MULTIDISCIPLINARY APPROACH TO BASIC AND CLINICAL SCIENCE

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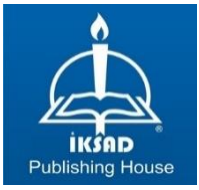

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PREFACE

This book covers the exciting discoveries of different disciplines in medicine and important topics that will shape the future of health. With a multidisciplinary perspective, the book aims to provide readers with scientific enrichment by covering a wide range of medical topics from basic to clinical. The chapters, written by expert authors, are designed to provide in-depth information without requiring readers to be familiar with the subject.

Comprising eighteen chapters, each of which has undergone a blind peer-review process, we hope that this work will be of interest to a wide readership as well as being a resource for medical students, physicians, health professionals and medical enthusiasts. Get ready to discover the infinite potential of medicine and the power of science to protect and improve human health.

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CHAPTER 1

HEAVY MENSTRUAL BLEEDING, MANAGEMENT AND PICTORIAL BLEEDING ASSESSMENT CHART

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INTRODUCTION

In a normal menstrual cycle, a woman will lose 30–40 ml of blood. Menstrual bleeding that is more than 80 ml is called heavy menstrual bleeding (HMB) (Duckitt and Collins 2012). HMB is also defined as an increase in volume that lowers the patient's physical, social, emotional, and economic quality of life ("National Collaborating Centre", 2007).

Heavy menstrual bleeding (HMB) is a specific type of abnormal uterine bleeding (AUB). AUB contains both the abnormal amount of menstrual bleeding that is the subject of HMB and pathologies in the duration or cycle of menstruation. In addition, the definition of HMB refers to a rise in menstrual bleeding during ovulatory cycles.

The period between the first day of menstruation and the first day of the next menstruation is a cycle. This cycle should not be less than 24 days or more than 38 days. Menstruation should last no less than four days and no more than eight days. A menstrual cycle shorter than 24 days, menstruation lasting longer than 8 days, and menstrual blood loss exceeding 80 ml indicate heavy menstrual bleeding (Munro et al. 2018). Heavy menstrual bleeding can be acute or chronic. Chronic bleeding is bleeding that has occurred within the last six months, whereas acute bleeding is defined as a bleeding episode severe enough to require intervention ("Diagnosis of Abnormal Uterine Bleeding," 2012).

Because they cause confusion, terms such as menorrhagia, metrorrhagia, polymenorrhea, hypermenorrhea, and dysfunctional uterine bleeding are no longer used (Woolcock et al. 2008). Table 1 shows the classification recommended by the International Federation of Gynecology and Obstetrics (FIGO) for uterin bleeding parameters (Munro et al. 2018):

Table 1. Parameters and definitions for uterine bleeding according to FIGO

Parameters for Menses	Descriptive Term	Definition
Frequency	Rarely	>38 days
	Normal	24-38 days
	Frequent	<24 days
Duration of menses	Shorten	<4 days
	Normal	4-8 days
	Prolonged	>8 days
Amount	Mild	<5 mL
	Normal	5-80 mL
	Heavy	>80 mL

Two billion people are affected by iron deficiency (ID) worldwide (Miller 2013). Menstrual blood loss is an additional common cause of ID in developed nations (Petraglia and Dolmans 2022). In this case, exceeding physiologic limits for HMB causes both ID and iron deficiency anemia (IDA). Despite the high prevalence of HMB among women (approximately 30%) and its impact on quality of life, ID and IDA are still underdiagnosed and undertreated among women of childbearing age (Petraglia and Dolmans 2022).

The investigation of the presence of HMB is crucial to determining the cause of ID, which can also result in significant economic losses. Therefore, regardless of whether HMB is described in female patients with ID, HMB should be ruled out categorically. If HMB is the initial clinical manifestation, ID should be investigated, and if ID is present in a female patient during the reproductive period, HMB should be investigated.

The phenomenon that brings HMB to the recognition of the clinician is related to the patients' perception of an increase in menstrual blood volume. However, awareness of HMB among patients, their family members, and medical professionals appears insufficient. Actual cases of HMB are sometimes viewed as the norm for the patient or as a normal process without treatment (Hallberg et al. 1966; Kamaludin, Zhang, and Shorey 2019).

In a survey with a large sample size, almost fifty percent of those surveyed said they did not know anything or very little about HMB. Moreover, 39% of participants in the same study believed that there is no cure for HMD (Bitzer, Serrani, and Lahav 2013).

On the other hand, certain social perceptions and/or social motives also contribute to HMB concealment (Dutton and Kai 2022). It has been reported that the reality of war-caused migration, which affects the entire world today, has a negative effect on the expression of HMB (Hawkey et al. 2017).

EPIDEMIOLOGY

The prevalence of HMB among women of childbearing age ranges from 10% to 30% (Liu et al. 2007). Up to fifty percent of perimenopausal women are affected ("Diagnosis of Abnormal Uterine Bleeding," 2012). It is one of the most prevalent reasons for admission to gynecology outpatient clinics, regardless of the patient's reproductive or postmenopausal status (Fraser et al. 2011). Again, 5% of women between the ages of 30 and 49 consult family physicians annually for this reason (Vessey et al. 1992). In the United Kingdom, HMB is the fourth most common reason for referral to gynecology services (Vo et al. 2013).

About two-thirds of HMB is due to organic causes. The remaining one-third of women experience dysfunctional uterine bleeding, which includes bleeding without an organic cause (Telner and Jakubovicz 2007). It accounts for 25% of gynecologic procedures (Matthews 2015).

ETIOLOGY

PALMCOEIN (Munro, Critchley, et al. 2011) is an acronym that defines the common causes of HMB. The classification system comprises nine main classes (Table 2).

Table 2. Factors that contribute to heavy menstrual bleeding

Organic Causes (PALM)	Non-organic Causes (COEIN)
Polyps	**C**oagulopathy
Adenomyosis	**O**vulatory disfunction
Leiomyoma	**E**ndometrial Causes
Malignancy and Endometrial Hyperplasia	Iatrogenic Causes
	Not yet classified

In general, the PALM group comprises structural abnormalities that can be detected using imaging techniques, whereas the COEIN group comprises non-structural pathologies that cannot be diagnosed using ultrasound and other imaging techniques (Harlow, Lin, and Ho 2000; Munro, Broder, et al. 2011). PALM includes organic causes such as polyps, adenomyosis, leiomyoma, and malignancy, whereas COEIN includes coagulopathy, ovulatory dysfunction,

endometrial causes, iatrogenic causes, and unclassifiable nonorganic pathologies (Marnach and Laughlin-Tommaso 2019; Munro, Critchley, et al. 2011).

There may be a single etiology or multiple etiologies contributing to HMB. Therefore, all potential causes should be thoroughly investigated and evaluated in patients.

In order to select the appropriate diagnosis and treatment strategies, the PALMCOEIN classification is utilized in conjunction with the patient's medical history as well as relevant imaging, histopathologic examination, or laboratory evaluations (Munro, Broder, et al. 2011; Sriprasert et al. 2017). These explanations are going to be dissected in detail under individual subheadings.

Organic Causes

- Endometrial polyps

One of the most common factors contributing to heavy menstrual bleeding is the presence of endometrial polyps. The growth of endometrial glands and stroma that results in the formation of a sessile or stalked protrusion from the surface of the endometrium is referred to as endometrial hyperplasia (Wong et al. 2017).

- Adenomyosis

Adenomyosis is the pathological condition that is characterized by the abnormal involvement of the endometrial gland and the stroma in the myometrium. Even though a histopathologic examination is necessary for a definitive diagnosis, a clinical diagnosis can be made with the use of ultrasound and magnetic resonance imaging (MRI) (Ferenczy 1998; Munro, Broder, et al. 2011).

- Leiomyoma

Leiomyomas are the most common type of pelvic neoplasm found in women. They are also referred to as fibroids and fibromas. They are tumors of benign smooth muscle and, in most cases, there are no symptoms associated with them. The incidence varies between 70-80%. They come in a variety of sizes, quantities, and locations (Baird et al. 2003). Studies have shown that

submucosal leiomyomas, in particular, have a greater likelihood of causing HMB (Galen, Isaacson, and Lee 2013).

- Malignancy and Endometrial Hyperplasia

Endometrial cancer and endometrial hyperplasia are both associated with estrogen exposure of the endometrium that is not balanced by progestin. This is one of the many risk factors for endometrial cancer. Increasing age, use of tamoxifen, early menarche, late menopause, nulliparity, polycystic ovary syndrome, obesity, diabetes mellitus, estrogen-secreting malignancies, and a family history of endometrial cancer are all factors that are considered risk factors (Epplein et al. 2008, 2009).

-Arteriovenous Malformations (AVM)

AVMs can occasionally be found in the uterine cavity. When looking into the causes of HMB that cannot be explained, an AVM should be suspected in a patient who experiences an increase in the amount of bleeding that occurs during dilatation and curettage.

Non-Organic Causes

- Coagulopathy

Coagulopathy refers to all conditions that impair hemostasis for any reason. In patients who present with heavy menstrual bleeding, coagulopathy should be considered, and patients should be evaluated accordingly. There is a 24% possibility that a genetic bleeding disorder is the underlying cause (Dragoman et al. 2018). Von Willebrand disease is the most common cause. The presence of severe bleeding, particularly after menarche, should alert a physician to coagulopathy (Vo et al. 2013).

- Ovulatory Disorder

Ovulatory dysfunction is the medical term for infrequent, irregular, or absent ovulation. To use this term, however, patients must not be taking any hormonal medications and must have had a menstrual period lasting between 7 and 9 days in the previous 6 to 12 months, depending on their age. Typically, these patients have menstrual irregularities and cycles with variable amounts and timing of bleeding (Prior et al. 2015).

- Endometrial Causes

Endometrial causes may explain abnormal uterine bleeding in women with regular menstruation and normal ovulatory cycles, if there is no other underlying cause. An increase in plasminogen activator, which disrupts hemostasis in the endometrium, and a decrease in endothelin-1 and prostaglandin F2 alpha are to blame. Endometritis is an additional source of abnormal bleeding. In these causes of abnormal uterine bleeding, ovulatory cycles are observed (Deneris 2016).

- Iatrogenic Causes

Increased vascularization, congestion, and degeneration of the endometrium may cause excessive menstrual bleeding, particularly in patients with intrauterine copper devices (Andersson, Odlind, and Rybo 1994; Munro, Critchley, et al. 2011). This group ("Drugs for Depression," 2020) also examines bleeding caused by pharmacologic agents such as anticoagulants and hormones previously administered to the patient.

- Unclassifiable

Conditions that may cause abnormal uterine bleeding but are insufficiently defined or infrequently encountered and therefore not covered under other headings are covered here. Under this heading, uterine scar defects and isthmocele can be evaluated. Typically, both of these conditions occur following cesarean delivery (Tower and Frishman 2013).

DIAGNOSIS

Patients of reproductive age and non-pregnant women who present with HMB should have their bleeding pattern, intensity, and frequency evaluated first. To understand the etiology, it is necessary to inquire about the family history, medications, and co-morbidities. Menarche age, menstrual history, and pattern should be determined. The date and duration of the last menstrual period should be inquired about. Gravidity, parity, miscarriage, abortion, and ectopic pregnancy must be identified. The presence of postcoital bleeding and method of contraception are also crucial factors to investigate (Fritz and Speroff 2010; Nebgen et al. 2016).

Patients must also be questioned about the presence of coagulopathy, as well as bleeding in other mucous areas (mouth, teeth, nose) and prolonged bleeding after skin incisions. If there has been a history of bleeding since childhood, genetic diseases, particularly hemophilias, should be considered.

It is also known from scientific literature conducted in the past that women's subjective estimates cannot accurately predict the volume of their menstrual flow. In contrast, very few patients who believe they have heavy bleeding actually have this condition (Hallberg and Nilsson 1964; Mansour, Hofmann, and Gemzell-Danielsson 2021). Utilized hygiene products and hygiene needs vary between women. There is not always a correlation between the number of pads changed and excessive bleeding. So, the patient should be tested with a method that can objectively measure the amount of menstruation (Zakherah et al. 2011). The self-perception of menstrual loss by patients has been deemed unreliable (Warner et al. 2004). This has necessitated the development of various objective methods for assessing menstrual bleeding. Although the Alkaline Hematin (AH) method is the most accurate of these techniques, it is impractical for use in outpatient clinic settings. Pictorial blood loss assessment charts (PBAC), which are recommended as a more practical alternative, are now being utilized. This method is simple for both the physician and the patient to employ.

Physical examination

Physical examination, particularly of vital signs, should be performed with care. Depending on the amount of HMB and active bleeding, low blood pressure and tachycardia may be detected.

With HMB, the presence of petechiae, purpura, and ecchymosis may be significant indicators of coagulopathies.

During pelvic examination, the primary consideration is whether the bleeding is due to uterine bleeding. Vaginal findings of trauma, foreign body, or infection should be investigated. The status of cervical polyp, lesion, and infection should be evaluated. Douglas, perineum, and rectum should be evaluated through a rectovaginal examination. Finally, an abdominal examination should be performed to evaluate the presence of possible comorbid conditions (Fritz and Speroff 2010).

Sampling of Tissue

Endometrium sampling is necessary to rule out endometrial hyperplasia and malignancies, which may be the underlying causes of HMB. In addition, polyps and intracavitary fibroids can be diagnosed with this sample. When presenting with abnormal uterine bleeding, women older than 45 years old should have an endometrial sample taken. In the presence of unopposed estrogen (obesity, PCOS), endometrial sampling is also recommended for patients younger than 45 (Armstrong et al. 2012).

Laboratory and Imaging

Methodology for diagnosing patients with HMB 1) Etiologic investigations and 2) ID/IDA detection should be conducted under two distinct headings.

The presence of pregnancy should initially be ruled out using beta-hCG. Additional hormone levels that may be ordered include thyroid function and prolactin levels.

Although the approach should be based on the patient's medical history, coagulopathy screening typically includes a baseline hemogram, peripheral smear, prothrombin time, and activated partial thromboplastin time. In instances of ongoing active bleeding, fibrinogen and d-dimer should be added to these initial tests.

In detailed and/or special coagulopathy tests, parameters such as bleeding time, aggregation tests, thrombin time, reptilase time, von Willebrand disease tests, platelet function tests, factor levels, antithrombin, Protein-C, Protein-S, and von Willebrand disease tests may be requested (İfran 2014).

In addition to hemogram, the ID/IDA investigation should order transferrin saturation (TSAT), ferritin, and c-reactive protein (CRP). A ferritin level between 20 and 30 ng/mL is sufficient to diagnose ID on its own. With the addition of a decrease in hemoglobin (HGB), it is now possible to discuss IDA. According to WHO, anemia is defined as hemoglobin below 12 g/dL in females (Cappellini and Motta 2015). If necessary or suspected, a peripheral smear must also be performed. Since ferritin may increase as an acute phase reactant in inflammatory conditions (high CRP, infections, chronic diseases,

etc.), the presence of normal ferritin in patients with HMB does not exclude the presence of ID. In such instances, TSAT scores below 20% are again indicative of ID.

