral resistance decreased with the increase in vascular compliance. Increasing cardiac output plateaued or continued to increase slightly in the second half of pregnancy (9).

Preeclampsia Added to Chronic Hypertension: Preeclampsia was added to the picture of a pregnant woman with chronic hypertension. The diagnosis of preeclampsia was determined by the emergence or increase of new proteinuria, increased blood pressure under control, increased liver enzymes, and thrombocytopenia.

Gestational hypertension: It is known as hypertension that occurs in the second half of pregnancy or within the first 24 hours postpartum, and returns to normal within 6-12 weeks after birth without proteinuria. Although the reason is unclear, it has been found that women are very likely to have HT in the future. It has been estimated that 15-25% of women with gestational HT will develop preeclampsia (37). In those who had gestational HT before 32 weeks, this rate approached 50%.

2.2. EFFECT OF MATERNAL OBESITY ON MACROZOMY

Clinically, macrosomia has been described in several ways. Although fetal weight over 4000-5000 gr is a generally accepted definition, it is considered more correct to talk about the baby according to the gestational age (20). The concept of LGA is different from absolute fetal weight, and the expected fetal weight according to gestational age has been achieved to be above the 90% percentile (11). Although not much, in some studies, fetuses with a ponderal index above 2.85 were evaluated as LGA (36).

Ponderal index = Weight (gr) .100 / height cm

In the Prevention of Macrosomia: Macrosomia observed especially in diabetes has been reported at a rate of 8-45% despite advanced perinatal medicine practices (44). The most important point in the prevention of fetal macrosomia has been the diagnosis of diabetic pregnant women. (4) Although patients were aware of their condition in diabetic cases, timely recognition of gestational diabetes, especially seen in risk groups, was required (26). Regardless of the type of diabetes, an appropriate metabolic balance has been maintained throughout pregnancy (23). It was stated that if insulin application will be applied for macrosomia prophylaxis, the last trimester should be selected and treatment should be started as early as possible and before 36th gestational week at the latest (10).

2.2. THE EFFECT OF MATERNAL OBESITY ON THROMBOEMBOLI DISEASES

Physiological and anatomical changes during pregnancy were important factors in venous stasis and decreased venous blood flow, uterine pressure on inferior vena cava and pelvic veins and immobility thrombus formation. While some coagulation factors increased during pregnancy, the protein level decreased (41). Age> 35, obesity, cesarean delivery, severe ovarian hyperstimulation, heart disease, DM, multiparity have been reported to increase the risk of pulmonary embolism (PE) (18).

Physiological changes during pregnancy cause PE-like symptoms causing diagnostic difficulties. Dyspnea, tachypnea, tachycardia and leg swelling occurred during pregnancy. The complaint of shortness of breath gradually increased during pregnancy. In particular, the unusual increase in dyspnea and the accompanying DNT symptoms were among the important clues for PE. The most common symptoms were listed as dyspnea, pleuritic, chest pain and cough (8).

Thrombolytic Treatment in Pregnant Women: When thrombolytic therapy in pregnant women is used in patients who are not pregnant, it carries a similar risk to the risk of bleeding (31). No large studies have been conducted on the use of pregnancy thrombolytic drugs. Therefore, experience in this matter has been limited (33). Tissue - plasminogen activator (tPA) has been recommended in pregnant women using thrombolytics in the recently published European PE guideline (22).

3.PREEKLAMPSİA AND EKLAMSİA

Preeclampsia has been a common occurrence in pregnant women. It has been characterized by protein in the urine accompanying high blood pressure. Women with preeclampsia have had swelling in their feet, legs, and hands. If preeclampsia is present, it may occur earlier, and it is usually seen in the second half of pregnancy, the last part of the second trimester, or the third trimester.

Eclampsia has been identified as the final and most severe stage of preeclampsia if left untreated. In addition to the previously mentioned findings of preeclampsia, it was found that women with eclampsia mostly had a stroke. Eclampsia caused the mother and the baby to go into a coma or even death. In addition, the disease was discovered before, during or after the birth of the child.

It has been stated that preeclampsia can cause your baby to be born very small and prevent the placenta from getting enough blood. It has also been the main cause of difficulties such as premature birth and accompanying learning defects, epilepsy, cerebral palsy, and hearing and

vision problems. It has been suggested that the only true cure for preeclampsia and eclampsia is the birth of a baby.

Other treatments included: It has been stated as intravenous magnesium injection to prevent strokes due to eclampsia, Hydralazine or another antihypertensive drug to relieve severe spikes in blood pressure, It has been reported as observing fluid intake (88).

3.1. THE RELATIONSHIP OF MATERNAL OBESITY AND PREEKLAMPSY

Preeclampsia has progressed as a pregnancy-specific syndrome affecting 3-5% of pregnancies. Preeclampsia was defined as the presence of new onset of hypertension and proteinuria or end-organ dysfunction or both in a woman who was normotensive before pregnancy (48). Severe hypertension and end-organ damage signs / symptoms have been accepted as the severe spectrum of the disease (49)

Eclampsia is a form of new onset, general, tonic clonic seizures or coma in a woman with preeclampsia, a convulsive sign of preeclampsia, and has been determined by various clinical data seen at the end of severe preeclampsia (50). It occurred in the second half of pregnancy (49). It has been suggested that it is a pregnancy-specific syndrome characterized by hypertension and proteinuria (47). It has been reported that it is responsible for approximately 70,000 maternal deaths and 500,000 infant deaths in the world every year (52) and preeclampsia and eclampsia are the second (17%) causes of maternal death, especially in our country (53). Risk factors for plexus are shown in table 1.

Table 1. PREEKLAMPSY RISK FACTORS (59, 63, 64, 65)

First pregnancy	Family history of preeclampsia
Preeclampsia in previous pregnancy	Prolonged birth interval (>10 years)
Antiphospholipid syndrome or	Type I Diabetes Mellituda Type Diabetes Mellitus
hereditary thrombophilia	
Chronic hypertension	High body mass index (29)
Multiple pregnancy	Systemic lupus erythematosus
Maternal age <18 and >40	Chronic kidney disease
Undisclosed fetal growth retardation	Vascular or connective tissue diseas
Fetal growth retardation in previous pregnancy	ablatio placenta or fetal loss Factors associated with the partner
Mole hydatidiform	Predisposition to genes
In vitro fertilization	Black race

3.2. INTERACTIONS OF PLEXUS RISKS

First pregnancy: It has been suggested that women with a first pregnancy may increase the risk of preeclampsia (48, 52, 55). Although it is not known exactly why the first pregnancy is a predisposing factor, one theory suggests that these women may have limited exposure to antigens from the father, and it has been claimed that this situation may play a role in the pathogenesis of the disease (57).

Preeclampsia history in the family: It has been determined that the medical history of first-degree close relatives increases the risk of preeclampsia from 2 times to 4 times in those with preeclampsia (51, 55). Although this situation suggests a hereditary mechanism, it has been suggested that the father of the baby may contribute to the increased risk or the father's contribution to fetal genes may have a role in insufficient placentation and subsequent preeclampsia (48).

Preeclampsia in previous pregnancy: It has been reported that women with a history of preeclampsia in their previous pregnancy increase

the risk of developing preeclampsia 7 times compared to those who do not (Relative Risk (RR): 7.19) (48, 51,55).

Prolonged birth interval (≥10 years): Too long a period between two pregnancies (10 years or more) increased the risk of preeclampsia (52, 53,54).

Multiple pregnancy: Preeclampsia is more common in multiple pregnancies (especially triplets and quadruplets) (48, 51, 55). RR in twin pregnancies: 2.93 (48). Triplet pregnancies have created a higher risk of preeclampsia development compared to twin pregnancy (51).

Maternal age <18 and > 40: Maternal age over 40 increased the risk of preeclampsia. (RR: 1.96 in multipara, RR: 1.68 in primipara). Older women tended to have more risk factors such as diabetes mellitus, obesity, and chronic hypertension. This has been explained by the increase in preeclampsia frequency among older women (48, 51, 55).

Diabetes Mellitus: RR in diabetes that exists before pregnancy: 3.56. Various factors such as underlying renal or vascular diseases, high plasma insulin level / insulin resistance, and abnormal lipid metabolism were thought to affect the risk of preeclampsia. (48,55).

Chronic kidney disease: The relative risk has changed in pre-pregnancy kidney disease, depending on the degree of reduction in glomerular filtration rate and the presence or absence of hypertension. Those with advanced chronic kidney disease (grade 47, 48, 49) were diagnosed with preeclampsia after the second half of pregnancy at a rate of 40-60%. (48, 55).

High diastolic blood pressure: Increased the risk of blood pressure ≥130/80 mmHg at the first prenatal visit (RR: 1.38). Women with the highest risk of superimposed preeclampsia were found to have diastolic blood pressure ≥110 mmHg (RR: 5.2) and ≥100 mmHg (RR: 3.2) before the 20th gestational week (48).

Other: Pre-existing hypertension (RR: 1.38) (52, 55), antiphospholipid syndrome (RR: 9.72) (59, 66) and BMI ≥35 (66) were among the other risk factors. It has been controversial that the risk of preeclampsia is higher in adolescents. It has been found that women who smoke are less likely to have preeclampsia than non-smokers. (48). The risk is increased in those who become pregnant with donor egg, embryo donation or donor insemination (52). It has been difficult to assess the racial differences in the incidence and severity of preeclampsia due to confounding socioeconomic and cultural factors (51).

3.3. MATERNAL AND FETAL EFFECTS OF PREECLAMPSIA

Preeclampsia has been an important problem affecting maternal and infant health. (58). In cases where preeclampsia developed, convulsion, pulmonary edema and ablatio placenta were seen. Women with preeclampsia were found to have a higher risk of hypertension (3.7 times), coronary artery disease (2.2 times) and stroke (1.8 times) in their later life (52). It has been observed that the changes in lipid metabolism of women with preeclampsia (such as increased total cholesterol and triglyceride levels) are greater than the changes in normotensive pregnant women (57, 59). One of the rare complications of preeclampsia is acute pancreatitis and can mimic preeclampsia (60, 61). Ablatio placenta is rarely seen (less than 1%) in women with preeclampsia without severe symptoms, while it has been reported that 3% of women have severe symptoms (48).

Fetal growth retardation and oligohydramnios were observed due to chronic fetal hypoperfusion in the fetus (48). Severe and early-onset preeclampsia resulted in a serious decrease in fetal birth weight compared to normotensive pregnancies (62). Fetal birth weight was found to be lower than expected for gestational age by 12% in severe preeclampsia and 23% in early-onset preeclampsia (52). Among the long-term effects of preeclampsia on children is an increased risk of stroke and hypertension (52). The frequency of neonatal diseases such as respiratory distress, intraventricular hemorrhage and necrotizing enterocolitis was similar to babies of preeclamptic pregnant women and babies of non-hypertensive women of the same age (48).

3.4. TREATMENT METHODS IN PREECLAMPSIA

According to the long held view, "childbirth" has been the only solution in preeclampsia treatment. In the management of preeclampsia, guided by evidence-based practices, early diagnosis of risk groups, prevention of multiple organ damage in the mother, and effective management of labor or ensuring the safe continuation of pregnancy for mother and baby until fetal lung maturity is achieved (54, 58, 63).

Women deemed to have a high risk of developing preeclampsia were directed to pre-pregnancy counseling in order to determine changeable risk factors before pregnancy. Being at the highest level of health before pregnancy has decreased the risk of developing preeclampsia (51, 52, 64, 65). It has been found that there is no ideal antenatal care program for women with a high risk of preeclampsia. However, when these women were diagnosed, antenatal management included more frequent follow-up, anomaly screening, regular blood pressure and urine controls,

and fetal growth assessments to evaluate fetal growth retardation every 4 weeks (52).

In mild preeclampsia cases, the mother's life is not in danger and the continuation of the pregnancy did not put the mother at serious risk and gained time for the baby (47). Maternal and fetal risks are well balanced when the decision is made for the delivery of a woman with preeclampsia. Generally, in this case, delivery at the 37th gestational week is recommended, but if the maternal and fetal condition is stable, a woman with mild preeclampsia has been followed closely. Laboratory evaluation twice a week and daily fetal monitoring are generally recommended for the management of all women with preeclampsia by hospitalization. If the woman was deemed suitable for outpatient follow-up, weekly prenatal visit and twice weekly fetal testing were provided (66). Initiating the emergency treatment of persistent severe blood pressure to reduce adverse vascular events has been the main factor in the treatment of severe preeclampsia (67). Another priority in the treatment of severe preeclampsia has been the prevention of eclampsia (51, 66).

All complications of preeclampsia have been observed especially in the first 48 hours after delivery (49). Women with hypertension throughout pregnancy were followed up after delivery until they were sure that the hypertension problem was completely resolved. Women who had preeclampsia during pregnancy have been told that their risk of cardiovascular and kidney disease increases in their later life. After discharge, the woman should be followed up regularly and her blood pressure was monitored closely. Those diagnosed with severe preeclampsia were more likely to recur in their next pregnancy (52).

3.5. INITIATIVES TO PREVENT PREECLAMPSIA

The fact that preeclampsia threatens maternal and infant health significantly, and the etiology and pathogenesis are not fully known, draw attention to the importance of preventive approaches. Determining the risky groups before the development of preeclampsia and taking the necessary precautions contributed to the improvement of maternal and infant health. In this context, the development of preeclampsia, which has widespread and serious consequences, can be stopped, slowed down, reduced in effect or treated early with some simple measures. To prevent the development of preeclampsia; Life style changes such as physical activity, dietary regulations, pharmacological methods, and avoidance of stress have been suggested (51, 68, 69).

Acetylsalicylic Acid: If there is an early onset of preeclampsia in the medical history, preterm birth before the 34th week or a history of preeclampsia in more than one pregnancy, it is recommended to start low dose acetylsalicylic acid (60-80 mg) at the end of the first trimester. In the

meta-analysis of randomized studies conducted for the prevention of preeclampsia by acetylsalicylic acid and involving more than 30,000 women, although long-term fetal effects could not be ignored, it was stated that it provided a small decrease in the rate and morbidity of preeclampsia and there was no evidence against an acute risk (70).

Antioxidant Supplements (With Vitamins C and E): Antioxidant support with vitamin C or vitamin E is not recommended for the prevention or treatment of preeclampsia. (70). High-quality evidence of ineffectiveness has been found. In two studies in which antioxidants were given to women with severe preeclampsia for treatment, no clinical benefit was found (71, 72). In order to prevent preeclampsia and reduce its complications; Although a pilot study found that supplementation of 1000mg of vitamin C and 400 IU of vitamin E daily during the second and third trimesters significantly reduced the risk of preeclampsia (73), it was broadly and in multiple, multicenter, randomized women with both low- and high-risk groups. No meaningful results have been achieved in studies (74, 75).

Folic Acid Supplement: Although it has been stated that folic acid reduces the risk of preeclampsia, there are studies reporting the opposite (48, 49, 51).

Antihypertensive Drugs: It has not been shown in controlled studies that lowering blood pressure with antihypertensive drugs reduces the risk of preeclampsia or ablatio placenta or improves fetal and pregnancy outcomes. However, treatment has decreased the frequency of moderate and severe hypertension (50).

Calcium Supplement: No benefit of routine calcium supplements has been observed for the prevention of preeclampsia in healthy nulliparous women whose dietary calcium intake is sufficient. It has been shown to be beneficial in the prevention of preeclampsia in the high-risk group, especially in societies where dietary calcium intake is low (64). World Health Organization (WHO) recommended 1500-2000 mg of calcium supplements per day to reduce the risk of preeclampsia for pregnant women who are at high risk for developing hypertension in places where calcium intake is low (51). In a randomized controlled study conducted by WHO in the female population (n: 8325) whose daily calcium intake is less than 600 mg per day; calcium supplementation provided a small statistically insignificant reduction in preeclampsia. Eclampsia and severe gestational hypertension were significantly lower in the calcium group. At the same time, the severe maternal mortality and morbidity index and neonatal mortality rates decreased in the group receiving calcium. As a result, 1.5 g calcium supplementation per day

cannot prevent preeclampsia, but its severity, maternal 1 morbidity and neonatal mortality have been reduced (65).