The pelvic USG should be the primary imaging technique because it is simple to perform and yields quick results. With pelvic USG, the uterine cavity should be thoroughly evaluated, and endometrial thickness should be measured. Thickness above 4 mm necessitates endometrial sampling (van Hanegem et al. 2016), especially in postmenopausal women.

If pelvic USG reveals no pathology and no cause for heavy menstrual bleeding is identified, pelvic MRI should be performed.

TREATMENT

The main goals of the management and treatment of HMB are to correct the underlying etiology, if present, to achieve a normal amount of bleeding (PBAC 100), to treat ID/IDA, to prevent endometrial hyperplasia or cancer, and to enhance quality of life ("Cervical Cancer Screening and Prevention," 2016; Benetti-Pinto et al. 2017).

The optimal time to begin HMB therapy depends on the patient. For patients whose daily activities are unaffected by HMB, treatment can be postponed until diagnostic approaches yield results. Alternatively, if daily activities are impaired and/or significant IDA is present, immediate treatment should be initiated while the diagnostic process is ongoing (Kaunitz 2023).

When evaluating treatment options, the following points should be considered:

- Patient preference

- Presence of comorbidity

- Presence of fibroids (including size, number, and location), polyps, endometrial pathology, or adenomyosis

- Presence of pain and pressure sensation

- Presence of pain and pressure sensation

The treatment options are medical or surgical. Medical therapies are classified as either hormonal or non-hormonal. Hormonal treatments include progestins, combined oral contraceptives, androgens, and GNRH analogs, whereas non-hormonal treatments include nonsteroidal anti-inflammatory drugs and fibrinolytics. Curettage, endometrial ablation, uterine artery embolization, and hysterectomy are surgical treatments for endometrial cancer (Bradley and Gueye 2016). The objective of treatment should be to select the most conservative and beneficial treatment for the patient.

The UK National Institute for Health and Care Excellence (NICE), which was updated in 2021, examines the management and treatment of HMB by dividing it into the presence or absence of fibroids smaller than 3 centimeters and fibroids larger than 3 centimeters ("Heavy menstrual bleeding," 2021). In Figure-1, the NICE recommendations are presented as an algorithm.

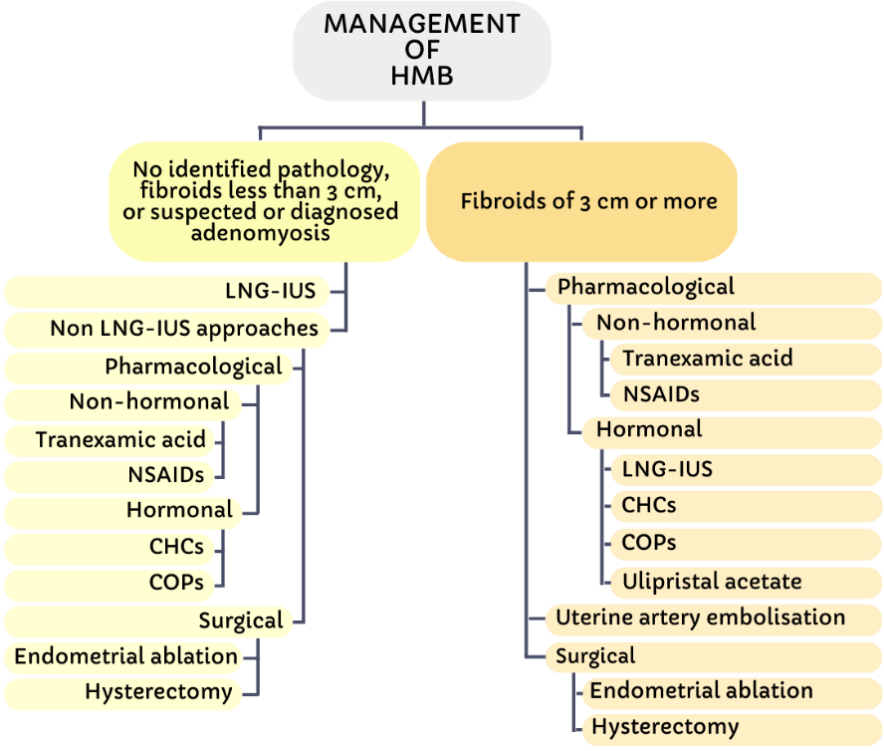

Figure 1. The NICE recommendations for HMB management. *Abbreviations.* LNG-IUS, levonorgestrel intrauterin device; NSAIDs, non-steroidal anti-inflammatory drugs; CHCs, combined hormonal contraception; COP, cyclical oral progestogen.

PICTORIAL BLOOD LOSS ASSESSMENT CHART (PBAC)

PBAC is a semi-quantitative method for the assessment of heavy menstrual bleeding, first described by Higham and Shaw in 1990 and modified by Janssen et al. in 1995 (Higham, O'Brien, and Shaw 1990; Janssen, Scholten, and Heintz 1995). In this method, scoring is done by looking at the amount of bleeding in pads or tampons used during a menstrual period. The specificity and sensitivity of the PBAC score was found to be 89% and 86%, respectively (Higham et al. 1990). However, in a systematic review of studies comparing PBAC with the AH method, sensitivity and specificity rates were reported to range from 58% to 98% and 7.5% to 97%, respectively (El-Nashar, Shazly, and Famuyide 2015).

Although PBAC is not yet a fully standardized method (Munro, Critchley, et al. 2011), it has been stated that it can be used due to its ease of use, stability in showing menstrual volume increase and complementarity in clinical use (Magnay et al. 2018).

Another finding showing the prevalence of the use of PBAC is that in 66 separate randomized controlled trials (RCTs) on HMB, PBAC was the most preferred method for measuring the amount of bleeding (Duffy and McManus 2016).

In a study of variation in PBAC conducted by Hald and Lieng in 2014 using 1049 menstrual cycles from 429 patients, it was determined that the variation in PBAC score between individuals was high (Hald and Lieng 2014). However, this variation was reported to be lowest for PBAC scores below 100. High PBAC scores were also found to be associated with Hb concentrations <12 g/dL, albeit with low sensitivity and specificity. The authors of this study noted that a cutoff value of 100 indicates normal or minimal bleeding, 80 mL blood loss, and normal hemoglobin levels. In another study supporting this article, 10% of patients with a PBAC score above 100 who were thought to have normal menstrual bleeding were found to have anemia (Ko, Lao, and Cheung 2021). Therefore, it may be deemed necessary to calculate the PBAC score in anemic patients even if they do not complain of heavy menstrual bleeding.

There are images of lightly, moderately, and heavily soiled pads and tampons in PBAC (Figure-2). Patients are asked to make a decision based on their use of sanitary pads or tampons and the amount of bleeding. Scoring should be performed independently. According to the amount of bleeding, 1,5,20 points are given out for the use of pads, while 1,5,10 points are awarded for tampons. In the presence of a clot in bleeding, 1 point is given for a small clot and 5 points are given for a large clot, with the final score being the sum of the points given.

Patients with a score greater than 100 must be evaluated for heavy menstrual bleeding (Higham et al. 1990). A 1995 study revealed that a score greater than 185 had a greater positive and negative predictive value. In most studies, this value was taken as 150 (Janssen et al. 1995).

PADS	BLEEDING DAYS											SCORE
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	
												.... x 1
												= .....
												.... x 5
												= .....
												.... x 20
												= .....
										TOTAL		.....

Figure 2. An example of PBAC with pads

By completing the PBAC scoring system, the anamnesis of a patient presenting with heavy menstrual bleeding can be strengthened. In addition to the patient's anamnesis and PBAC score, a physical examination and laboratory and imaging techniques as necessary should be requested. Due to the fact that the patient performs a retrospective evaluation, the evaluation is occasionally inferior. It would be appropriate for the patient to complete this chart during her menstrual cycle for a more accurate evaluation.

The PBAC score is a method that directs the physician in both treatment and diagnosis. By recalculating the PBAC score in post-treatment controls, the physician can determine the effectiveness of the administered treatment. It has been demonstrated that a decrease in the score is associated with an improvement in quality of life (Herman et al. 2016). This demonstrates that the PBAC score is an effective method for both diagnosis and post-treatment

monitoring (Higham et al. 1990). The PBAC score is believed to prevent unnecessary drug treatment or surgery (Fritz and Speroff 2010), in addition to being a practical and applicable method in the clinic.

The development of websites or mobile applications that calculate the PBAC score so that patients can evaluate their own bleeding may be effective for evaluating the patient's bleeding and submitting an application to a healthcare facility. This will aid in early detection (Ko et al. 2021).

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CHAPTER 2

COMMON GYNECOLOGICAL EMERGENCIES

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1. ECTOPIC PREGNANCY

1.1. Introduction, Definition and Risk Factors

Ectopic pregnancy refers to the implantation of a fertilized egg outside of the uterus after fertilization. Normally, a fertilized egg travels through the fallopian tubes towards the uterus and implants there. However, in some cases, the fertilized egg may implant outside of the fallopian tubes, in the ovaries, cervix, abdominal cavity, or other rare locations.

Although the exact cause of the development of ectopic pregnancy is not fully understood, several theories have been proposed. Ectopic pregnancy may result from a combination of factors such as abnormalities in the fallopian tubes, hormonal imbalances, and difficulties in the transportation of the embryo.

Damage or scarring of the fallopian tubes, caused by infections, surgical interventions, or other factors, can hinder the normal progression of the fertilized egg from the fallopian tubes into the uterus, resulting in the implantation of the embryo in the fallopian tube or in other locations outside the uterus (Barnhart, 2009).

Any factor that hinders or delays the transportation of the fertilized egg from the fallopian tubes to the uterus, such as abnormalities in the cilia of the fallopian tubes or muscular contractions, can increase the risk of ectopic pregnancy (Bouyer et al., 2002).

Ectopic pregnancies account for approximately 1-2 % of all pregnancies and are the most common type of pregnancies that implant outside the uterus (de Bennenot et al., 2012).

- Previous ectopic pregnancy
- Pelvic inflammatory disease
- Sexually transmitted infections
- Tubal surgeries
- Assisted reproductive techniques
- Smoking
- Advanced maternal age
- Contraceptive failure (e.g., failure of intrauterine devices or contraceptive implants)

1.2. Diagnosis

Serial serum β-hCG measurements and transvaginal ultrasound are used to make early and accurate diagnosis of ectopic pregnancy. Although the incidence of ectopic pregnancy has been on the rise, the mortality rate associated with this condition has been steadily declining. Patients typically seek medical care during the early weeks of pregnancy due to symptoms such as pain and abnormal vaginal bleeding. If an ectopic pregnancy has ruptured, patients may present to the hospital with hypovolemic shock.

Heterotopic pregnancy refers to the concurrent occurrence of an intrauterine pregnancy and an ectopic pregnancy. Although spontaneous heterotopic pregnancy is a rare occurrence, the incidence of heterotopic pregnancies has significantly increased, estimated to be around ten times higher, with the utilization of assisted reproductive techniques (Luna Lugo et al., 2011).

The presence of decidual tissue and Arias-Stella reaction in the endometrial curettage specimen without fetal tissues and placental appendages is significant for the diagnosis of ectopic pregnancy (Jurkovic et al., 2011).

1.3. Management

The management of ectopic pregnancy can be followed and treated medically or surgically depending on the patient's clinical condition. The treatment plan is determined based on factors such as the location, size of the pregnancy, patient's symptoms, overall health status, and whether the pregnancy has ruptured or not. For example, small-sized ectopic pregnancies in stable patients may be managed medically, while ruptured or large-sized pregnancies may require surgical intervention. Laparoscopy or laparotomy can be performed as surgical treatments (salpingostomy, salpingotomy, or salpingectomy).

When a stable hemodynamic condition is observed in patients with diagnosed unruptured ectopic pregnancy, medical treatment should be considered as the first choice. In the medical management of ectopic pregnancy, the most frequently employed approach is the administration of intramuscular

methotrexate (MTX) at a dose of 50 mg/m^2 as a single injection (Davenport et al., 2022).

The administration of MTX as a treatment option for ectopic pregnancy is preferably considered when the β-hCG concentration is below 5000 mIU/ml and there is no fetal cardiac activity detected. MTX is a folate antagonist that is used in the treatment of various diseases. Before administering MTX to the patient, a complete blood count, liver function tests, serum creatinine, Rh determination, and chest X-ray should be obtained. Following MTX treatment, β-hCG levels may initially show an increase for a few days, but then gradually decline (Sivalingam et al., 2011).

After treatment for ectopic pregnancy, close follow-up with health care providers is crucial to monitor the recovery process and ensure that β-hCG levels return to normal. Serial β-hCG measurements and ultrasound examinations are typically performed to confirm the resolution of the ectopic pregnancy and to rule out any residual or recurrent ectopic pregnancies (Brady, 2017).

The prognosis of ectopic pregnancy depends on various factors, including the location of the ectopic pregnancy, the timing of diagnosis and treatment, the extent of damage to the fallopian tube, and the overall health of the patient. Early diagnosis and appropriate management can significantly improve the prognosis. However, ectopic pregnancy can still be a serious and potentially life-threatening condition if not promptly diagnosed and treated.

In conclusion, ectopic pregnancy is a condition where the fertilized egg implants outside of the uterus, typically in the fallopian tubes. It can cause serious complications if not diagnosed and treated promptly. Close monitoring, early diagnosis, and appropriate management are crucial in improving the prognosis for women with ectopic pregnancy. It is vitally important that patients seek immediate medical attention if they have signs of an ectopic pregnancy, such as abdominal pain or abnormal vaginal bleeding. (Sivalingam et al., 2011).

2. TUBO-OVARIAN ABSCESS

2.1. Introduction, Definition and Risk factors

Tubo-ovarian abscess (TOA) can be defined as a serious complication of untreated pelvic inflammatory disease (PID). It most commonly affects women of reproductive age, and more than half of women with TOA are nulliparous (Rosen et al., 2009).

TOA has a high morbidity and can be a potentially fatal disease, with death rates of up to 10% in cases of sepsis. Clinically, it is characterized by increased inflammatory markers and pelvic mass. Abscess drainage or excision may be performed as treatment, but there is no consensus on the optimal timing of surgery. Complications of TOA include infertility, ectopic pregnancy, and chronic pelvic pain (Chappell et al., 2012).

Tubo-ovarian abscess can be caused by various organisms, with a significant portion of TOAs resulting from sexually transmitted microorganisms. TOAs can rarely be caused by the transmission of atypical microorganisms or by direct or hematogenous spread of intra abdominal infections such as appendicitis, diverticulitis, or pyelonephritis. Nearly half of the cases are poly microbial (Chan et al., 1995). There are many risk factors for tubo-ovarian abscess (Halperin et al., 2013).

- Intrauterine device
- Pelvic inflammatory disease
- Early onset of sexual intercourse
- Multiple sexual partners
- Immunosuppression

2.2. Diagnosis

If women experience pelvic pain and discomfort, PID should be considered as a possible diagnosis. In severe cases, signs of sepsis such as tachycardia, hypotension, and increased lactate levels may be present. High CRP (C reactive protein) levels are the most sensitive indicator of TOA. The absence of fever or leukocytosis does not rule out TOA. Diagnosing TOA may not always be easy. Differential diagnosis should include appendicitis, endometriosis, ovarian cyst, ovarian cyst rupture, ovarian torsion, ectopic

pregnancy, diverticulitis, or malignancy should be considered (Demirtas et al., 2013).

TOA can present as a pelvic mass and can be diagnosed with ultrasound. Pyosalpinx can be seen as a partially septated and thick-walled, elongated, fluid-filled mass. The presence of septate within the tubes is a sensitive indicator for tubal inflammation and abscess. The presence of "cogwheel" sign, caused by thickened endosalpingeal folds, can be observed. This sign is specific to acute tubal inflammation. Ultrasound is the first step in diagnosis and treatment. Magnetic resonance imaging (MRI) and Computed tomography (CT) can be used in complex cases (Dupuis et al., 2015).

2.3. Management

The initial treatment for a woman with suspected TOA is determined based on clinical findings and ultrasound. If there is acute abdomen, emergency surgery may be considered. If the woman is systemically stable, surgical intervention is deferred and antibiotic therapy is initiated.

Antibiotic treatment is effective in approximately 75% of patients with tubo-ovarian abscess (TOA); however, the recurrence rate of this treatment is high. Successful antibiotic treatment relies on the ability to penetrate the abscess and be effective against the most commonly encountered pathogens. Vital signs should be monitored closely, fluid balance and urine output should be carefully observed. White blood cell count and C-reactive protein levels should be assessed daily. Compression stockings should be applied to prevent venous thromboembolism, and low molecular weight heparin may be initiated if necessary. Patients who become afebrile and show clinical improvement after receiving antibiotic treatment for 48-72 hours may be discharged with oral treatment (Goharkhay et al., 2007).

There is no established optimal timing for surgery in cases where antibiotic treatment is ineffective or there is no clinical improvement. Drainage or surgical intervention may be considered for patients who do not respond to antibiotics or do not show clinical improvement. Drainage can be performed under Ultrasound or Computed tomography guidance, with a high success rate (Karakulak et al., 2008).

TOA surgery is technically challenging due to the presence of necrotic tissues, which can easily fragment and cause bleeding. Visualization of intra-abdominal organs becomes difficult. Adhesions may increase, leading to a higher risk of visceral organ injury. Depending on the patient's fertility desires, fertility-preserving surgery or total abdominal hysterectomy with bilateral salpingooophorectomy may be planned. Tissue specimens must be examined pathologically to rule out the possibility of malignancy.

3. OVARIAN TORSION

3.1. Introduction, Definition and Risk factors

Over torsion is the disruption of blood flow to the ovary due to complete or partial rotation of the ovary around its ligaments. If the fallopian tube is also involved in torsion along with the ovary, it is referred to as adnexal torsion. Isolated fallopian tube torsion is quite rare (Shrager et al., 2012).

The most common factors leading to ovarian torsion in adults are functional cysts or neoplasms of the ovary. As the size of the mass increases, the likelihood of torsion also increases. However, masses that progress with adhesions (endometriomas, TOAs) are less likely to undergo torsion. Torsion can occur in normal-sized ovaries, although the exact mechanism is not fully understood (Celik et al., 2005).

Torsion can affect women of all ages, but it is more commonly seen in adolescent girls. Torsion of the right ovary is more frequent than that of the left ovary, which is thought to be due to the longer right uteroovarian ligament. The sigmoid colon being located on the left side is considered to be one of the reasons for the lower occurrence of torsion on the left side (Huchon et al., 2010).

Infundibulopelvic ligament rotation causes compression on the ovarian vessels, disrupting arterial, venous, and lymphatic flow of the ovary. Initially, venous flow is compromised while arterial flow continues. Due to the persistent arterial flow, edema, increased size, subsequent ischemia, and necrosis occur in the ovary.

The greatest risk factor for ovarian torsion is particularly cysts with a diameter of up to 8 cm (Asfour et al., 2015). Risk increases during pregnancy

and in patients undergoing ovulation induction therapy for infertility. The majority of torsioned masses are benign in nature as malignant masses are more prone to fixation. In post menopausal ovarian torsions, the proportion of malignant masses increases and can reach up to 20% (Yousefi et al., 2015).

3.2. Diagnosis

The definitive diagnosis of ovarian torsion is made during surgery by visualizing the torsioned ovary. The decision for surgery in cases of ovarian torsion is based on clinical pre-diagnosis, taking into consideration the symptoms, physical examination findings, and ultrasound findings.

The classical symptom of ovarian torsion is sudden onset of moderate to severe pelvic pain, which may be accompanied by nausea, vomiting, and adnexal mass. However, the clinical presentation can vary. Pelvic USG is the initial imaging modality for suspected ovarian torsion, as it is cost-effective and has similar diagnostic accuracy compared to CT and MRI. In cases where USG is inconclusive, MRI can be useful. MRI findings in ovarian torsion typically show an enlarged and edematous ovary, similar to USG findings. "Whirl pool sign" may be visualized on contrast-enhanced MRI (Lourenco et al., 2014).

3.3. Management

The main principle of treatment for ovarian torsion is to correct the torsion. In reproductive-age women, the primary goal should be to preserve the ovary. Unless there is obvious necrosis in the torsioned ovary, after detorsion, the ovary usually returns to its normal function. Studies have reported that even in cases where the ovary appears cyanotic or necrotic, after detorsion, the function of the ovary can recover and follicular development can continue.

In cases where the normal anatomical structure is lost and the ovary appears gelatinous, oophorectomy may be considered. If there is suspicion of malignancy or if the patient is postmenopausal, oophorectomy may be selected as a treatment option (Bozdag et al., 2014).

4. OVARIAN CYST RUPTURE

4.1. Introduction, Definition and Risk factors

Ovarian cyst rupture is the bursting of a cyst (a fluid-filled sac) in the ovary. In this condition, the fluid, blood, or other contents of the cyst can spill outside the ovary and irritate the surrounding tissues. Ovarian cyst rupture can cause symptoms such as abdominal pain, bleeding, and other signs, and it typically occurs in females of reproductive age.

In the regular menstrual cycle, physiological rupture of follicular cysts, which are usually smaller than three cm, happens with each ovulatory cycle. This natural occurrence is usually symptom-free or may be associated with mild mid-cycle pain, also known as Mittelschmerz, likely due to a small amount of blood being released during the rupture of the follicular capsule (Farghaly et al., 2014).

Serous fluids are generally not irritating, and patients with rupture of a simple cyst may not experience symptoms even if a large amount of intra peritoneal fluid accumulates. On the other hand, rupture of a hemorrhagic cyst often causes pain, which may be due to blood accumulating in the ovary and stretching the ovarian cortex, or blood flowing into the abdomen and irritating the visceral peritoneum. It's worth noting that not all patients may perceive pain associated with ovulation, and some may not even notice small amounts of hemoperitoneum. In cases where a dermoid cyst ruptures and sebaceous material spills, it can cause a significant granulomatous reaction and chemical peritonitis, which is usually quite pain full (Kim et al., 2014).

The exact incidence of ruptured ovarian cysts is not accurately known. Hospital admission rates where benign ovarian cysts are diagnosed can provide some data for estimating the incidence, but these rates may be overstated as they may also include admissions for other complications of ovarian cysts, such as hemorrhage or torsion. Ruptured ovarian cysts commonly occur in patients who are of reproductive age. However, there have also been reports of ovarian cyst rupture in postmenopausal patients (Yamakoshi et al., 2016)

- Ovulation induction
- History of ovarian cysts

- Known cyst
- Vaginal intercourse
- Thrombocytopenia
- Anticoagulation therapy

4.2. Diagnosis

Rupture of an ovarian cyst can either be asymptomatic or may present with pelvic and/or abdominal pain. The classic symptoms include the sudden onset of unilateral lower abdominal pain, which may occur after strenuous physical activities such as sexual intercourse or exercise. If a patient has a history of ovarian cysts or is known to have a current ovarian cyst, it should raise suspicion for the possibility of ovarian cyst rupture.

The nature of the pain associated with ovarian cyst rupture is usually sharp and localized, with varying degrees of intensity ranging from moderate to severe. In cases of significant hemorrhage, patients may also experience shoulder or upper abdominal pain due to blood extravasation under the diaphragm. Some patients may also report increased pain with sitting, possibly due to irritation of the psoas muscle.

If there are any signs of hemodynamic instability, such as low blood pressure or rapid heart rate, prompt evaluation for bleeding and potential surgery should be prioritized. However, in young and otherwise healthy patients, vital signs, including changes in posture, may appear normal initially even in the presence of significant bleeding, due to compensatory mechanisms. It's important to note that some patients with acute hemo peritoneum and hypotension may not exhibit tachycardia and may even display bradycardia (Jansen et al., 1978).

Although significant hemorrhage leading to shock is uncommon, it's crucial to re check hemoglobin levels if there is suspicion of ongoing blood loss, even if the patient presents as hemodynamically stable initially.

Palpation of the lower abdomen may reveal tenderness on one side, typically the right lower quadrant, in cases of ovarian cyst rupture. Rupture of a simple cyst usually results in mild to moderate tenderness sup on deep

palpation. However, in cases of a large volume of intra peritoneal blood, patients may experience more pronounced tenderness and peritoneal signs, such as rigidity of the abdominal wall and rebound tenderness, due to overt peritonitis caused by the release of sebaceous material or blood into the abdomen. Intra-abdominal hemorrhage may also be associated with Cullen's sign, which is a clinical finding characterized by periumbilical ecchymosis (bruising), indicating blood in the peritoneal cavity (Kaplan et al., 2007).

Laboratory findings in cases of ovarian cyst rupture may vary, and abnormalities may not always be present. Hemoglobin levels may be low due to hemorrhage, although the initial values could be normal or only mildly decreased with acute blood loss (Sharp, 2019). Severe thrombocytopenia can exacerbate bleeding associated with cyst rupture. The white blood cell count is typically normal or only mildly elevated in cases of cyst rupture, and therefore leukocytosis or left shift should raise suspicion of an infectious or necrotic process as the potential cause of symptoms, rather than cyst rupture (Bhavsar et al., 2016).

Pelvic ultrasound is the main stay of evaluation as it is readily available, inexpensive, is a sensitive method for detecting ovarian cysts, and does not expose a patient to radiation. Pelvic ultrasound may reveal an adnexal mass and presence of fluid in the pelvis, although fluid can also be present without a ruptured cyst. It is important to note that a small amount of an echoic fluid in the pelvis is considered normal in reproductive-age patients. However, if the fluid appears to have debris within it, it may be suspicious for blood (from a ruptured cyst or ectopic pregnancy) or infection. Pain may be elicited upon scanning with the vaginal probe in the region of the mass.

In cases of cyst rupture, the amount of peritoneal fluid is typically small, but larger amounts of fluid may be present if hemorrhage accompanies the rupture. If ultrasound reveals bloody-appearing fluid extending beyond the upper margin of the uterus, further evaluation of the upper abdomen using abdominal ultrasound should be considered (Bottomley et al., 2009).

In the differential diagnosis, ectopic pregnancy, torsion, appendicitis, pelvic inflammatory disease (PID), tubo-ovarian abscess (TOA), and ovarian

hyper stimulation syndrome (OHSS) should be considered as primary considerations.

4.3. Management

The majority of patients experiencing ovarian cyst rupture generally has a straightforward clinical course, maintaining hemodynamic stability and may be managed with observation. However, complicated cases, such as those with hemodynamic instability, significant or ongoing blood loss, signs of infection, or suspected malignancy, may necessitate inpatient management and/or surgical intervention (Bottomley et al., 2009).

5. OVARIAN HYPER STIMULATION SYNDROME

5.1. Introduction, Definition and Risk factors

OHSS is a potentially dangerous condition that can occur as a complication of controlled ovarian hyper stimulation (COH) used in assisted reproduction. COH involves the use of medications to stimulate the growth and maturation of multiple ovarian follicles, which can lead to enlarged ovaries and the release of fluid into the abdominal cavity, resulting in abdominal distension, discomfort, and other complications related to increased fluid accumulation. OHSS can range from mild to severe, and in severe cases, it can be life-threatening. Overall, OHSS is a serious concern for those undergoing COH and requires close monitoring and medical management (Wang et al., 2021)

5.2. Diagnosis

OHSS is a condition that can be caused by medical intervention and has the potential to be life-threatening. It primarily affects women who are young and in good health, and is a known risk associated with controlled ovarian hyper stimulation (COH) used in assisted reproductive technologies.

Although the precise mechanisms behind OHSS are not entirely understood, its primary characteristic is an increase in capillary permeability resulting in fluid loss into the third space. OHSS is triggered by the administration of β-hCG in susceptible individuals who are undergoing final follicular maturation and ovulation induction. This process causes the over expression of vascular endothelial growth factor (VEGF) in the ovary, leading

to the release of vaso active and angiogenic substances, which in turn increase vascular permeability and the loss of fluid into the third space. These events ultimately culminate in the development of full-blown OHSS (Roberts et al., 1997).

5.3. Management

Conservative management focused on addressing symptoms and signs of deterioration are the recommended approach for treating established OHSS, and many affected women can be safely managed on an outpatient basis. However, if a woman presents with severe or critical OHSS, she may require hospitalization and, in some cases, intensive care unit (ICU) admission. While OHSS is a self-limiting condition, the presence of an ongoing pregnancy can prolong the duration of symptoms. Therefore, close monitoring and appropriate interventions are necessary for the safe and effective management of OHSS, particularly in severe cases (Mathur et al., 2015).

The majority of OHSS cases is considered mild or moderate and can typically be treated on an outpatient basis. These cases are generally self-limited and can be managed conservatively, with a focus on providing symptomatic relief. The goal of management for mild OHSS is to alleviate discomfort and other symptoms associated with the condition. Close monitoring and follow-up with a health care provider may be necessary to ensure that symptoms do not progress to a more severe form of the condition.

It is important to note that mild OHSS has the potential to progress to moderate or severe OHSS, particularly if the patient becomes pregnant. Therefore, women with mild OHSS should be closely monitored for at least two weeks, or until the onset of menstrual bleeding, for signs of worsening symptoms such as increasing abdominal pain, weight gain of more than 1 kg per day, and increasing abdominal girth. Early detection and management of worsening symptoms can help prevent the development of more severe forms of OHSS (Levin et al., 2002).