Fish Oil: There are studies indicating that fish oil supplements have a protective effect on the vessels, lowers systemic blood pressure, reduces the incidence of preeclampsia and pregnancy-induced hypertension (52, 53). On the contrary, fish oil supplementation has no There have been many studies stating that it has no effect (54, 55, 56).

Nitric Oxide: There is no sufficient level of evidence that nitric oxide prevents preeclampsia (35).

Other Dietary Interventions: There have been only a few small randomized studies investigating the role of diet in preventing preeclampsia. No beneficial results were found from dietary recommendations such as protein and energy supplements, protein and energy restriction (in obese women), magnesium supplementation, and salt restriction (21, 55, 58, 59).

Vitamin D Supplementation: Some observational studies indicate an association between vitamin D deficiency and increased risk of preeclampsia (38) and early-onset severe preeclampsia (61), while no association was found in a prospective cohort study of women with high-risk preeclampsia (76). As with other nutritional interventions, evidence was insufficient to prevent preeclampsia (70).

Weight Loss: Although there is an increased risk between maternal obesity and preeclampsia, few studies have evaluated the effect of weight loss on risk. In cohort studies of patients undergoing surgery for weight loss, weight loss was reported to significantly reduce the risk of preeclampsia (76). Additionally, in a cohort study, it was determined that the risk of recurrent preeclampsia was reduced in women with preeclampsia who lost weight between pregnancies (62).

Rest and Physical Activity: There is no strong evidence that exercise or physical activity affects the risk of developing preeclampsia. (43). Restriction of bed rest or other physical activities is not recommended primarily in the prevention of preeclampsia and its complications. (80). It has been hypothesized that regular exercise prevents preeclampsia by improving vascular functions. Although bed rest is recommended as a preventive strategy, it has been reported that the evidence for this is limited. Only two small studies evaluated bed rest as a protective strategy, but did not evaluate perinatal-maternal morbidity and mortality and adverse effects of bed rest (44, 45).

Moderate activity has been found to reduce the risk of hypertension and cardiovascular disease in non-pregnant women. In normal pregnancy, 30 minutes of moderate activity is not recommended on

most days of the week, if not every day. (46). It has been postulated that moderate activity also stimulates placental angiogenesis and maternal endothelial dysfunction. A few small clinical studies have evaluated the effects of moderate exercise in preventing preeclampsia, but data have been limited to make realistic interpretations of the results (47). In addition, long bed rest during pregnancy increased the risk of thromboembolism (65). The American Collage of Obstetricians and Gynecologists (ACOG) has not recommended strict bed rest without severe symptoms for women with gestational hypertension or preeclampsia (70).

Coping with Stress: According to Çelik and Özdemir (70), prolonged stress situations caused a constant high blood pressure. The stress that will be experienced during pregnancy also caused blood pressure changes. Hypercortisolemia caused by maternal stress affected pregnancy by affecting many factors affecting the placenta and fetus. (71). Studies have shown that maternal stress leads to the delivery of low birth weight babies and an increased risk of preeclampsia (72, 73). The stimulating effect of stress on the sympathetic nervous system causes changes in peripheral vascular resistance during pregnancy as well as in the immune system, and it has been found to be associated with the development of preeclampsia (52). Shamsi et al. (64) reported that maternal stress experienced during pregnancy increased the risk of preeclampsia. Vollebregt et al. (72) also reported that the risk of developing preeclampsia and hypertensive disorders increased approximately twice in working women compared to non-working women in relation to the tensions experienced in working life.

Coping with stress, which is among the lifestyle changes that are effective in hypertension, has been done with an effective stress management. In stress management, relaxation techniques such as deep breathing, muscle relaxation exercises and mental relaxation were included in addition to finding a solution to the cause of the stress. It was found that blood pressure decreased with the application of deep breathing exercises. Respiratory exercises have relaxing effects on the cardiovascular system, and mental relaxation has positively affected blood pressure by affecting the sympathetic nervous system. (53). It is thought that a similar effect can be seen in preeclampsia that may develop during pregnancy. Because rhythmic and regular breathing calms the nervous system, people can cope with stress more easily by deep breathing in stressful situations. As the abdominal muscles are used during deep breathing, the tension in the neck and shoulders is also reduced (54).

4. GESTATIONAL DIABETES

Gestational diabetes mellitus (GDM) has been described as carbohydrate intolerance of various degrees, which started during

pregnancy or was first diagnosed during pregnancy (23). This definition did not exclude the possibility of diabetes, which was also present before conception but was unknown until the first examination during pregnancy.

Although the American Obstetrics and Gynecological Association still uses the same terminology, in recent years, the International Diabetic Working Groups Association (IADPSG), the American Diabetes Association (ADA), and the World Health Organization (WHO) and others were first diagnosed during pregnancy but probably previously thought to be diabetic. stated that transient diabetes due to pregnancy-related insulin resistance should be differentiated (17). These organizations have used the term gestational diabetes emerging in the second half of pregnancy: overt diabetes or diabetes mellitus during pregnancy, and the terms for diabetes, which is recognized by standard non-gestational criteria in early pregnancy, when insulin resistance is less (45).

4.1. PATHOPHYSIOLOGY OF GESTATIONAL DIABETES

A subclinical metabolic dysfunction is thought to occur in women who have normal glucose tolerance during normal pregnancy but develop GDM in the late period of pregnancy. The 60% decrease in insulin sensitivity that occurs during normal pregnancy has led to clinical hyperglycemia / GDM in these women. Maternal obesity, which is frequently associated with gestational diabetes, has been associated with increased inflammation in maternal white adipose tissue and placenta. Adipokines and cytokines such as leptin, adiponectin, TNF-a, interlokin-6 were secreted from white adipose tissue (5). The placenta showed a similar cytokine gene expression profile except for adinopectin. It is thought that inflammation caused by secreted cytokines may be related with increased insulin resistance in pregnant women with gestational diabetes (20). If the maternal pancreatic B-cells cannot provide enough insulin to meet the increasing insulin need, GDM has developed (2).

4.2. FETAL AND MATERNAL EFFECTS OF GESTATIONAL DIABETES

It is known that both the mother and the fetus are at many risks associated with diabetes during pregnancy. There was an increase in risks (12). While negative consequences in the mother may be short-term (hypertension, preeclampsia), they have also been long-termed, such as the increased risk of Type 2 DM in later life (21). Although postpartum glucose levels return to normal in women with GDM, it has been known that the risk of developing Type 2 diabetes mellitus (DM) increases 7 times in later life (3, 62).

GDM history has also been found to be a determinant for increased cardiovascular risk and early atherosclerosis (14). Hypertensive

complication rates were found to be higher in diabetic pregnancies compared to normal pregnancies (48). It was found that the risk of preeclampsia increased from 5-7% to 15-20% depending on glycemic control, GDM severity and pre-pregnancy body mass index (BMI) (12). In women with GDM, the risk of developing type 2 DM in the later years of life increased by 20-80% (24). Glucose can pass freely from mother to fetus, but maternal insulin could not. Maternal glucose in high concentrations passing through the placenta stimulated insulin secretion in the fetus, increasing growth factors and causing macrosomia (5).

4.3. BIRTH COMPLICATIONS AS A RESULT OF MATERNAL OBESITY AND INCREASE IN CAESARY DELIVERY

Maternal obesity has been a recognized risk factor for serious obstetric complications, which are becoming more common worldwide and include hypertensive disorders, preeclampsia, gestational diabetes, thrombophlebitis, urinary tract infections, chorioamnionitis, preterm labor, fetal macrosomia, shoulder dystosis, and intrauterine fetal death (7).

Obese women have a high risk of intrapartum complications, and it is thought that various factors may have an effect on this situation. Ineffective uterine activity comes first, and macrosomia, which is also a fetal event, reduces the progression of labor; Difficult assessment of fetal position during delivery in obese women made it difficult to define the breech presentation precisely (10). Spontaneous pregnancy loss and neural tube defect in the first pregnancy period; gestational diabetes in the second timester; In the third trimester, the risks of gestational hypertension, difficulty in fetal follow-up, anesthesia complications, macrosomia, stillbirth and cesarean section increased. In the postpartum period, obese women have been reported to have bleeding, infection, wound opening, and venous thromboembolism risks (25). At the beginning of the difficulties experienced by obese women during childbirth, changing position is the first thing that comes to mind. There have been difficulties in inserting an epidural catheter due to excessive adipose tissue. Transporting the woman to the operating table can also become difficult, and excessive neck, abdominal, and chest weight have made intubation and mechanical ventilation more difficult and complex. Due to the increasing surgical deliveries, prenatal anesthesia consultation was recommended (19).

Although there is a relationship between BMI over 30 and prolonged delivery, techniques that stimulate contraction such as nipple stimulation and walking were used. It was important to follow up the follow-ups for uterine contractions and fetal heart rate just after the induction use. The ability to palpate and record uterine interactions using

excessive abdominal adipose tissue is limited by using a tachodianometer. Calculation of fetal heart rate with dops is also limited in obesity. Therefore, the most accurate monitoring was performed with fetal scalp electrode and internal pressure catheter (19). The first and second stages of macrosomic deliveries are prolonged, the risk of intervention vaginal birth, third degree perineal trauma, emergency cesarean section, postpartum hemorrhage, and apgar score below 4 is increased. It was found that he stayed in the hospital for a longer period of time (38).

Studies have shown that shoulder dystocia is more common in women who are obese and gain excessive weight during pregnancy. It was understood that women with a BMI above 40 had a five-fold higher risk of uterine rupture at delivery, prolonged labor and increased risk of fetal distress during this period, therefore healthcare professionals might have reservations about trying vaginal delivery after cesarean in obese patients (19).

Although there is a relationship between prolonged birth and being on it, techniques that stimulate contraction such as nipple stimulation and walking were used. It was important to follow up the follow-ups for uterine contractions and fetal heart rate just after the induction use. The ability to palpate and record uterine interactions using excessive abdominal adipose tissue is limited by using a tachodonometer. In obesity, calculation of fetal heart rate with dops is also limited. Therefore, the most accurate monitoring was performed with fetal scalp electrode and internal pressure catheter (19). In a study, the rate of those who had medical problems or birth complications during pregnancy was 44.7%, and 30.7% of them were found to be obese / obese before becoming pregnant (21). It has been deemed extremely important that obese women are not encouraged in terms of access to antenatal service and continuity in service. Counseling and quality follow-ups led by healthcare professionals from the early stages of pregnancy have had important effects on intrapartum and neonatal outcomes at birth (10).

In a retrospective study of 100 obese women with single pregnancy in the United Kingdom, obesity was examined in three categories. These; Group A: BMI 30-34 (n = 39), group B: BMI 35-39.9 (n = 43); Group C: BMI> 40 (n = 21), spontaneous onset vaginal delivery rate was 60% in non-obese women, 62% in group A, 49% in group B and 24% in group C. The use of induction is increased in moderately and severely obese women, with rates being 37% and 38%, respectively, and 28% in non-obese women. When the groups are compared according to delivery types, spontaneous vaginal delivery is 60% in non-obese patients, 44% (7139 and 9143) in group A and B and 62% in group C (7). Simic et al. (2010) stated that the frequency of posttherm births increased in obese mothers, while a

delay of at least 7 days was 31.9% in women with a BMI of 30 and above, while this rate was 23.7% in women with normal weight (41).

5. PRETERM BIRTH

In determining whether maternal obesity is a risk factor for preterm delivery, it was found that optional preterm births and spontaneous preterm births should be examined separately. In general, cohort studies have found that obese women have a high risk for voluntary preterm birth, but miscarriage risk for spontaneous preterm delivery. However, the relationship between maternal obesity and preterm birth risk is complex and potentially influenced by age, parity, smoking and other factors including ethnicity.

The mechanism that obesity reduces the risk of spontaneous preterm birth is not fully explained, but it has been stated that there may be a decrease in the level of spontaneous uterine activity in obese women compared to normal weight and thin women. More than 3000 preterm births were analyzed in the Danish National Birth Cohort study (n = 62,167 women); premature rupture of membranes and spontaneous preterm delivery are more common in obese women; It has been found that the risk of spontaneous preterm birth is low with intact membranes in obese women (32).

MCP-I (Monocyte Chemolactic Protein-I) was significantly increased in overly obese mothers. In recent studies; He suggested that the relationship between obesity and inflammation in pregnancy is partially related to the increased risk of pregnancy complications observed in obese women. Inflammation, infection, and associated cytokines that are increased during pregnancy have been reported to be associated with risks for the fetus such as preterm birth, intraventricular hemorrhage, periventricular leukomalacia and brocopulmonary dysplasia (28).

In a systematic review and meta-analysis of 84 studies consisting of 64 cohorts and 20 case-control studies in order to examine the relation of obesity and obesity in mothers in singleton pregnancies in developed and developing countries with preterm birth and low birth weight babies, obese and obese women were It was found that they were at high risk for preterm labor and preterm delivery with induction before 37 weeks. (14) In a systematic review of 49 studies, it was stated that the risk of preterm birth increased as BMI increased, respiratory distress occurred in babies, and this situation increased resuscitation practice (39). Between 2000 and 2008, it was determined that the risk of preterm labor increased significantly among obese women in women with 417 twin pregnancies in Japanese maternity hospital and the independent risk factor in preterm labor developing in dichorionic twin pregnancies was maternal obesity (39).

While cesarean delivery was observed in one case out of four obese pregnant women at 30 weeks of gestation due to severe preeclampsia, in the other 3 cases, unavoidable preterm delivery following EMR was performed without preeclampsia. Chorioamnionitis was added to the picture in the next three cases. The incidence of chorioamnionitis was higher in obese women who had a preterm delivery compared to other women (43).

In another study, the relationship between maternal obesity uterine contraction frequency and spontaneous preterm birth risk was evaluated. 20-36. 253 women who had one or more spontaneous preterm birth or second trimester vaginal bleeding in their current pregnancy between weeks of gestation were connected to uterine activity monitor twice a day from 22 weeks to 34 weeks of gestation. It was found that obese and obese women who were at risk of preterm labor with external tocometer had less frequent uterine contraction and overweight women were at risk of spontaneous preterm birth before 35 weeks. Analyzes after controlling for other factors, 22-24 or 31-32. No significant relationship was found between contraction frequency and SPD at 27-28 weeks. no such relationship has been shown in weeks. In conclusion, obese women who showed contraction frequency similar to normal and thin women when screened for preterm labor risk stated that they were especially at risk for delivery before term (12).

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CHAPTER VIII

AN EXAMINATION OF THE RELATIONSHIP BETWEEN SMOKING DURING-BEFORE PREGNANCY AND NEWBORN BIRTH WEIGHT/PLACENTA WEIGHT AND ITS HISTOPATHOLOGICAL IMPACTS ON THE PLACENTA TISSUE

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1. INTRODUCTION:

The fact that smoking is quite common in the society leads to 90% of the smokers to start this habit before the age of 20 and whereas the number of male smokers continues to decrease, the increase in the number of smoking women results in an increase in smoking during pregnancy [1]. Increasing cigarette use among female population results in lung cancer, coronary obstructive lung disease, coronary diseases, osteoporosis, late pregnancy, early menopause, menstrual issues, infertility and cancers [2, 3].

It was determined based on the Turkey Population and Health TPHS-2008 data, these values decreased to 11,4% for pregnant women and 16,5% for nursing mothers [4]. Worryingly, then the global prevalence of smoking duringpregnancy is 1.7% with up to 8.1% of women smoking during pregnancy in Europe and 250 million women smoking during pregnancy worldwide[5, 6] . All these findings indicate that smoking among pregnant women is a frequently encountered behavior and an important health issue. The increase of smoking among the female population in a community results in an increase in smoking related pregnancy risks [7].