The treatment of severe ovarian hyper stimulation syndrome (OHSS) aims to preserve intravascular blood volume, correct fluid and electrolyte imbalances on the one hand, and prevent thromboembolic events and monitor signs of infection on the other.Hospitalization is mandatory for women with

severe OHSS who meet certain criteria, such as a hematocrit>55 percent, leukocytes>25,000/L, and creatinine>1.6 mg/dL, or who have severe abdominal pain, intractable vomiting, severe oliguria/anuria, tense ascites, dyspnea or tachypnea, hypotension, dizziness or syncope, severe electrolyte imbalance, or abnormal liver function tests (Abramov et al., 1998).

To manage severe OHSS, isotonic crystalloid solutions such as normal saline or Ringer's lactate are typically used for intravenous hydration, and prophylaxis for thromboembolic events is recommended for all hospitalized patients with OHSS. Patients should also be monitored for signs of infection, and broad-spectrum empiric antibiotic therapy may be used if bacterial infection is suspected.

In addition, critical OHSS cases should be managed in an intensive care unit (ICU), and routine ICU care should be provided, including monitoring of fluid balance, weights and abdominal circumference, CBC, electrolytes, blood urea nitrogen (BUN), creatinine, serum β-hCG measurements, invasive monitoring of central venous pressure, and pelvic ultrasound as needed. For prophylaxis of thromboembolic events, low molecular weight heparin or heparin is commonly used, and an intermittent pneumatic compression device may be recommended for patients in whom bed rest is suggested. Management of severe complications such as massive hydrothorax, pericardial effusion, arterial thrombosis, pulmonary embolism, sepsis, acute renal failure, acute respiratory distress syndrome (ARDS), and disseminated intravascular coagulation (DIC) are reviewed in the irrelevant topics (Dulitzky et al., 2002).

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CHAPTER 3

APPROACH TO ABDOMINAL PAIN IN CHILDREN, SURGICAL VIEW

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INTRODUCTION

The abdominal pain is one of the most common symptoms which referred in the pediatric emergency outpatient clinic. Approximately 15% of school-age children apply to the pediatricians with the complaint of abdominal pain (Moir CR., 1996).

Abdominal pain in childhood is seen mostly by the reasons that resolve spontaneously. The rate of surgical reasons among all pediatric abdominal pains are 5% (Özcan R., Emre S., 2010). Appendicitis is the most common cause which requires of emergency surgery in children. While the overall incidence of appendicitis is 7-8% and this rate decreases to 2.5% in children.

The evaluation should be done carefully because of the diseases that causes abdominal pain might require urgent surgery. That is why the approach to abdominal pain is one of the most important practices to be learned. The majority of diseases that cause abdominal pain in children are benign and do not require surgical intervention.

In this section, acute abdominal pain and the approach to abdominal pain especially requires emergency surgery will be explained from the perspective of pediatric surgery department.

The causes and approaches of acute surgical abdominal pain in children changes according to age. For this reason, we divided the reasons to common causes of abdominal pain as newborn and infant, preschool, school age and adolescent age (Table 1).

Table 1. Most often diagnosed pediatric abdominal pain reasons by the age groups

Age Group	**Infancy (<2 year)**	**Preschool (2-5 year)**	**School Age (5 year<)**	**Adolescent**
Non-Surgical	*Infantile Colic *Gastroesophageal Reflux *AGE *Constipation	*AGE *Constipation *Urinary tract infection *Functional abdominal pain *Pneumonia *Mesenteric lymphadenitis	*Constipation *Gastritis *Functional abdominal pain * AGE *Urinary tract infection *Pneumonia *Mesenteric lymphadenitis *Pancreatitis	*Constipation *Gastritis *Functional abdominal pain *AGE *Urinary tract infection *İnflammatory bowel disease

			*Henoch-Schonlein purpura	
Surgical	*Intussuception *Incarcerated Hernia *Volvulus *Infantile Hypertrophic pyloric stenosis	*Intussuception *Appendicitis *Ovarian/testicular torsion *Meckel's diverticulum	*Appendicitis *Blunt trauma *Ovarian/testicular torsion	*Appendicitis *Blunt trauma *Ovarian/testicular torsion *Cholecystitis

HISTORY

Pediatric history taking is the most important step for evaluation of acute abdominal pain. History taking is started from the first seen of child. If the child looks healthy, plays with toys, walks by himself, it means presumably the reason is non-surgical (Table1).

It is important to use open questionnaire to understand the child's prior complaint. Another importance of open questioning is not to skip parts that are not mentioned by the child or family but may help in the diagnosis.

The following questions may be useful to understand more details about the abdominal pain;

- Where is the pain?
- When did the problem started?
- İs it food related? Trauma related?
- How long have you been experiencing the pain?
- Did the pain come on suddenly or progressively?
- Is the pain permanent or does it come and go?
- Does anything seem to make pain better or worse?
- Are there any accompanying symptoms (fever, nausea, diarrhea, constipation, cough)?
- Does anything make the pain worse or better?
- Have you experienced such pain before?
- Do you have a chronic disease?

Pain Localization:

If the baby cries constantly pulling her/his feet up to her tummy, the reason may be probably infantile colic. School age and older severe abdominal

pain especially in the right lower quadrant is specific for appendicitis and mesenteric lymphadenitis. Abdominal right upper quadrant pain is typical for gallbladder. Lumbar region pain is brought to mind the diseases such as kidney stones and urinary tract infections. Urinary tract infection, ovary/testicle torsion, inguinal lymphadenitis should be considered in the inguinal-pelvic region pain.

Type of Pain:

Whether the pain is blunt or colic, very important in the diagnostic approach. The colic pain, we think of the pain of hollow organs in the foreground. These are organs such as the intestine, kidney, gall bladder. Gastroenteritis, urinary tract infection, mesenteric lymphadenitis, intussusception, constipation should be considered frequently in colic pain in children. Surgical pain comes to mind more often in blunt pains. Appendicitis, ovary/testicular torsion is one of them.

Accompanying Symptoms:

It should be kept in mind that the symptom of biliary vomiting can be intestinal obstruction in any infant or child. Reduction of pain with defecation should bring to mind conditions such as constipation and gastroenteritis and the decrease in pain after biliary vomiting should bring to mind the pathologies of the upper gastrointestinal tract. Constipation and abdominal bloating is an accompanying symptom that must be questioned. Specific findings such as redcurrant jelly stool due to intussusception should be kept in mind in the diagnostic approach. Dysuria is an accompanying symptom that must be questioned in the approach to abdominal pain. Families may not associate urinary tract infection with abdominal pain. Cough, fever, sore throat should be questioned. Abdominal pain can be observed in these diseases, especially in lower lobe pneumonias and tonsillitis.

History of Pain Episodes:

If the patient has recurrent acute abdominal evaluations in the patient's history, they should be evaluated in terms of autoimmune/rheumatic diseases like Familial Mediterranean Fever (FMF).

PHYSICAL EXAMINATION

Pediatric examination begins at the first sight of the patient. When a pediatric surgeon first sees the child, they usually decide that they may need to be hospitalized and operated on. Nutritional status, level of consciousness, toxic or distressed, cyanosis, hydration, developmental delay, mental state should be evaluated at the first time. Evaluation of vital signs is essential after the first look; Heart and respiratory rate, blood pressure, temperature, weight, height/length should be evaluated. Head and neck examination is important to rule out possible upper respiratory tract infections, and auscultation of the lungs is important for exclusion of pneumonia.

Examination of Abdomen

The abdominal examination position should be with the knees pulled to the abdomen in order to break the defense of the abdominal muscles. Abdominal examination, like all system examinations, begins with inspection. It is often said that the abdomen should be exposed from nipples to knees. Abdominal diffuse/regional distension, scar, stretch marks, rash, umbilical/inguinal hernia, testicular redness/swelling should be evaluated on inspection. Before starting palpation, the child should be talked to, calmed down, and voluntary guarding defense should be defeated. Abdominal palpation should start from the place furthest from the location where the child described the pain. During the examination, calming, distracting conversations should continue. Palpation in all quadrants should be followed by a gentle palpation for mass evaluation first, followed by deep palpation. The 3 most important findings of acute abdomen are rigid abdomen, abdominal guarding and rebound tenderness. Abdominal rigidity is tightness of the muscles in the belly area. Guarding is the voluntary or involuntary tensing of the abdominal muscles. Rebound sign is evaluated in the right lower and left lower quadrants. Rebound sign refers to the presence of pain when pressure is removed, not when abdominal pressure is applied. Specific to appendicitis, if it is positive in the right lower quadrant, it is considered direct, and if it is positive in the left lower quadrant, it is considered indirect rebound. Murphy's sign is performed by palpating the subcostal region during inspiration. If pain is elicited and the patient suddenly stops their inspiratory effort, a positive Murphy's sign has been elicited. A positive Murphy's sign can be seen with acute cholecystitis

(Musana KA., Yale SH., 2005). In a study published by Kenneth Yen et al., it was determined that the most important difference in examinations between pediatric emergency doctors and pediatric surgeons was in abdominal tenderness and defense (Yen K. et al., 2005).

The third step of the abdominal examination is auscultation. Auscultation is listening to bowel sounds in 4 quadrants. Normal bowel sounds are heard once every 5-15 seconds. Below this level it is called hypoactive, above it is called hyperactive. Hypoactive or inaudible bowel sounds should bring to mind diseases such as constipation, ileus, acute abdomen. Gastroenteritis is the first disease that comes to mind in hyperactive bowel sounds.

SURGICAL DIAGNOSES

Appendicitis

The incidence of acute abdominal pain in children visiting pediatric and emergency departments is about 5% and, among all acute causes, appendicitis has an incidence of 12.7%, representing the most common reason for abdominal surgery (Caruso AM., 2017).

Table 2. Alvarado scoring; 1 to 4 is discharged, 5 to 6 is observation and admission, 7 to 10 is gone to the surgery. Pediatric appendicitis score (PAS) over 6 is considered significant for appendicitis

Clinical Variables	Alvarado Score	PAS
Migration of pain	1	1
Anorexia	1	1
Nausea or vomiting	1	1
Right lower quadrant tenderness	2	2
Rebound pain	1	
Elevated temperature*	1	1
Leukocytosis (≥10,000/μL)	2	1
Shift of WBC count to the left (≥75% polymorphonucleocytes)	1	1
Cough/percussion/hopping cause pain in the RLQ		2
Total	**10**	**10**

(Ebell MH., et al., 2014).

The most common age is 11-12 years. The most common cause in its etiology is lymphoid hyperplasia, fecaloid, intestinal parasites and systemic infections. Two scorings are used in the diagnostic approach. Alvarado scoring system and pediatric appendicitis scoring (Table 2).

Appendicitis is most commonly encountered in the retrocecal region (64%). The second most common is pelvic appendicitis (32%).

Symptoms:

1. Constant blunt abdominal pain especially in right lower quadrant.
2. Accompanied by loss of appetite.
3. Onset of nausea and vomiting after abdominal pain.

Physical Examination:

1. It is observed that the child cannot walk upright at first sight.
2. Mc-Burney sign (Pain on palpation of the right lower quadrant)
3. Rebound tenderness
4. Guarding of abdomen
5. Rovsing's sign (Pain referred to the right lower quadrant when the left lower quadrant is palpated.)
6. Psoas sign (Pain increases when the patient lie on his or her left side and the right thigh is flexed backward.)

Diagnosis:

1. Blood test (white blood cell, c-reactive protein, sedimentation)
2. Urine test
3. Imaging tests (Ultrasound, computerized tomography, MR-I)

Treatment:

The first choice in the treatment of appendicitis has been appendectomy for many years. However, in recent years, the conservative approach has come to the fore in non-complicated appendicitis. In a recent meta-analysis, children and adolescents with uncomplicated appendicitis who were treated conservatively became free of symptoms in 92% of cases, although 16% (10–22%) went on to undergo appendectomy because of a recurrence (Téoule P. et al., 2020).

Intussusception

Intussusception is interpenetration of one segment bowel into a distal segment. The estimated global rate of intussusception is 74 cases per 100,000 children under one year of age (Myat TW., et al., 2021).

Intussusception typically occurs between 6 and 36 months (80%) and is one of the most common causes of intestinal obstruction in this age group. There is a leading point that initiates invagination in 5-8% of cases. The most common leading points are Meckel's diverticulum, and hemangioma, lymphoid hyperplasia, ectopic tissues, and polyps.

Symptoms:

1. Paroxysmal periodic abdominal pain in the form of cramps that come every 15-20 minutes, followed by relief periods (83%).
2. Non biliary vomiting in the early period is followed by bile vomits in the late period (85%).
3. Redcurrant jelly stool (rectal bleeding)
4. Symptoms related to electrolyte imbalance are seen.

Physical Examination:

1. Palpable mass in the abdomen (sausage-shaped mass)

Diagnosis:

1. Ultrasound (target sign, pseudo-kidney sign)
2. Computerized tomography (bull's-eye sign)

Treatment:

1. Begins with the treatment of fluid-electrolyte imbalance.
2. Hydrostatic or pneumatic reduction
3. Open/Laparoscopic manual reduction or resection anastomosis (If hydrostatic or pneumatic reduction has failed).

Meckel’s Diverticulum

Meckel’s diverticulum is a remnant of the omphalomesenteric duct. It is the most common congenital anomaly of gastrointestinal (GI) system. It is present in about 2% of the population.

The rule of two's: Meckel diverticulum, 2% incidence of the population, 2% symptomatic, mostly in children < 2 years, affects males twice, is located 2 feet proximal to the ileocecal valve, is ≤ 2 inches long, and can have 2 types of mucosal lining (pancreatic, gastric).

Symptoms:

1. Painless, episodic lower GI bleeding (especially under age of 5).
2. The clinic may vary according to its complications. (Intussusception, Volvulus, Diverticulitis)

Physical Examination:

1. Examination findings vary according to complications.

Diagnosis:

1. Technetium scan scintigraphy
2. Colonoscopy

Treatment:

1. Replacement therapy should be performed according to the severity of the bleeding.
2. Laparoscopic/Open diverticulectomy

Incarcerated Hernia

A hernia is caused by the movement of the abdominal organs into an open space which is outside the natural anatomy. The herniation into two spaces (umbilical and indirect inguinal) is encountered most frequently in children. Those 2 areas of incarceration, that should be evaluated in the approach to abdominal pain. Indirect inguinal herniorrhaphy is one of the most frequently performed surgical procedures in children. Approximately 0.8% to 4.4% of all children will develop an inguinal hernia, with a positive family history in about 11.5%. The incidence of bowel incarceration in premature infants is significantly increased (Brandt ML., 2007).

Umbilical hernias are common, occurring in 10% to 20% of all children. The majority of umbilical hernias close spontaneously by the age of 3-4 years old.

Apart from these two common hernias, it can rarely be seen in epigastric, femoral and direct hernias.

Symptoms:

1. Abdominal pain (For inguinal hernia especially groin or lower abdominal pain.)
2. Nausea, vomiting.
3. Crying, restlessness by pulling the feet towards the tummy in the baby.

Physical Examination:

1. During abdominal examination, swelling and hardness palpation is visibly noticed in the umbilical region or inguinal areas.
2. Cannot be reduced on examination.

Diagnosis:

1. Generally, diagnosis is made on examination.
2. In cases where the examination is uncertain, ultrasound can be used for diagnosis.

Treatment:

The first intervention in incarceration is to apply gentle pressure to the swelling in the direction of the navel with the fingertips, after calming the child (sedation can be used). Approximately 80% of incarcerated inguinal hernias can be reduced using this technique (Brandt ML., 2007).

In hernias that are reduced by this intervention, it is recommended that the patient be hospitalized and the hernia repair should be performed after the tissue edema disappears (24-48 hours). In non-reduced patients, emergency surgery should be planned. It is important not to reduce the hernia under anesthesia before the incision in order to inspect the incarcerated bowel for evidence of strangulation. Generally, the incarcerated tissue in the sac is the intestine, but it should be kept in mind that ovarian tissue may also be present in girls. Recently, laparoscopic repair is preferred over open repair in incarcerated hernia.

Volvulus

Volvulus is one of the diseases that require urgent surgical intervention in pediatric surgery. Unlike volvulus in adults, volvulus in children is usually experienced due to congenital anomalies. Acute midgut volvulus is the most common cause of volvulus in children. Normally, the duodenojejunal segment and the cecocolic segment rotate 270 degrees around the SMA and fixation occurs. Disruptions in this process cause rotation anomalies.

A population based study estimated an incidence of approximately 15 per 1 million in children less than 1 year-old and 10 per 1 million in children aged 1 to 2 years, 2 with a decreasing incidence after that. Associated congenital abnormalities are found in approximately 30% to 60% of cases and may include intestinal atresia or web (the most common associated anomaly), Meckel diverticulum, intussusception, Hirschsprung's disease, mesenteric cyst, and anomalies of the extrahepatic biliary system (Langer JC., 2017).

Symptoms:

1. It usually begins with a sudden onset of bilious vomiting in a clinically healthy infant.
2. Lethargy,
3. Hematochezia, irritability, pain, peritonitis,
4. Septic shock

Physical Examination:

1. Scaphoid abdomen (The abdomen is not distended initially because the obstruction is very proximal.)
2. Abdominal wall erythema
3. İn older children, intermittently colic abdominal pain

Diagnosis:

1. Plain abdominal radiography (A double bubble and a paucity of distal air, multiple dilated bowel loops with air-fluid levels.)
2. The gold standard for the diagnosis of a rotation abnormality is upper gastrointestinal contrast radiography (A corkscrew sign).
3. Doppler Ultrasonography (A whirlpool sign)

Treatment:

The first 6-8 hours are very important in the decision of surgery. This period is the golden hour for intestinal ischemia, necrosis and resection.

1. Ladd procedure surgery.

Ovarian/Testicular Torsion

Ovarian torsion is common in children, especially between the ages of 9 and 14 (Childress KJ., Dietrich JE., 2017). Ovarian torsions are usually caused by an underlying ovarian pathology in children. These are often ovarian cysts (especially over 5 cm), polycystic ovarian syndrome, dermoid cysts, ovarian tumors.

Symptoms:

1. Abdominal pain, especially lower abdomen region and intermittently colic pain.
2. Nause, anorexia, vomiting.

Physical Examination:

1. Localized defense, tenderness in the right or left lower quadrant abdomen.
2. Pouch of douglas should be palpated on rectal exam.

Diagnoses:

1. Most laboratory findings are normal in patients with adnexal torsion.
2. Pelvic doppler ultrasonography to evaluate blood flow to the ovaries.

Treatment:

1. Surgery, laparoscopic surgery is the best therapeutic approach.

Cholecystitis

Gallbladder disease is a rare condition in children. Acute acalculous cholecystitis (AAC) is the most frequent form of acute cholecystitis in children (50-70%). Among the most common causes of AAC; post-operative, autoimmune/immune-mediated diseases, viruses, bacteria, yeasts and parasites.

Cholelithiasis (CL) is one of the rare diseases in children. Hemolytic diseases, premature, prolonged parenteral nutrition, repeated use of ceftriaxone are risk factors for cholelithiasis. Due to the increase in the incidence of obesity, it has been observed that the incidence of CL has increased recently (4%) (Murphy PB., 2016).

Symptoms:

1. Abdominal pain (right upper quadrant)
2. Jaundice
3. Vomiting and nausea
4. Fever

Physical Examination:

1. Murphy's sign (Severe pain on deep palpation of the right upper quadrant)

Diagnoses:

1. Blood test: Biochemical abnormalities (plasma bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase), leukocytosis
2. Abdominal ultrasonography (Thickening of the gallbladder wall is the most reliable single criterion)
3. Computerized tomography

Treatment:

1. The treatment of cholecystitis is generally conservative, unlike adults. Hospitalization is strongly recommended. Broad-spectrum antibiotics, intravenous fluid replacement, controlled or complete cessation of oral intake, and pain relief are recommended.

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CHAPTER 4

THERAPEUTIC IMPORTANCE OF SMALL HEAT SHOCK PROTEINS AND THEIR INTERACTION WITH OTHER PROTEINS

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INTRODUCTION

Sudden fluctuations in the environment can disturb cellular homeostasis and have a detrimental effect on the organism's survival. One of the big challenges of living systems is to cope with protein denaturation and aggregation under stress conditions (*e.g*., pH, oxidative, heat stress)(Macario et al., 1999). Proteasomes and major chaperone systems, which are part of the protein control system in the cell, take a crucial role in decreasing protein aggregation and assisting protein refolding. Small heat shock proteins (sHSPs) are the important member of the protein homeostasis system(Reinle et al., 2021). They are the molecular chaperones available in the three domains of life and they are distinguished by their low molecular mass (12-43 kDa)(Mogk et al., 2019). Different from other well-known chaperone machines such as Hsp100, Hsp90, Hsp70 and Hsp60, sHSPs have ability to hold the damaged proteins soluble by ATP independent way, which is defined as holdase activity. The sequestrated client proteins can be refolded by help of the ATP dependent chaperones like HSP70/HSP90 (Hu et al., 2022).

sHSPs are made up of tripartite domains: Highly conserved Alpha Crystallin Domain (ACD) and variable N-terminal Domain (NTD) and C-terminal Domain (CTD). These three regions have distinct features with respect to their amino acid composition. The ACD of sHSPs consists of 90-100 residues that form an immunoglobulin fold with the organization of six or seven beta strands (Haslbeck et al., 2019). The key structural characteristic of sHSPs is forming oligomers and the oligomerization begins with the interaction of the two ACDs; therefore, ACD is the basic building block of sHSPs dimers (Haslbeck & Vierling, 2015). The divergent NTD contains mostly hydrophobic amino acids (*e.g*., tryptophans and phenyalanines) and responsible primarily for substrate interactions (Haslbeck et al., 2019). On the other hand, the highly disordered CTD is enriched with charged and polar residues that are supposed to contribute solubilization of sHSPs in tissues (*e.g*., eye lens). This region has a role in the formation of higher-order oligomers by interacting with the ACDs (Janowska et al., 2019). All these three parts of sHSPs are required for oligomer formation by making weak and inter-subunit interactions (Riedl et al., 2020).

sHSPs have ability to bind variety of unfolded substrates through their exposed hydrophobic surfaces and to decrease the accumulation of aggregates

(Jaya et al., 2009). This feature makes the sHSPs vital in preventing several diseases associated with protein aggregation (*e.g.*, neuromuscular, cardiac and neurodegenerative diseases) (Carra et al., 2017).

Moreover, they have multifunctional activities in the cell, which are inhibition of inflammation, prevention of apoptosis, regulation of cell cycle, differentiation and carcinogenicity (Boelens, 2020; Kannan et al., 2012). Human have ten members of small heat shock proteins, HSPB1-HSBP10, and malfunction of sHSPs are linked to the development of severe diseases (Tedesco et al., 2022). Since sHSPs have remarkable physiological roles, they are supposed to be important therapeutic targets for treatment of human diseases (Haslbeck et al., 2019).

1. ROLE of sHSPs in DISEASES

1.1. Cataract

In human body, eyes can expose to several stress factors including osmotic stress, high temperature, oxidative stress, and UV light. It was suggested that small heat shock protein with its cytoprotective action can protect the eye from ocular stress by increasing its expression (Sooraj et al., 2022).

As molecular chaperones in the eye, two human sHSPs, HSPB4 (αA crystallin) and HSPB5 (αβ-crystallin), have been well described. The alpha crystallins are capable of inhibiting protein aggregation and maintaining the transparency of the eye lens (Phadte et al., 2021). In the eye lens, αA crystallin exert its chaperone activity by making a hetero-oligomer structure with αβ-crystallin. The amount of αA crystallin and αβ-crystallin can be variable in different organisms. Srinivas *et al*., (2008) found that a 3:1 ratio of αA crystallin /αβ-crystallin in vitro conditions were associated with chaperone activity. An alteration in the concentration can unbalance the equilibrium, and in turn promote aggregation, which would decrease the lens clarity by increasing the turbidity (Tikhomirova et al., 2017). A cataract is a severe eye disease implicated by vision impairment. This disease obstructs the transmission of light onto the retina and causes blindness (Kumar & Reddy, 2009). One of the proposed mechanisms for the formation of the disease has been stated that decrease solubility and an enhanced rate of nucleation of the crystallins lead to

cataract formation (Meehan et al., 2004). The second mechanism illustrates that cataract arise from the unfolding of the lens crystallins. Therefore, high molecular mass aggregates form due to the precipitation of the eye crystallins, which results in light scattering and reduction in transparency (Harding, 1998). Cataract-causing mutations were identified in human small heat shock proteins. Single amino acid change within the protein altered the structure and chaperone activities. One of the well-known mutations in human αβ crystallin is R120G mutation. The substitution of the highly conserved arginine 120 by glycine at the dimer interface altered the network of interactions (*e.g*., salt bridges) which led to the formation of defective chaperones with less thermal stability and a high tendency to precipitate. Aggregation of the crystallins interfere the scattering of visible light (Bova et al., 1999; Clark et al., 2011). The R116H and R116C single mutations, which are linked with cataract in αA crystallin (HSPB4) were equivalent to the R120G mutation (Boelens, 2020). Another defined gene mutation, G98R, in human αA crystallin was associated with onset of cataract in an Indian family (Santhiya et al., 2006). The G98R mutant had the features of a high oligomeric mass, low chaperone activity and impairment of structural stability. Those properties were thought to contribute cataract formation in affected people (Singh et al., 2006). In addition to the mutations, post-translational modifications (e.g., oxidation, acetylation, deamination, and phosphorylation) have been observed in specific residues of αA-crystallin and αβ-crystallin from cataractous lenses (Budnar et al., 2022).

Until now, surgical removal of the lens and replacing an inocular lens are the available treatment for the cataract and the surgery provides good outcomes for the disease; however, possible complications can occur after surgery. For example, the optical qualities of an artificial lens may not meet the requirements of a normal lens. Also, not all people over the world have an opportunity for surgery due to low economic status. Therefore, understanding chaperone action and finding pharmacological methods for maintaining the transparency of the lens is much more required (Kumar & Reddy, 2009). For this reason, mini-chaperone peptides produced from human αA and αβ crystallins were taken into consideration regarding therapeutic intervention. To optimize mini-chaperone peptides against protease degradation, several modifications of the selective residues (*e.g*., acetylation) and the addition of D- amino acid (*e.g*., D-Asp) were

done (Raju et al., 2016). Injected short peptides by intrapertioneally can pass the blood aqueous barrier and then enter the lens and protect the retina from cataract. Moreover, mini peptides are more effective than native full length peptide with respect to the easy delivery to the specific organs (Nahomi et al., 2013). In another experiment, human fetal retinal pigment epithelial cells (hfRPE) were protected from oxidation induced cell apoptosis in the presence of mini- αA and mini-αβ chaperones (Raju et al., 2016). In a similar study, the mini-αA chaperone prevented the retina from injuries caused by sodium iodate (Zhang et al., 2015). Not only short peptides, but sterols were also found beneficial in decreasing the negative effects of cataract. Small molecule lanosterol was found to interact with human sHSPs and reduce protein aggregates in cell culture and maintaining lens transparency in animal models. It was stated that the lanosterol can cover hydrophobic region of the huge aggregates by its amphipathic property and can return aggregates to soluble form (Strauch & Haslbeck, 2016; Zhao et al., 2015). Therefore, such chaperone based strategies could be important for treatment of the cataract.

1.2. Cancer

In recent years, understanding sHSPs mechanisms, their role in cancer and their dysfunction linked to the cancer development in human will improve methods for effective therapeutic targets. Therefore, this is the current issue of scientists. Expression of small heat shock proteins in several type of cancers is important for tumorigenesis, progression, metastasize, cell growth, death and chemo/radio resistance. sHSPs are expressed in some tumors in an enhanced level (*e.g*., breast cancer (HspB5, HspB1, and HspB2), testis (HspB9), liver (HspB5)) ; whereas, in some tumors, decreased expression of small heat shock proteins is identified (*e.g*., pancreatic cancer (HspB4), renal cancers (HspB7)(Xiong et al., 2020). Small heat shock proteins have dual roles in cancer. They can either promote tumorigenesis or suppress the tumor development. For example, human HspB1 was found to accelerate breast cancer development, but the same sHSP has role in regression of the testis tumor (Treweek et al., 2015; Xiong et al., 2020). Recently, reports have revealed that small heat shock proteins have pivotal role in prevention of apoptosis by inhibiting the activity of pro-apoptotic factors (Tedesco et al., 2022). Arrigo & Gibert, (2014) analyzed the potent carcinogenic role of

HspB1(Hsp27), HspB5(αβ Crystallin), and HspB4 (αA crystallin) and their interaction with client proteins. These sHSPs were found to follow their specific rules to knock down the pro-apoptotic factors such as Bax, caspase 3, cytochrome c, and BclXs and to activate the anti-apoptotic activity of other proteins (*e.g*., PEA-15, XIAP). Inhibition of apoptosis due to small heat shock protein expression blocks the anti-cancer response of cells, so aggressive cancer development, and metastasis could be inevitable. In order to cope with proliferation of cancer cells, small heat shock protein based strategies are under development. HspB1 (Hsp27) can be potential therapeutic agent in cancer since accumulated data showed that enhanced expression level of it is associated with various types of cancer (*i.e*., glioma, gastric, breast, liver, melanoma, prostate and kidney). Therefore, inhibition of HSP27 expression could be the effective method in cancer theraphy. There are three main clinical trials for inhibition of the Hsp27 activity. In the first strategy, small inhibitors, Quercetin, Brivudine (RP101), and cross-linker interact with Hsp27 protein and block its activity. Second strategy is based on alteration of the oligomeric structure of HSP27. Using peptide aptamers (PA11 and PA50) interfere the dimeric and oligomeric structure of HSP27, which in turn have a negative effect on its chaperone function. Last strategy focuses on decreasing the transcript level of HSP27 by anti-sense oligonucleotide (OGX-427), so the amount of the protein is decreased (Seul-Ki et al., 2019). This method gave promising results for the treatment of prostate, breast, bladder and lung cancer (Treweek et al., 2015; Zoubeidi & Gleave, 2012). All these show the importance of sHSPs in the treatment of various cancer types, but further research are necessary for understanding of their activities.