Fetal growth and development is characterized by fetal tissue and organ differentiation, maturation and growth[8]. Fetal genetic structure, uteroplacental function and maternal environment are among the primary factors with impacts on fetal growth and development. A healthy fetus completes its intrauterine somatic growth under conditions for which all

these factors are proper. Fetal growth and development may be affected adversely when conditions are not proper. Abnormal maternal, fetal and placental factors may have adverse impacts on fetal growth and development either separately or as a whole [9, 10]. Even though there are many factors that play a role on fetus growth and development, smoking and being subject to cigarette is quite important due to their frequency and the fact that they can be prevented [11, 12] . Smoking among women continues to increase rapidly especially in developing countries despite all efforts to prevent it. It was presented in various studies that pregnant women continue smoking at varying ratios subject to their socioeconomic status. Smoking is one of the agents that has been well studies due to its relation with intrauterine growth retardation (IUGR) the impact of which has been put forth via dose-response curves [13].

Smoking during pregnancy results in many different complications in pregnancy in addition to IUGR. Moreover, it has a lot of negative effects which will have an impact on both the postnatal periods of babies as well as their later ages. Major impacts of smoking during pregnancy are; growth retardation, increased abortus risk, ealy membrane rupture, premature birth, placenta previa, decollement placenta, sudden infant death syndrome and childhood period impacts. Countless studies have been carried out on this subject which put forth the adverse impacts of smoking and many new studies are being carried out for indicating the presently unknown impacts of smoking [14].

It is also very important how much the fetus is affected from the mother's smoking or being subjected to the smoke from the mother's cigarette. It was observed in well-documented dose-response curves that fetal weight decreased with increasing number of cigarettes smoked by the mother[13]. In addition, there are also various publications indicating that similar results have been observed in the babies of mothers who are not smokers but who have smokers around them [15].

Placenta has an indisputable importance for the development and growth of the embryo and the fetus as an organ that takes on all metabolic functions during the intrauterine period which are actually carried out by many different organs during postnatal life.

The mechanisms with which smoking during pregnancy affects the coordination of intrauterine growth retardation, placenta proliferation and differentiation are unknown. Thus, understanding the histopathological changes in human term placenta may provide much valuable information in this subject. The purpose of this study was to examine both the placenta and newborn weights as well as to determine the relationships between the histopathological changes in the placenta tissue.

2. MATERIALS AND METHOD:

2.1. Newborn and Placenta Weights and Tissue Selection

The present study was carried out at the Manisa Merkez Efendi State Hospital during October 2017-April 2018 for a period of about 6 months by taking the necessary ethical and official permissions. It was aimed to evaluate the relationship between the newborn weights and weights of placentas acquired from pregnant women who have started smoking at least 5 years before pregnancy and who have continued smoking throughout their pregnancies (n:35) and from pregnant women who have not smoked at all (n:35) in addition to examining the histopathological changes in the placenta tissues. It took about 6 months between October 2017-April 2018 to collect the placentas and record the data. In addition to questioning smoking addiction in all pregnant women giving normal birth in this study, the presence of pathologic, anatomic findings which may have an impact on fetal growth and development were also examined. The acquired placenta tissues were measured one by one in every group. The weights in kilograms of the babies of all pregnant women included in the study were also recorded.

2.2. Histochemical Staining

The placenta tissues separated from the mothers were weighed rapidly. Following these processes, a sample tissue with dimensions of 1 cm^3 was taken from the larger placenta tissue which was then placed in 10% formaldehyde and fixed for 24 hours. They were then subject to dehydration processes and embedded in paraffin blocks. The embedded tissues were transferred as 5 μ sections onto a microscope slide via a microtome (Leica RM 2135; Bensheim, Germany). The sections were left to dry for 1 night on the slide after which they were kept for 1 night in a drying oven for deparaffinization. The tissues were treated with xylene the next day and passed through decreasing alcohols of 90%,80,70,60 after which they were kept for 2 min in hematoxylene (Cardiff RD 2014) and 2 min da Eosin (Cardiff RD 2014) (H&E) stain. The tissues that were exposed to 80% and 90% alcohols were kept for 1 hour in xylene and mounted with Entellan (UN 1866, Merck, Darmstadt, Germany). They were made ready for examination under the microscope (Olympus BX-40-Tokyo 163-0914, Japan).

2. 3. Statistics

The data were analyzed via SPSS software version 20.0. Independent T test (student T test), F test (Anova) and Pearson correlation test were used for data analysis. p values of <0,05 were accepted as statistically significant.

3. RESULTS:

In this study, pregnant women who started smoking at least 5 years ago and who did not quit smoking throughout their pregnancy periods made up the study group, while those who have not smoked throughout their lives made up the control group. According to the statistical results, the age average of the pregnant women who participated in the study was 26,97 for smokers and 27,05 for non-smokers. The age average of all participants was 27,01. While 48,6% of the pregnant women who participated in the study were aged 25 and below, 42,9% were between the ages of 26-35 and 8,6% were aged 36 and above. Normality assumption was taken into consideration with regard to the use of parametric or non-parametric tests for data analysis. The accordance of the data with normal distribution can be evaluated via Q-Q Plot[16]. In addition, the normal distribution of the data used is subject to the values of skewness and kurtosis to be between ±3 [17].. In the light of all these data, independent T test (student t test) was applied in SPSS 20.0 software for the analysis of data related with placenta weight and newborn weight taking into consideration that the number of our subjects was above 30 and that all groups were suited for normal distribution, while Anova test was applied for Hematoxylene Eosin pathological findings. According to this test; the birth weights for non-smokers (3456,71 gr) was observed to be greater than the birth weights of smokers (3093,71 gr). Newborn weight decreased by 10.5% in smokers. Similarly, the placenta weights of non-smokers (648,14 gr) was observed to be greater than the placenta weights of the smokers (577 gr). Placenta weight decreased by about 10.9% in smokers (Table 1).

When the Independent T test table is examined, it should be $p>0.05$ for homogeneous variance. Since the sig values for birth weight and placenta weight were above >0.05, sig 2 values corresponding to the first row are examined. Variance is homogeneous in all groups and a statistically significant difference was observed between smoker and non-smoker groups with regard to both the placenta weight and newborn weight ($P<0.05$) (table 2).

Independent sample t-test was performed for determining whether there is a statistically significant difference between the average birth weights of the babies of mothers who smoke and those who do not. According to the test results, the birth weight variable variances are homogeneous for the smoker and non-smoker groups ($p>0,05$). It was determined based on the independent sample t-test results that there were difference between the birth weights of the babies of smoker and non-smoker mothers ($p<0,05$). Independent sample t-test was carried out for determining whether there are any statistically significant differences between the placenta weight averages for the babies of smoker and non-smoker mothers. One of the fundamental assumptions of the independent

sample t-test which is the homogeneity of the variances was tested via F-test. The test results put forth that the variances are homogeneous for the birth variable of the smoker and non-smoker groups (p>0,05). It was put forth based on the independent sample t-test results that there were differences between the birth weights of the babies of smoker and non-smoker mothers (p<0,05).

The correlation coefficient for all data was calculated as r= 0,598 upon examining the relationship between birth weight and placenta weight. There is a moderate and positive relationship (p <0.05) (Table 3) between newborn birth weight and placenta weight according to the correlation table. The correlation coefficient was calculated as r: 0.566 when the relationship between birth weight and placenta weight was evaluated only for smoker mothers, this ratio was r: 0.0508 for non-smoker mothers. A moderate and positive relationship was determined between the birth weights and placenta weights of smoker and non-smoker mothers (p<0.05).

Table 1: Descriptive statistics of variables in the research.