1.3. Neurological Diseases

Development of the neurological diseases is caused by the alteration of the protein homeostasis that leads to the abnormal aggregation of the misfolded proteins. Current knowledge in this field supported that synaptic loss and neural death occurred due to the presence of aggregated proteins. Immediate diagnosis is essential for the prevention of further neural damage. Molecular chaperones are thought to be promising therapeutic targets for discarding huge protein aggregates in the cell (Maiti et al., 2014). Many neurological diseases such as Lewy bodies dementia (LBD), Parkinson’s Disease (PD), Alzheimer's Disease

(AD), and Huntington's Disease (HD) are identified by accumulation of lewy bodies in LBD, tau or amyloid β protein in AD, huntingtin in HD and α-synuclein or tau in PD (Maiti et al., 2014).

Seidel *et al.*, (2012) analyzed the effect of chaperone complex HspB8 with co-chaperone Bag3 on protein conformation disorders; Alzheimer's disease, Parkinson's disease, Spinocerebellar Ataxia Type 3 (SCA3) and Huntington's disease. Results of this research indicated that expression of HspB8-Bag3 was up-regulated in astrocytes which in turn activated the autophagy pathways and facilitated the clearance of the misfolded proteins. In this respect, such chaperone complex can be valid medical tool for regression of neurological diseases. Similar to HspB8, HspB6 has been documented for its vital role in the regulation of autophagy by interaction and stabilization of the specific protein, *i.e.*, beclin, for autophagosome formation (Webster et al., 2019).

In another study, recombinant human αB-crystallin suppressed the amyloid fibril formation of Aβ (1-40) and protected the cells from toxicity. It was supposed that the sHSP prevented the fibril formation by making a stable complex with the amylogenic protein or/and sHSP masked the complementary surface of the amyloid fibril so that it could not nucleate the growth of fibril. Such a mechanism could be important for understanding the role of sHSPs in the prevention of Alzheimer's disease (Raman et al., 2005). In addition to these, Hsp27 (HspB1) prevented the neural toxicity of α-Synuclein fibrils by inhibiting their elongation and decreasing their relative hydrophobicity, so interaction with cell membranes were decreased. Since α-Synuclein are especially characteristic of dementia and Parkinson's disease, sHSP can have high potency in targeted therapy(Cox et al., 2018).

Furthermore, a recent review about the therapeutic role of sHSPs gave new insights that the use of HSPBs loaded with extracellular vesicles can stimulate repair signaling against multiple sclerosis lesions and can exert their activity during neuroinflammation (Van den Broek et al., 2021).

Although all these reports showed the importance of sHSPs in the prevention of neurodegenerative diseases, more work is still needed to complete understanding of their effects.

1.4. Cardiac Diseases

Some cardiac diseases, such as heart failure, cardiac hypertrophy, and ischemia/reperfusion are caused by the presence of misfolded proteins. Cardioprotective roles of small heat shock proteins against diseases were investigated (Tedesco et al., 2022). Studies illustrated that the human sHSP, CRYAB, inhibited the development of cardiac hypertrophy. Furthermore, an enhanced expression level of the HSP20 was found to protect cardiomyocytes and suppress the ischemia/reperfusion injury by activating autophagy. Protection against oxidative stress caused by ischemia/reperfusion injury in the presence of HSP20 was identified. It is expected that increasing the expression level of sHSPs could be one feasible way to deal with cardiac diseases. Statins, *i.e*., lovastatin and simvastatin, can potentially protect the heart by inducing small heat shock protein levels. However, these drugs' use has still been searched in human studies (Willis & Patterson, 2010; Zilaee et al., 2014). Furthermore, heat shock protein 27 (HSP27) has been supposed to be a remarkable protective function in atherosclerosis in type II diabetic patients. Clinical case reports showed that patients with carotid atherosclerosis have decreased serum level of HSP27 compared to the healthy control groups. The one proposed mechanism of HSP27 to protect against atherosclerosis is related to their antioxidant ability. This sHSP can reduce reactive oxygen species (ROS) by increasing the level of glutathione. The other mechanism blocks acetylated low-density lipoprotein uptake by interacting with scavenger receptor-A (SR-A) and preventing foam cell formation. Therefore, the serum level of this sHSP can be linked to the diagnosis of atherosclerosis in the patient (Wang et al., 2020). Atrial fibrillation (AF) is another cardiac disease characterized by rapid and uncontrolled contraction of atrial cardiomyocytes. This situation damages cells, disrupts proteostasis, and increases oxidative stress and calcium levels. Expression of HSPB1 and other sHSPs (*e.g*., HSPB6, 7, and 8) have beneficially affected the sinus rhythm by remodeling actin fibers and stabilization of the cytoskeletal elements (Christians et al., 2012). Moreover, HSPB8 (HSP22) utilizes complex mechanisms to protect cardiomyocytes. Not only the sHSP protects the mitochondrial function, but also it encourages myocardial cell survival and energy metabolism in animal models. Increased glycogen metabolism was associated with the required

energy source under stress conditions (Hu et al., 2022). Together, these findings indicate that sHSPS may be a therapeutic target for treatment of cardiac diseases.

1.5. Autoimmune Diseases

Different from chaperone function of small heat shock proteins, they can also have roles in induction of the immune response and immunoregulatory roles in the nature (Moudgil et al., 2013).

Effect of small heat shock proteins on the prevention of autoimmune uveoretinitis was investigated in the animal model. The mutant mice, which lacked the αA-crystallin (null aA -/-), were more prone to retinal damage than the wild type. Intravenous injection of the sHSP protected the mice against inflammation due to an autoimmune response and cell death in the experimental autoimmune uveoretinitis (EAU) model. Following administration of the αA-crystallin, the Th1 cytokines, *e.g*., IL-12, TNF-α, decreased in the retina and the spleen. Furthermore, IL17, which is characterized by the severity of the inflammation in EAU was reduced in the presence of the chaperone. This data showed that αA-crystallin is crucial for suppressing both innate and adaptive immune systems. Therefore, this small heat shock protein may provide a therapeutic way to treat uveitis or related immune retina disorders (Rao et al., 2012).

Rheumatoid arthritis (RA) is another autoimmune disease and the joints are adversely affected due to sustained inflammation. The diverse inflammatory roles of the heat shock protein 27 (HSP27) were determined in recent years. The HSP27 was found to have dual roles in the inflammatory response. While HSP27 increased the production of IL-8, which is the pro-inflammatory cytokine, in astrocytes and in smooth muscles and causing neurogenic inflammation, the same sHSP can decrease inflammation in RA by inducing the release of inhibitory mediators (*e.g*., IL-10 and thrombospondin-1) which in turn decrease the T cell activation (Fouani et al., 2022). The other anti-inflammatory role of the HSP27 was proven in the acute kidney injury mouse model such that the sHPS took a central role in protecting the kidney injury and neutrophil infiltration (Hu et al., 2022).

In addition, supplementation of the immune cells with HSPB5 in MRL/lpr mice decreased the production of pro-inflammatory cytokines. In contrast, it increased the anti-inflammatory cytokines such as IL-10, which improved renal pathology in the animal model of Lupus nephritis (LN). Treatment of this disease is complex because there is no specific antigen for targeting. In these respects, using immunomodulators and regulating inflammation could be one ideal therapeutic intervention to deal with such diseases (Berg et al., 2022).

In conclusion, sHSPs have a wide variety of roles in the cell. Their superior chaperone activities, physiological roles, and tissue-specific expression could be important for designing new therapeutic interventions. Future research regarding their structure, function, and mechanism of action will facilitate progress in medicine.

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CHAPTER 5

ATTENTION DEFICIT HYPERACTIVITY DISORDER AND SLUGGISH COGNITIVE TEMPO

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1. ATTENTION AND HYPERACTIVITY DISORDER AND SLUGGISH COGNITIVE TEMPO

Attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders with a prevalence of 3-5% in school-age children (Polanczyk et al., 2007). ADHD is characterized by symptoms of hyperactivity, impulsivity, and inattention (Fassbender et al., 2015). ADHD is a common neurodevelopmental disorder and has a particularly important relationship with male gender. ADHD symptoms have a heterogeneous nature and clinician opinion is of great importance. Individuals with ADHD may show symptoms more or less and have subtypes based on the presence or absence of symptoms.

ADHD inattention type is the type where there are symptoms of inattention and no symptoms of hyperactivity. ADHD hyperactivity type is the type where there are no symptoms of inattention, whereas hyperactivity and impulsivity are dominant. The third type is the combined type, which includes both inattention and hyperactivity symptoms (Wood et al., 2009).

Attention deficit and hyperactivity disorder was first defined as a childhood hyperkinetic reaction in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-II (APA, 1975). It is named as attention deficit and hyperactivity disorder in DSM-III-R. Today, it is included in the title of neurodevelopmental disorders in DSM-5 under the name of attention deficit and hyperactivity disorder. According to DSM-5 diagnostic criteria, symptoms should persist for at least 6 months, at least a few symptoms should start before 12 years of age, symptoms should occur in at least two settings (e.g., at home and at school or work) and should cause deterioration in functionality in order to diagnose ADHD (APA, 2013).

According to DSM-5, inattention diagnostic criteria are as follows, and the individual should have at least 6 of the following (5 of them for 17 years of age and over) in order to meet the inattention criterion (APA, 2013);

a) Often does not pay attention to details or makes inattentive mistakes at school, work or during activities.
b) Often has difficulty maintaining attention while working or playing.
c) Often seems not to listen when spoken to directly.

d) Often does not follow the given instructions and cannot complete school duties, ordinary daily tasks, and responsibilities.
e) Often has difficulty in arranging work and activities.
f) Often avoids work that require constant mental effort, does not like/doesn't want to engage in such work.
g) Often loses required objects for work/activity.
h) Easily distracted by external stimuli frequently.
i) Frequently forgetful in daily activities.

According to DSM-5, the diagnostic criteria for hyperactivity/impulsivity are as follows, and at least 6 of the following (5 of them for 17 years of age and above) must be present in order to meet the inattention criterion (APA, 2013);

a) Often squirms or taps hands or feet or moves while seating.
b) Often gets up when expected to sit down.
c) Often runs around or climbs in unsuitable places.
d) Often unable to silently participate in leisure activities or play quietly.
e) Often "on the move", pretending to be "pedal to the metal".
f) Often runs on.
g) Most of the time, gives answer before the question is completed.
h) Unable to wait turn most of the time.
i) Often cuts in or interrupts others.

ADHD is a disorder that can cause important academic, social, and psychiatric problems, and its adverse effects can last a lifetime. ADHD is found in 3-7% of school-age children (APA, 2013). In a study conducted in Turkey, the prevalence of ADHD was found to be 12.7% (Ercan et al., 2013).

It is known that ADHD is more common in boys than in girls. In a large-sample study, it was found that ADHD is seen 2.5 times more in boys than in girls (Polanczyk et al., 2007). Barkley correlated ADHD with lack of inhibition, working memory, emotional regulation, speech internalization, and behavioral sequencing. As a result, he emphasized that movements, behaviors, and speech cause inconsistency with the context (Barkley, 1997). ADHD affects functionality and quality of life adversely. It is claimed that this negative effect is mostly caused by the inattention symptom (Sroubek et al., 2013). Medication

and behavioral interventions are used in the treatment of attention deficit hyperactivity disorder.

2. SLUGGISH COGNITIVE TEMPO

Sluggish cognitive tempo (SCT) is an attention disorder seen in childhood, which is characterized by motor and cognitive components. SCT is a cluster of symptoms that includes an attention disorder that is different from ADHD, and it affects daily life negatively (Camprodon-Rosanas et al., 2019). Recently, it is also called as cognitive disengagement syndrome (Frederick & Becker, 2023). Cognitive problems in SCT include symptoms such as confusion and daydreaming. Motor symptoms are associated with slow movement. In another definition, SCT is defined as a symptom cluster such as confusion, slow thinking and responding, blank staring, excessive daydreaming, and getting lost in one's own thoughts. SCT shows frequent comorbidity with ADHD (Becker et al., 2016). There are studies showing that ADHD and SCT are completely different from each other (Milich et al., 2001). SCT is different from ADHD with symptoms such as slow mobility, mental confusion and lethargy.

There is a correlation to some degree between the SCT symptom cluster and inattention (Fassbender et al., 2015). SCT rises with impairments in many functional areas (Becker et al., 2016). Especially in SCT and ADHD comorbidity, serious problems and impairments in most of the functional areas are reported (Barkley, 2013). There are studies reporting the connection between SCT and social withdrawal (Frederick & Becker, 2023). In addition to being related with daydreaming and slow motor movements, it was found that SCT is also related with lower academic grades (Frederick & Becker, 2023).

2.1. History and diagnosis

The initial characterization of SCT is characterized by attention deficit and the clinical picture is similar to ADHD. In attention disorder, which is defined secondly, lack of energy comes to the fore. It has been suggested that the second characterization is more compatible with SCT. The problem in SCT is associated with lethargy and fatigue. It has been reported that this state of lethargy and fatigue may lead to the inability of the patient to communicate with friends, apathy to the environment, and shyness (Saxbe & Barckley, 2014).

Attention disorder in DSM-III is classified as with hyperactivity and without hyperactivity. In the group accompanied by hyperactivity, destructive behaviors are often at the forefront. In the group without hyperactivity, symptoms such as shyness, academic failure and anxiety are more common. The features most commonly associated with SCT have been reported as sluggishness, daydreaming, and drowsiness (Lahey et al., 1985). A "sluggish cognitive tempo factor" was identified in the group without excessive inattention and hyperactivity. This "sluggish cognitive tempo factor" is characterized by slow movements and lethargy (Lahey et al., 1988).

In a 2001 study comparing differences in ADHD subtypes, it was suggested that ADHD combined type and ADHD predominantly inattentive type are distinct disorders. In this study, it was stated that there were significant differences between subtypes (Milich et al., 2001). In another study, it was suggested that ADHD and SCT are distinct disorders (Becker et al., 2016). However, it is not included in SCT classification systems (Saxbe & Barkley, 2014). Instead, Barkley listed the below most common symptoms in SCT (Barkley, 2013);

a) Daydreaming,
b) Difficulty staying awake and taking action,
c) Blurred and confused mind,
d) Staring with empty eyes,
e) Being confused and seem to be somewhere else
f) Being lethargic,
g) Being less active than usual,
h) Moving slowly and inactivity,
i) Inability to understand questions correctly or quickly,
j) Having a sleepy appearance,
k) Being indifferent or shy,
l) Getting lost in own thoughts,
m) Difficulty in completing given tasks,
n) Low initiative and lack of effort.