Groups		Birth weight	Placenta weight	HE
Smokers (n=35)	Mean	3093,71	577,00	2,51
	Standard deviation	384,439	92,285	0,612
	Minimum value	2550	415	2
	Maximum value	3980	770	4
Non-smokers (n=35)	Mean	3456,71	648,14	0,60
	Standard deviation	446,572	101,352	0,497
	Minimum value	2560	415	0
	Maximum value	4850	865	1
Total (n=70)	Mean	3275,21	612,57	1,56
	Standard deviation	452,232	102,673	1,112
	Minimum value	2550	415	0
	Maximum value	4850	865	4

Table 2: Independent Sample T Test according to Birth weight and Placenta weight.

Variables	Groups	Mean±SD	Test of Equality of Variance		t test			
			F	Sig.	t	df	p value	Means difference
Birth weight	Smoker	3093,71±384,43	0,085	0,772	-3,645	68	0,001*	-363,000
	Non-smoker	3456,71±446,57						
Placenta weight	Smoker	577,00±92,28	0,049	0,825	-3,071	68	0,003*	-71,143
	Non-smoker	648,14±101,35						

Table 3: Pearson correlation analysis for all data.

Pearson correlation		Birth weight	Placenta weight
Birth weight	r	1	
	p value		
Placenta weight	r	0,598**	1
	p value	0,000	

Table 4: Statistical analysis of histopathological concentrations (Hematoxylin-Eosin) of smokers and non-smokers.

	Group	N	$\bar{X}$	SD
H&E	Smokers	35	2,5143	,61220
	non smokers	35	,6000	,49705

Histopathological Findings:

Majority of the placentas in the non-smoker group were terminal villus based on our histopathological findings (Figure 2a). The villuses were located at intervillous intervals with sporadic maternal blood cells in between. While fetal veins and connective tissue were observed in the villuses, rare cytotrophoblast cells and syncytial cells and knots were observed on the outside (Figure 2b). Normal structure mesenchymal connective tissue and fibroblasts were observed in root villuses (Figure 2c,d). Intravillous deposits at the tertiary villuses and fibrinoid deposits on the intervillous areas were observed when the structural differences and cellular average were evaluated for the placentas of the smoker group in addition to an increase in syncytial knots (Figure 3a,b). The connective tissue of root villuses lost its normal structure, necrotic and inflammation areas were determined different than the non-smokers (Figure 3c,d). The presence of histopathological findings in smokers increased by 76.09 % (Table 1). In addition, a statistically significant difference was determined between the smoker and non-smoker groups with regard to fibrinoid deposits at the intravillous and intervillous areas and necrotic and inflammation areas in the term placenta ($p < 0.05$) (Figure 1,2,3) (Table 4).

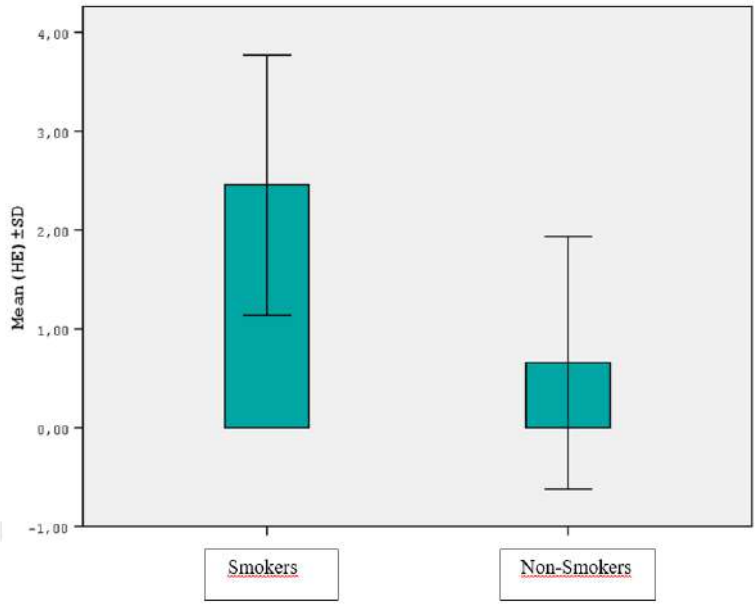

Figure 1: Statistical analysis of histopathological concentrations (Hematoxylin-Eosin) of smokers and non-smokers. Values are expressed as mean ± SD.

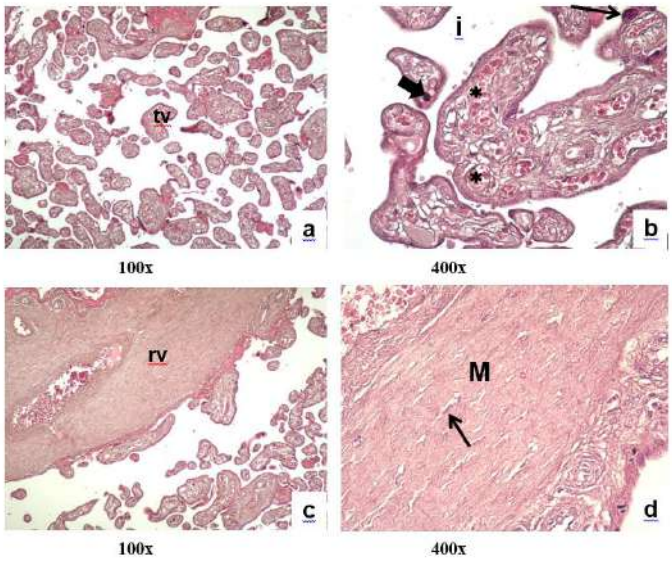

Figure 2. Placenta histopathology in non-smoker group. H&E. **tv:** tertiary villouses ✱:Fetal veins, ➡: Cytotrophoblast cells, →: Syncytial knots, **i:** Intervillous gap (a,b) **rv:** root villuses, **m**: mesenchymal connective tissue, →: fibroblasts (c,d)

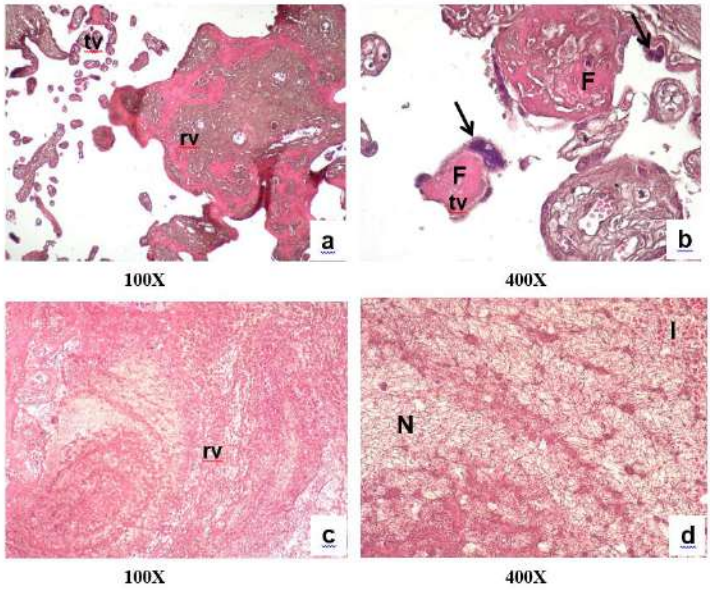

Figure 3: Placenta histopathology in smoker group. H&E. **tv:** tertiary villouses, **F**:Fibrinoid deposits, **➔:** Syncytial knots, (a,b) **rv:** root villuses, **N:** Necrotic regions, **I:**Inflammation (c,d).

4. DISCUSSION AND CONCLUSION:

Smoking is one of the most important factors that affects the fetus during pregnancy. There are various findings indicating that the harmful substances and toxins in the cigarette are transferred to the baby via the placenta [7, 18]. Various studies have been carried out which indicate that cigarette is not only effective on the development of the baby but has an impact on placenta weight and newborn weight as well. In the present study, the placenta and newborn weights were examined in addition to the status of histopathological changes in the placenta tissue.