The presence of SCT may be suspected in patients with attention problems detected in the psychiatric evaluation and who report symptoms such as absent-mindedness, slowness, and confusion instead of hyperactivity. Using

assessment tools for SCT together with the scales that evaluate ADHD helps to differentiate two disorders (Barkley, 2013). There are 14 items in a scale developed to evaluate SCT symptoms in children, and the total score of the scale and 3 subscale scores are evaluated. Subscales of the assessment tool are dreaminess, slowness, and sleepiness (Penny et al., 2009).

2.2. Relationship between attention deficit and hyperactivity disorder and sluggish cognitive tempo

Although SCT and ADHD are different disorders, they are related to each other (Saxbe & Barkley, 2014) In a study that included 1,800 children aged 6-17 years, ADHD comorbidity was reported in 59% of children with SCT symptom cluster. Similarly, SCT comorbidity has been reported in 39% of children diagnosed with ADHD (Barkley, 2013).

In a study examining ADHD and SCT symptoms over a 10-year period, it was noted that hyperactivity and impulsivity were more strongly associated with inattention than with SCT. In the same study, it was reported that ADHD and SCT follow different developmental instructions. Also, it was stated that the rates of hyperactivity and impulsivity decreased in the developmental process, and inattention remained constant. A slight increase in SCT symptoms has been reported in the developmental process (Leopord et al., 2016).

In a study that investigated the relationship between ADHD and SCT and included 322 children and adolescents, it was reported that SCT symptoms were often in the ADHD inattentive type. In addition, it was reported that SCT symptoms were also present in the non-ADHD group (Garner et al., 2010). SCT is not a subtype of ADHD. In another study involving 78 children aged 6-12 years, the ADHD rate was reported as 40% in children with severe SCT symptoms. In the same study, the ADHD rate of children with mild SCT symptoms was reported as 29% (Markovich-Pilon et al., 2017).

In a study that included adults aged 18-96 years, it was found that SCT and ADHD are different disorders, however, they are comorbid at a high rate (Barkley, 2012). The most important feature in differentiating SCT from ADHD's inattentiveness is slow movement (Fredrick & Becker, 2023).

2.3. Etiology

796 children aged 8-17 years were included in a twin study examining the etiological relation of SCT and ADHD subtypes. A moderate heritability was found for SCT as a result of the study. In the study, it was reported that there is a large genetic aspect for ADHD subtypes, whereas unshared environmental factors are more important than genetics in SCT (Moruzzi et al., 2014).

It is known that prenatal intense alcohol exposure has a place in the ADHD etiology. In a study examining the relationship between prenatal alcohol exposure and SCT, higher SCT symptom scores were determined in children exposed to alcohol (Graham, 2013).

In a study examining the relation of SCT and ADHD symptoms with sociodemographic characteristics in children aged 7-10 years, smoking during pregnancy and postpartum passive smoking were reported to be associated with severe SCT symptoms. In addition, paternal unemployment and low maternal education were also associated with severe SCT symptoms. In this study, the presence of high SCT symptoms was associated with attention problems, academic problems, and peer relationship problems (Camprodon-Rosanas, 2016).

In an fMRI study conducted with young people aged 12-17 years, hypoactivity was found in the left superior parietal lobe in patients with SCT symptom cluster. It was stated that the increase in symptoms and attention problems in SCT may be related to hypoactivity in the superior parietal lobe. On the other hand, hypoactivity in the supplementary motor area and right superior parietal lobe was reported in ADHD (Fassbender et al., 2015).

In a cross-sectional MRI study conducted in Spain with 178 children aged 8-12 years, SCT symptoms were associated with abnormal frontal area volume increase. It was also reported that SCT differs from the classical neural substrates in ADHD (Camprodon-Rosanas et al., 2019). It is known that there is a negative relationship between intelligence and SCT (Becker et al., 2016). Increasing neuroimage studies related to SCT will also be positive for the treatment , contributing to the etiology of SCT. In addition, studies on SCT will be important in finding protective factors such as intelligence.

2.4. Comorbidity

The most frequently reported comorbid disorder in children with SCT is ADHD with a rate of 27.4-39% (Barkley, 2023 & Burns, Becker, 2021). These rates are based on parental comorbidity reports. It has been reported that adult patients with SCT and ADHD have more problems in getting organized and problem solving than adults with only ADHD (Barkley, 2012). In a study conducted with 131 children aged 6-16 years, inattention-dominant ADHD was found to be positively associated with hyperactivity, impulsivity, behavioral problems, and peer relationship problems. In contrast, SCT was reported to be negatively associated with hyperactivity, impulsivity, and behavioral problems. In the same study, it was stated that SCT was associated with internalization problems and learning difficulties (Cortes et al., 2017).

In a study conducted with inpatient children, SCT was reported to be positively correlated with internalization problems. It was found that SCT is more frequently associated with depression and less frequently with anxiety disorders (Becker et al., 2014). The Sluggish Cognitive Tempo Scale is a 14-item scale inquiring SCT symptoms (Jacobson et al., 2012).

A large-sample study from Spain reported higher levels of oppositional problems in children with only ADHD than children who met only SCT criteria. In contrast, children who met only the SCT criteria reported higher depression scores than those with only ADHD. In the same study, no increase in ADHD and depression was found in 28-46% of those with high SCT symptoms (Servera et al., 2018).

In a recent study conducted with Turkish children, SCT was shown to be associated with a much higher rate of depression and anxiety than ADHD inattention type. On the other hand, it was reported that SCT was less associated with oppositional defiant disorder and academic impairment compared to both ADHD groups (inattention type & hyperactivity and impulsivity type) (Basay, 2021).

In a study conducted with students aged 6-13 years, it was reported that SCT symptoms predicted future internalizing problems such as depression and anxiety. On the other hand, it was found that this relationship was not bi-directional (Becker et al., 2021). Similarly, in a study with adolescents, it was

reported that SCT predicted future depressive symptoms. In this study, it was suggested that peer bullying may be a cause of depression in adolescents with SCT (Frederick et al., 2022). In a study conducted with primary school children, it was found that SCT may be associated with cognitive problems and excessive sleeping. It was also reported that there was a relationship between SCT symptoms and somatic complaints (Mayes et al., 2021).

In a study conducted with preschool students, it was found that both SCT and ADHD inattention type had more emotional reactivity when compared to ADHD hyperactivity and impulsivity type. In addition, SCT symptoms have been associated with somatic complaints (Lee et al., 2017). As a result, ADHD and SCT cause functional impairment independently (Willcutt et al., 2014). Patients with additional SCT symptoms to autism spectrum disorder were examined, and it was reported that patients with a high percentage of SCT symptoms showed internalization disorders and psychosocial disorders compared to those with less SCT symptoms (Reinvall et al., 2017).

2.5. Treatment

In a study conducted with children with a diagnosis of attention deficit and hyperactivity disorder-inattentive type, responses to 1 month of methylphenidate treatment were examined. No significant difference was reported in response to methylphenidate treatment between the patient group with and without SCT symptoms as a result of the study (Ludwing et al., 2009).

The effect of SCT symptoms on methylphenidate responses of ADHD combined type and ADHD inattentive type patients aged 7-11 years was investigated. Sluggish and sleepy symptoms were reported to be associated with a lack of response to methylphenidate (Froehlich et al., 2018). There are studies showing the positive effect of atomoxetine treatment on SCT symptoms (Wietecha et al., 2013 & McBurnett et al., 2017).

ADHD predominantly inattentive type Child Life and Attention Program activity was analyzed. In this study, it was reported that SCT symptoms improved through behavior modification (Pfiffner et al., 2007). It has been reported that methylphenidate, lisdexamfetamine and atomoxetine are effective in the treatment of SCT or cognitive dysengagement syndrome (Wiggs et al.,

2023). Besides, there are studies showing that psychostimulants are not effective on SCT symptoms (Saxbe & Barkley, 2014).

It has been reported that lisdexamfetamine has positive effects in adult patients with ADHD and SCT (Adler et al., 2021).

In a study conducted with children with ADHD in Turkey, it was reported that SCT symptoms in addition to ADHD reduced the response in teacher analysis for ADHD total scores to psychostimulant treatment (Fırat et al., 2021). In particular, children with ADHD who had higher SCT-daydreaming scores had a lower level of treatment response for ADHD symptoms. It needs to be investigated why SCT symptoms cause lower methylphenidate treatment response in n teacher analysis. There is no enough clinical evidence on this issue.

3. CONCLUSION

SCT is a cluster of symptoms that is similar to ADHD especially because of the attention problems it contains, and it is different from ADHD with symptoms such as slow mobility, mental confusion, and lethargy. Along with these symptoms, SCT is characterized by daydreaming and hypoactivity.

ADHD and SCT cause functional impairment independently. Since SCT is new, new studies on diagnosis, etiology and treatment are needed. Psychometric assessment tools and scales showing SCT severity need to be developed. Future research on SCT will facilitate a multidimensional understanding of the factor structure of SCT. Defining the SCT diagnostic criteria more comprehensively will make it easier to differentiate it from ADHD. Thus, it helps better to identify areas of functional difficulty in children with SCT and ADHD. Also, there is a need to examine SCT during longer developmental periods and to investigate comorbidities in detail.

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CHAPTER 6

SOCIAL SKILLS IN AUTISM SPECTRUM DISORDER

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1. INTRODUCTION

The term social skill is a multidimensional concept. Stephens classified social skills into 4 subgroups in 1978 as self-related behaviors, task-related behaviors, environmental behaviors, and interpersonal behaviors (Merrell & Gimple, 2014). Later, Callederalla and Merrel (1997) reviewed social skills in children and adolescents in five dimensions. These dimensions are as follows: Peer relations, self-management, academic, compliance, and assertion (Cardarella & Merrell, 1997).

1) Peer relations skills: These are the skills that support communication with friends, such as approving friends, asking for and/or offering help when necessary, being willing to play games together, not having problems in establishing friendships, and exchanging ideas.

2) Self-management skills: Anger control, understanding and applying social rules correctly, maintaining common sense in the face of problems, being able to reach an agreement and being open to criticism are the skills that help the person to understand herself/himself correctly.

3) Academic skills: These are the skills that increase the success rates of individuals such as working individually, acting within the framework of the given directions, using their spare time qualifiedly, and asking for help when needed.

4) Compliances skills: There are behaviors that enable the individual to be accepted in the eyes of others, such as following the instructions, obeying the rules, being sharing about sharing goods, awareness of their responsibilities.

5)Assertion skills: There are skills such as being willing to communicate, acting together while playing games, expressing oneself and emotions correctly in social environments.

Merrell and Gimple classified social behaviors into three groups. These are interpersonal relationships, behaviors related to self and behaviors related to responsibilities (Merrell & Gimple, 2014).

Many definitions of the concept of social skills have been made. Behaviors in a social context and interpersonal interactions are at the core of the concept of social skills (Cordier et al., 2015).

Students lack of social skills experience more social isolation and more loneliness. Lack of social skills and insufficient development cause students to have more problems with their peers. In addition, it is known that children with good social skills have higher academic success than their peers who do not have (Miller, 2010). In addition, effective social interaction depends on social cognitive characteristics, which are defined as the ability to understand people's emotions and their minds. Social cognition is the mechanisms underlying social behavior. It includes coding of social cues, representation and interpretation of cues, recognizing emotions from faces and prosody, understanding theory of mind, empathy, and humor (Uekermann et al., 2010).

Whatever the reason for deficiencies in social skills, they are associated with depression and stress. Social skills regulate the relationship between depression and school stress as well as being negatively related to depression. Social skills are preventive factors against childhood depression (Jaureguizar et al., 2018).

There is a relationship between social skills and mental health. In a study conducted with university students, it was found that social skills of individuals were associated with anxiety, depression, and loneliness (Moleller & Seehuus, 2019).

The school environment is one of the areas of life that the lack of social skills in young people and children negatively affects. Students with a lack of social skills are also challenging for their parents and teachers and cause a chaotic school and home environment (Gresham, 2016).

Lack of social skills also reduces access to social support networks. Thus, individuals with a lack of social skills cannot benefit from the mental protective feature of social support networks and may experience psychological problems (Segrin et al., 2016). Also, some psychopathologies also cause a lack of social skills. Autism spectrum disorder (ASD) is one of the disorders that cause social skill deficits.

2. AUTISM SPECTRUM DISORDER

Autism spectrum disorder is a neurodevelopmental disorder that begins early in life and is characterized by social and communicative deficiencies or

limited, repetitive behaviors and interests (APA, 2013). Epidemiological studies conducted in recent years show that its frequency is increasing rapidly, and it is a public health problem. In the report published in 2021, the prevalence of autism was reported as 1/44 (Maenner et al., 2021). In clinical samples, the incidence of ASD in boys is 4 times higher than in girls (Giarelli et al., 2010).

Although there is no major gene in the etiology of autism, there are genetic factors (Chakrabarti & Fombonne, 2001). Siblings of children diagnosed with ASD have a 22 times higher risk of autism (Lauritsen et al., 2005). Besides, the significant difference in the male/female ratio and the 4% probability of recurrence among siblings show that autism is a multifactorial disease (Kumar et al., 2016). ASD is a heterogeneous disorder in which gene-environment interaction is important.

Since the autism clinic appears heterogeneous in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5, evaluating autism within the spectrum was found appropriate. The number of clustered areas of ASD symptoms has been reduced from three to two.

The core symptoms of ASD in the DSM-5 are grouped under 2 main titles as marked deficiencies in the communicative area and stereotypical repetitive behaviors (APA, 2013). Children with ASD cannot perform the social communication that is expected of their age, and they have deficiencies in non-verbal skills. For the diagnosis of ASD, all three criteria in the field of "social interaction/communication deficiencies", at least two of the four criteria in the field of "limited and repetitive interests, behaviors and activities" must be met (APA, 2013).

Although the symptoms of ASD are often recognized between the ages of one and two years, some of the symptoms can be seen before the age of one. Symptoms seen before the age of one such as inability to show positive or negative affect, not looking at the pointed object, insufficient social smile, dislike to be hugged, not babbling, squeaking in a loud voice should raise suspicion for autism (Mukaddes, 2013).

There are some signs that the diagnosis of ASD should be evaluated during development process. One of these symptoms is the inability to make eye contact. In addition, babies with ASD may focus on only one object instead

of exploring many objects and may have stereotypical behaviors such as rocking and clapping. Even if these babies focus on an object or toy, they may lack pointing at it (Wimpory et al., 2000). Children with ASD may have difficulty in orienting to natural stimuli (Dawson et al., 1998).