The number women who have started smoking before pregnancy and who have not been able to quit throughout their pregnancy period is quite high in the world. Since cigarette contains addictive substances, it is not very easy to quit during pregnancy. We carried out a study on the women who applied to the delivery room of the Merkez Efendi State Hospital in the province of Manisa and limited the sample group with 35 smoker and 35 non-smoker pregnant women in two groups in order to be able to carry out pathological examinations on all placenta tissues obtained.

One of the inclusion criteria in a study by Ender Durualp et al. on smoking during pregnancy at the Çankırı State Hospital in 2011 was that the pregnant mothers should have applied to the hospital during a certain period of time. Of these pregnant women, 130 were included in the study and it was observed that 23.9% (n: 19) are smokers and 76.1% are non-smokers (n:99). Newborn weights were also recorded in this study. The analyses carried out in the study put forth a statistically significant difference between the smoking status of the mothers and the birth weights of the babies (χ^2=37.631, sd=6, p<0.01)[7]. Similar to our study, this study also presents a directly proportional difference in newborn weights of smoker mothers.

Sandra Larsen et al. published a study in Norway in 2018 in which the pregnant women hospitalized during 1999-2014 were classified into 2 groups as smokers, non-smokers and those who quit during pregnancy. According to this retrospective study, it was presented that 12.6% of 698 891 women smoke and that of these women 29.6% quit during the first trimester and that 70.4% continued smoking throughout their pregnancy. There were differences between the placenta weights and birth weights among the groups of women who smoke 11 or more cigarettes a day and non-smoker groups[18]. Our study includes women who started smoking 5 years before pregnancy. The results of our study are in accordance with the results of the aforementioned study.

According to the Turkey Population Census and Health Study (TPHS-2008) results; it is indicated that 30.5% of married women smoked before, 24.1% are still smoking and that the starting age for smoking was 19.6 with an average of 11 cigarettes per day. An increase is observed in comparison with the TPHS-2003 results for smoker women (28%). TPHS-2008 results put forth that 26.2% of the pregnant women were smoking before, 11.4% are still smoking and that the starting age for smoking was 17.4 with 10 cigarettes per day. While the ratio of smoking before among nursing women was 26%, the ratio of those who are still smoking was 16.5%, the starting age for smoking was 18 with 8 cigarettes smoked daily. Both the TPHS-2008 results as well as the results of studies carried out in the world and in our country indicate that smoking is an important issue among both nursing women as well as other women[19-21].

A statistically significant relationship was observed between smoking and the birth weights of newborns in all studies carried out on the subject. Smoking doubles the risk for a women to give birth to a baby with less birth weight. Published a report in 1985 in which it was indicated that smoking is the most important factor playing a role in the development of babies with low birth weight[22].

Placenta is an organ that clinicians and pathologists do not generally show the required interest in. The fact that the number of placentas is high and that majority do not contain any pathology in addition to the lack of any relation between various placenta pathologies and perinatal mortality and morbidity have resulted in clinicians and pathologists to not evaluate the placenta sufficiently[23].

The Nicotinic acetylcholine receptor (nAChR) expression in the placenta of pregnant women who are smokers and who have preeclampsia were examined immunohistochemically as a result of which it was determined in various histopathological studies in literature as well as in the study published by Machaalani R et al. in 2018 that the cells in the decidua and the villuses, external villouses at the trophoblast are stained positively[24]. Reijnders IF et al. published a study in 2018 in which they evaluated 82 studies on the impact and function of maternal life style on the biomarkers and clinical characteristics of placenta development as well as function. Findings of these studies have indicated that smoking in pregnancy affects the lower first trimester placental vascularization flow index, that the uterine and umbilical arteries are more resistant in the second and third trimesters and that the middle cerebral arterial resistance is lower. Even though it was expected to determine that smoking has a negative impact on placenta weight, the result was not that definitive[25]. Zhao F et al. Published a study in 2018 in which the protective effects of hydrogen sulfide were examined rather than the impacts of smoking during pregnancy. The protective effects of hydrogen sulfide against oxidative damage on the placenta throughout the pregnancy were evaluated. In addition to the reactive oxygen species (ROS), total antioxidant capacity (T-AOC) and Glutathione (GSH) levels, superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), catalase (CAT) and gluta- thione peroxidase (GPx) activities were examined. Nuclear factor erythroid 2 related factor 2 (Nrf2) was analyzed via immunohistochemical staining. Moreover, realtime PCR and Western Blot applications were also included. In the light of all these findings, it was thought that the protective effect of H2S on the placenta in cases of exposure to cigarette is due most probably to the decrease of the imbalance in the Nrf2 pathway[26]. Heidari Z et al. published a study in 2018 in which they examined the placenta tissues according to Cavalieri's point counting method. Statistical analysis was carried out in the method section after applying Masson's Trichrome staining and a statistically significant difference was observed. While a significant difference was determined between the placenta weights of smoker and non-smoker groups in the findings section, statistically significant differences were observed between the total volume in the placenta, intervillous gaps, fibrine and syncytiotrophoblasts ($p<0.05$)[27]. Based on the histopathological findings, a statistically significant increase was also observed in our study in fibrinoid deposits in the intravillous and

intervillous areas as well as necrotic and inflammation areas in the term placenta.

In conclusion, it was determined that there are mothers who smoke and are subject to cigarette smoke before and during their pregnancy, that smoking has adverse impacts on the mother, fetus/newborn and the placenta. It was put forth as a result of the present study that there is a statistically significant relationship between smoking of mothers before and during their pregnancy and the birth weights of the newborns, that smoking decreases birth weight and placenta weight while also resulting in adverse changes in placenta histology.

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Informed consent: Informed consent was needed due to being a study.

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CHAPTER IX

EFFECTS OF LAVENDER OIL ON WOUND HEALING

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INTRODUCTION

Wound healing is one of the oldest topics in medicine. Throughout history, mankind has sought ways to cope with the wound, and this search still continues. In the 19th century, with advances such as infection control, hemostasis and necrosis control, an important progress has been achieved in this field. However, chronic wounds still constitute an important problem.[1]

Numerous methods have been described and many studies have been conducted in the field of wound care and treatment. It is important to know the basics of these methods in order to obtain the desired results in clinical practice.

THE WOUND HEALING PROCESS

Wound healing consists of many processes that are intertwined, that trigger or inhibit each other. Many agents play a role in the activation or inhibition of all these processes.[2] Wound healing phases are defined as hemostasis and inflammation, proliferation and remodeling.[3]

The primary purpose of the hemostasis phase is to stop bleeding, and this is achieved by clot formation.[4] The clot not only stops bleeding but also acts as a barrier against microorganisms as well as a matrix rich in cytokines and growth factors for inflammatory cells, stem cells and progenitor cells.[5] The inflammation phase begins as the clot captures neutrophils in the bloodstream. In recent studies, it has been shown that neutrophils can produce structures that are called neutrophil extracellular traps (NET) and that enable them to capture microorganisms like a network, apart from their phagocytosis functions.[6] Monocyte derived macrophages, which become dominant on day 3 to 5 of injury, play a role in fibroblast proliferation and angiogenesis by releasing cytokines, growth factors and angiogenic factors in addition to their phagocytosis function.[7]

Macrophages are highly malleable and can transform into both proinflammatory and anti-inflammatory cells through different environmental mediators.[8,9] Although mast cells mainly play a role in allergic responses, their importance in immune response regulation in wound healing has been shown in recent studies.[10]

The proliferative phase progresses intertwined with the inflammatory phase and is characterized by granulation tissue formation, angiogenesis, epithelization, and collagen deposition. Fibroblasts and endothelial cells fill the gap opened by the degredation of the fibrin plug.[3] Macrophage-derived growth factors, mainly vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), play an important role in angiogenesis.[2] It has been shown that basal membrane pericytes involved in angiogenesis are able to act as mesenchymal stem cells and transform into bone, adipose tissue and cartilage.[11] Epithelialization process is characterized by the proliferation and migration of epithelial cells. Migration is the rate limiting step of this process; migration disorders result in a chronic non-healing wound. [12]

The function of protein-coding genes is important in the wound healing process. The production of all protein structures takes place through these genes. In recent studies, it has been shown that the transcription of mRNA from DNA and protein translation from mRNA takes place under the control of microRNAs (miRNA). miRNAs are shortchain RNAs found in all eukaryotic cells and not used in coding.[13] miRNAs have begun to be recognized as the most important regulators of the entire wound healing process. In the future, as the specific tasks of miRNAs are better understood, it is thought that miRNA-based therapies can be identified and may be particularly useful in chronic wound treatment.[12]

Wound closure for chronic wounds is defined as epithelized skin that does not require dressing and does not show leakage in 2 control examinations performed 2 weeks apart. There should be no recurrence for at least 3 months to accept the treatment as successful.[12] Recent studies have shown that biofilm-infected wounds may remain functionally open, despite visual closure, and fail to fulfill their barrier function. This condition is called high transepidermal water loss (TEWLhi) and is associated with the increase of biofilm-induced miRNAs. For this reason, measurements such as TEWL in addition to visual evaluation will provide more objective results for evaluating wound healing.[14]

WOUND DRESSING

The ideal dressing method should keep the wound moist, absorb exudate, avoid contamination and prevent dehydration while not traumatizing the wound.[15] For this purpose, many materials have been

developed to be used in the treatment of different wound types and studies in this area continue rapidly. Materials such as films, foams, hydrocolloids, hydrogels and alginates have long been used as dressings. Recently, it has been observed that hydrogels give more satisfactory results by adding antibacterial and antifungal properties or using them as drug carriers .[16,17] The most commonly used topical agents in wound treatment are antibiotics and growth factors to stimulate healing. However, some natural products of herbal origin that were used since the ancient times have been gaining importance because of their antioxidant, anti-inflammatory, antibacterial properties and cost effectiveness.

LAVENDER AND ITS ESSENTIAL OIL

Figure 1: Bunch of lavender, Kocaeli, Turkey

Lavandula species (Lavender) belongs to the Lamiaceae family.[18] Lavender name derives from the Latin word “lavare” which means “to wash” depicting antique usage in washing clothes and baths. Essential oils are used in phytotherapy since ancient times. Lavender essential oil is constituted from monoterpenoids (linalool, camphor etc.) and sesquiterpenoid alcohols, esters, oxides, and ketones mixtures.[19] There are different administration routes of essential oils. They can be administered orally, transdermal and via inhalational route. These administration routes are studied in various therapeutic indications from insomnia to wound healing. European Medicine Agency, herbal medicine products on the medicinal uses committee documented lavender oil therapeutic use in sleep disorders, temporary insomnia, mental stress and mood disorders based on long-standing traditional use.[20] One of the traditional use of lavender in wound healing is not included in the registered indications currently in

EMA. Wound healing and related factors were studied in many in-vitro, in-vivo and clinical studies.

IN-VITRO STUDIES

Lavender essential oil is studied for antifungal properties and resulted with antifungal efficacy in Candida, Cryptococcus neoformans, dermatophyte and Aspergillus strains.[21] A different species of lavender is shown to have anti-leishmanial and antibacterial activities.[22] Bouyahya et al. not only showed these activities they also showed antioxidant activity with the essential oil. A constituent in lavender oil; Terpinen-4-ol, is found to inhibit in vitro growth of human melanoma cell line.[23] In many in-vitro cancer studies anti-proliferative effect is shown.[24] In vitro scratch assay or wound healing assay with a prostate cancer cell line, in parallel resulted reduced healing with lavender essential oil.[24] Perillyl alcohol, a monoterpene constituent in the lavender essential oil is showed to have antiangiogenic effect via VEGF.[25]

Abe et al. evaluated the anti-inflammatory effects in neutrophil adhesion method with various essential oils including lavender oil and reported that the main component in the lavender EO linalool has antiinflammatory effect in TNF-α -induced neutrophil adhesion in-vitro.[26] Even the concentration is higher than the expected concentration of transdermal exposed dose of lavender EO, they supported in-vivo interpretation cannot exclude the potential anti-inflammatory role.

IN-VIVO AND CLINICAL STUDIES

Several clinical studies have been conducted investigating the effects of lavender oil in wound healing.[27] It is well known to have antioxidant, antibacterial, antifungal and analgesic effects. It is also known to accelerate wound contraction.

Hartman and Coetze investigated the effect of lavender and chamomile essential oils in their study on 8 patients with chronic wounds. Complete closure was achieved in 4 of the five patients who were applied the essential oils, and recovery was not possible in 3 patients in the control group until the end of the study. Although it is not possible to make a statistical comparison in this small sample group, it would not be wrong to say that the essential oils used were effective.[28]

Kerr compared the effects of various essential oil blends created in aloe vera gel base on simple wounds and concluded that the most effective combination was lavender, German chamomile, myrrh and tea tree essential oils in aloe vera gel. He reported that this preparation reduced odor, prevented redness and inflammation, and shortened the healing time.[29]

Altaei evaluated the effect of lavender essential oil on oral mucosa ulcers in a 4-step study conducted on both animal and human subjects. Aphthous ulcers treated with lavender essential oil healed faster in both rabbits and humans. In the safety / toxicity study conducted on mice, the LD value for the mice was found to be 6.5 gm / kg and no evidence of dermal irritation was found. In the antibacterial activity study, which is another pillar of the study, effectiveness has been demonstrated against all bacterial strains studied.[30]

The effect of lavender essential oil on wound healing has been evaluated in many studies in the gynecological patient group. In these studies, not only the anti-inflammatory effect of lavender oil but also its analgesic effect were emphasized. Although Vakilian et al. reported that lavender oil applied to episiotomy incisions reduced erythema but had no significant effect on pain,[31] the following studies by Sheikhan et al. and Marzouk et al. have shown that it is associated with less pain, as well as reducing erythema, edema, ecchymosis and discharge.[32,33]

Koca Kutlu et al. showed that lavender essential oil significantly increased EGF and FGF expression compared to the control group in their study comparing the efficacy of several wound treatment methods. EGF is an agent thought to play a role in wound contraction and epithelization. It has become a popularized treatment modality recently, although its topical use is quite expensive. The results of this study suggested that lavender oil could be a cost-effective alternative to EGF application. The authors also reported that no edema, discharge and local infection findings were observed in the lavender oil applied group.[34]

Ben Djemaa et al. concluded that in animals treated with lavender oil, wounds were closed faster, covered with a thinner epidermal layer similar to normal skin, and showed a better epidermal and dermal structure on histopathological examination in their experimental study conducted on rats.[35]

In their 2016 study, Mori et al. showed that topical application of lavender essential oil reduced the wound area earlier, increased the amount of TGF- β and increased the amount of both type I and type III collagen on the excisional wound model in the rat. TGF-β plays a role in wound contraction by inducing fibroblast proliferation and its transformation into myofibroblasts. Type III collagen is the first type of collagen produced during the proliferation phase of wound healing and then matures and is replaced by Type I collagen. In the same study, the fact that the amount of type III collagen was close to the control group, but the amount of type I collagen was higher in the group in which lavender oil was applied on the 7th day measurement, was interpreted as collagen maturation occurred faster.[36]

SAFETY DATA

Safety information is important for herbal medicinal products. During pregnancy and lactation safety data has not been established for lavender essential oil. In the absence of the sufficient data, rationally the use during pregnancy and lactation is not recommended. EMA reminds its potential on the impairment of the ability to drive and use machines. Children below 12 years-age are excluded from the eligible data. In a multicenter clinical study linalool and its hydroperoxide fraction were found to be common contact allergens in dermatitis patients.[37] Moreover prepubertal gynecomastia is associated with lavender oil topical exposure in three cases and in vitro cell line studies indicated lavender oil had estrogenic and antiandrogenic activities.[38]

CONCLUSION

Lavender oil is a traditional medicinal product that has been used for many years in wound healing. With recent experimental and clinical studies, its antioxidant, antimicrobial, anti-inflammatory and analgesic effects have been revealed and details of its mechanism of action have been brought to light. In the future, with the help of more detailed and extensive studies it may be possible to develop standardized treatment protocols and gather more safety data for wound healing.

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