Children with ASD experience an inability to develop mutual social relationships with their peers. On the other hand, they may misunderstand social messages from body language and give inappropriate answers and behave inappropriately (Church et al., 2000). Even if children with ASD have learning skills based on memorization, there is often a lack of reading comprehension (Church et al., 2000). Some children with ASD may have stereotypical behaviors at school, such as clapping their hands, turning around, and self-talk and humming in anxious situations. These children may be hyperverbal and intrusive when talking to their peers. Thus, children with ASD may not fully understand their roles during a conversation and there may be misunderstandings (Church et al., 2000).

ASD is a disorder with deficits in communication and social interaction. There are difficulties in social reciprocity in ASD. Social skills difficulties in ASD are related to social cognition and social interaction (APA, 2013). The gap between skill and expectation may increase due to the high expected social skills, especially in adolescence. Since the majority of children with ASD are not at the same skill level as their typically developing peers, they experience serious compliance problems in school, both academically and socially.

ASD is a neurodevelopmental disorder accompanied by stereotypical behaviors where there are deficiencies in interpersonal social communication and interaction. These basic features of ASD seriously affect their social lives by disrupting their communication and interaction with their peers and other people around them.

Social skills are the skills that enable individuals to establish healthy communication at home, at work, and in their relationships with their families. In ASD, communication with other people is limited due to deficiencies in social skills (Scattone, 2007).

The avoidance of peers to interact with the child with ASD causes further delays in the emotional, cognitive, and social development areas of the child

with ASD. Also, the lack of adequate communication with peers negatively affects the school life of individuals (Hume et al., 2005).

One of the most important and fundamental deficiencies of individuals with ASD is social skills. In order to continue life, initiating communication, having a mutual conversation, social communication with the people around and understanding the feelings and thoughts of the other person are important steps. Individuals with ASD have inabilities in these steps (Sani-Bozkurt and Vuran, 2014). Children with ASD have difficulty in initiating social communication and maintaining it even if they have started (McConnell, 2002).

Problems in social communication also affect academic and professional life negatively. To overcome this difficulty, individuals with ASD need effective social skills training (Radley et al., 2014). Individuals with ASD have varying degrees of social and motor skill deficits. Social isolation is associated with depression and adverse health outcomes.

There are limitations in eye contact and deficiencies in solving social problems in ASD. Also, individuals with ASD have difficulty in responding to the emotions of others and understanding nonverbal communication.

Symptoms and level of functioning in ASD are heterogeneous due to the nature of the disorder. Autistic patients with good expressive language skills may have difficulties in different parts of the social area. The repetitive behaviors of these patients can be associated with their daily routine. Although some individuals diagnosed with ASD in childhood do not meet the diagnostic criteria for ASD in adult life, they experience some difficulties in social life and are at risk for comorbid psychiatric disorders (Horwitz et al., 2020). On the other hand, ASD patients with significant impairment in expressive language have difficulties in social life (Hattier et al., 2011).

Children with ASD direct themselves to non-social stimuli due to the nature of autism, and this may disrupt both the language and social development of the individual (Gale et al., 2019). In a study conducted with primary school children with high functioning autism, it was reported that students with autism were less accepted by their friends and had less mutual friendships (Chamberlain et al., 2007). It is about making sense of the social stimuli

experienced by students with autism at school. Inclusion systems in schools should be more structured rather than being random (Luddeckens, 2021).

Students with autism are more likely to rejected school than their typically developing peers (Munkhaugen et al., 2017). The frequency of peer bullying is high for students with autism (Skafle et al., 2020).

3. SOCIAL SKILLS TRAINING PROGRAMS

Intervention studies and social skills training programs aimed at increasing social skills for individuals with autism have a positive effect on the treatment of individuals' mental disorders (Wolstencroft et al., 2018).

Interventions for social skills training in ASD are gaining more and more importance day by day. Below are examples of interventions to improve social skills;

1-Parent-facilitated social skills training program (Radley et al., 2014).

2-Group-based social skills training (SST) (Dubreucq et al., 2022). The lack of social reciprocity in ASD demonstrates the importance of group-based interventions (White et al., 2007). SST has been shown to have positive effects on social responsiveness in adults with ASD (Dubreucq et al., 2022). Group social skills interventions are a common treatment modality for children with high-functioning autism.

3-Social story intervention is one of the interventions that can be used to improve social communication skills in children with ASD (Adams et al., 2016). Social stories make it easier for children with ASD to adapt to education. In this way, new routines are created at home and at school by explaining the reasons for the behavior of other individuals (Gray & Garand, 1993). The social story intervention is effective in reducing maladaptive behaviors in society (Adams et al., 2016). It has been reported that the social story intervention reduces problematic behaviors and increases verbal communication for the expression of emotions in ASD (Adams et al., 2016).

4-Virtual reality training programs cause positive effects to improve social skills in ASD (Howard & Gutworth, 2020). Virtual reality programs are based on artificial environments that imitate everyday scenarios. These programs benefit the development of social and emotional skills. Virtual reality

programs make children with ASD feel more comfortable and calmer in their real-life experiences (Newbutt et al., 2020).

5-School-based social skills interventions (Bellini et al., 2007). The school settings provide effective social programming for children. School provides a social environment for interaction with peers. Social skills interventions in the school setting, a side skills intervention, a peer-mediated intervention , child- specific intervention and comprehensive intervention are used.

In addition, animal-supported physical activities in ASD allow children to develop communication and relationships with their peers, accompanied by a fun activity with their peers (Movahedi, 2013).

4. CONCLUSION

Social skills provide the individual to gain a place in society and are important for feelings of belonging and trust. ASD is a neurodevelopmental disorder with deficiencies in interpersonal social communication and interaction, accompanied by stereotypical behaviors. Children with ASD who have insufficient social skills may experience problems in interpersonal relationships, academic, emotional, and behavioral areas. The inability in social skills is also carried over to the future life. It is necessary to increase the social skill levels of these children and ensure their integration with the society. For this purpose, social skills training gains importance. Children with autism need treatment methods and research for better social communication and interaction. It is important to identify the social skills difficulties of children and adolescents with ASD and to develop interventions to improve these skills.

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CHAPTER 7

ORGANOTYPIC HIPPOCAMPAL AND BRAIN SLICE CULTURE MODELS IN NEUROSCIENCE

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1. INTRODUCTION

Organotypic slice cultures, particularly those involving the hippocampus and other brain regions, have emerged as a powerful tool for studying the structure and function of the central nervous system (CNS) in a more physiologically relevant context (Gähwiler, 1981; Gähwiler, Capogna, Debanne, McKinney, & Thompson, 1997; Humpel, 2015). These cultures offer numerous advantages over traditional in vitro models, such as dissociated cell cultures, as they preserve the native cytoarchitecture and cell-cell interactions of the tissue (Humpel, 2015). In recent years, organotypic slice cultures have been employed in a wide range of studies, including investigations of synaptic function, neuronal plasticity, and the mechanisms underlying various neurological disorders (Gogolla, Galimberti, DePaola, & Caroni, 2006b; Noraberg et al., 2005; Stoppini, Buchs, & Muller, 1991).

This book chapter aims to provide a comprehensive overview of the techniques, applications, and challenges associated with organotypic hippocampal and brain slice cultures. We will discuss the history and background of these cultures, detailing the various methods used to prepare and maintain them, and exploring their advantages and limitations compared to alternative models. Furthermore, we will examine their applications in neurobiology research and drug discovery, highlighting the potential for future advancements in this area.

2. BACKGROUND ON HIPPOCAMPAL AND BRAIN SLICE CULTURES

The development of organotypic hippocampal and brain slice cultures can be traced back to the early 20th century, when early pioneers such as Harrison and Trowell first demonstrated the feasibility of maintaining living nervous tissue in culture (Harrison, 1910; Trowell, 1959). However, it was not until the 1970s and 1980s that Gähwiler and colleagues developed the roller tube technique and other methods that allowed for the long-term maintenance of brain slice cultures (Gähwiler, 1981; Gähwiler et al., 1997).

Organotypic slice cultures differ from traditional dissociated cell cultures in that they preserve the native cytoarchitecture and cellular organization of the tissue, including cell-cell interactions and extracellular matrix components

(Humpel, 2015). This feature has made slice cultures particularly well-suited for studying aspects of brain function that depend on the complex interplay between multiple cell types, such as synaptic transmission, plasticity, and circuit dynamics (Gogolla et al., 2006b; Noraberg et al., 2005; Stoppini et al., 1991).

Hippocampal slice cultures have been especially popular in neuroscience research due to the well-characterized anatomy and connectivity of the hippocampus, as well as its central role in learning and memory processes (Gähwiler et al., 1997; Humpel, 2015). Over the years, various techniques have been developed to prepare and maintain organotypic hippocampal slice cultures, including the roller tube method, the membrane interface method, and the air-liquid interface method (Gähwiler, 1981; Noraberg et al., 2005; Stoppini et al., 1991). Each of these techniques has its own advantages and limitations, but all aim to provide an optimal environment for preserving the structure and function of the cultured tissue.

In addition to hippocampal slices, organotypic cultures of other brain regions have also been developed and utilized in neuroscience research. These include cultures of the neocortex, cerebellum, striatum, and spinal cord, among others (Humpel, 2015). These cultures have been employed to investigate a wide range of topics, from the development and organization of neural circuits to the mechanisms underlying various neurological disorders (Gähwiler et al., 1997; Noraberg et al., 2005).

The diverse range of applications for organotypic brain slice cultures is a testament to the versatility and physiological relevance of this model system. Researchers have used these cultures to study various aspects of brain development, such as neuronal migration, axon guidance, and synapse formation (Humpel, 2015). Furthermore, organotypic slice cultures have been employed to model and investigate the pathophysiology of various neurological disorders, including Alzheimer's disease, Parkinson's disease, epilepsy, and stroke (Noraberg et al., 2005).

Recent advancements in imaging and electrophysiological techniques have further expanded the utility of organotypic slice cultures in neuroscience research. For instance, researchers can now perform long-term live imaging of neuronal circuits in organotypic slice cultures, enabling the real-time observation of dynamic processes such as synapse formation, remodeling, and

plasticity (Gogolla, Galimberti, DePaola, & Caroni, 2006a). Additionally, the combination of organotypic slice cultures with advanced electrophysiological methods, such as multielectrode arrays, has facilitated the study of neuronal network activity and the effects of various pharmacological interventions on circuit function (Gähwiler et al., 1997).

In summary, organotypic hippocampal and brain slice cultures have emerged as valuable tools in neuroscience research, providing a more physiologically relevant context for studying the complex structure and function of the CNS. These cultures have been used to address a wide range of research questions, from basic neurobiology to the development of novel therapeutics for neurological disorders. As new techniques and methodologies continue to be developed, it is likely that organotypic slice cultures will remain an important model system for the foreseeable future.

3. ORGANOTYPIC HIPPOCAMPAL SLICE CULTURE: TECHNIQUES AND APPLICATIONS

Organotypic hippocampal slice cultures have become a popular model system in neuroscience research due to their ability to maintain the native cytoarchitecture and cellular organization of the hippocampus. In this section, we will discuss the most commonly used techniques for preparing and maintaining organotypic hippocampal slice cultures and highlight some of their key applications in neurobiology research.

3.1 Techniques for Preparing And Maintaining Organotypic Hippocampal Slice Cultures

There are several methods for preparing and maintaining organotypic hippocampal slice cultures, including the roller tube method, the membrane interface method, and the air-liquid interface method (Gähwiler, 1981; Noraberg et al., 2005; Stoppini et al., 1991).

The roller tube method, developed by Gähwiler (1981), involves placing the brain slices on a porous membrane and rotating the culture tubes in a humidified incubator. This method allows for the exchange of nutrients and waste products between the culture medium and the tissue, while the rotation prevents the slices from sticking to the membrane.

The membrane interface method, introduced by Stoppini et al. (1991), involves placing the brain slices on a porous membrane that is supported by a metal grid and immersed in culture medium. This method allows the tissue to be exposed to both the liquid and gas phases, which facilitates nutrient exchange and gas diffusion.

The air-liquid interface method, described by Noraberg et al. (2005), involves placing the brain slices on a porous membrane that is suspended at the air-liquid interface in a culture dish. This method provides a more physiological environment for the tissue, as the slices are exposed to both the liquid and gas phases, similar to the in vivo situation.

Each of these methods has its own advantages and limitations, but all aim to provide an optimal environment for preserving the structure and function of the cultured tissue (Humpel, 2015).

3.2 Applications of Organotypic Hippocampal Slice Cultures in Neurobiology Research

Organotypic hippocampal slice cultures have been employed in a wide range of studies, including investigations of synaptic function, neuronal plasticity, and the mechanisms underlying various neurological disorders (Gogolla et al., 2006b; Noraberg et al., 2005; Stoppini et al., 1991).

One key application of organotypic hippocampal slice cultures is the study of synaptic transmission and plasticity, which are critical for learning and memory processes. These cultures have been used to investigate the mechanisms underlying long-term potentiation (LTP) and long-term depression (LTD), two forms of activity-dependent synaptic plasticity (Gähwiler et al., 1997). Additionally, researchers have employed organotypic hippocampal slice cultures to examine the role of various neuromodulators, such as dopamine and serotonin, in the regulation of synaptic function (McKinney, Debanne, Gähwiler, & Thompson, 1997).

Another important application of organotypic hippocampal slice cultures is the modeling and investigation of neurological disorders, such as Alzheimer's disease, Parkinson's disease, epilepsy, and stroke (Noraberg et al., 2005). For example, researchers have used organotypic hippocampal slice cultures to study the effects of amyloid-beta peptides on synaptic function and neuronal viability,

providing insights into the pathophysiology of Alzheimer's disease (Harkany et al., 2000).

Furthermore, organotypic hippocampal slice cultures have been employed in drug discovery efforts, serving as a physiologically relevant platform for testing the efficacy and safety of novel therapeutics for neurological disorders (Noraberg et al., 2005).

Organotypic hippocampal slice cultures have also been utilized to study the effects of various environmental factors, such as hypoxia, oxidative stress, and inflammation, on neuronal function and survival (Noraberg et al., 2005; Zimmer & Gähwiler, 1984). For instance, researchers have investigated the mechanisms underlying neuronal cell death and neuroprotection in response to ischemic insults, which can provide valuable insights into the development of novel therapies for stroke and other ischemic disorders (Choi, Maulucci-Gedde, & Kriegstein, 1987).

In addition to their applications in basic neurobiology research, organotypic hippocampal slice cultures have also been employed in the study of neurodevelopmental processes, such as neuronal migration, axon guidance, and synapse formation (Humpel, 2015). These cultures provide a valuable model system for investigating the molecular and cellular mechanisms underlying the development and maturation of the nervous system, which can inform our understanding of various neurodevelopmental disorders, such as autism and schizophrenia (Müller, Jacobi, Sakimura, Malinow, & von Engelhardt, 2018).

Organotypic hippocampal slice cultures have also played a crucial role in understanding the cellular and molecular mechanisms underlying the actions of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), on neuronal survival, differentiation, and synaptic function (Bamji, Rico, Kimes, & Reichardt, 2006; Dijkhuizen & Ghosh, 2005). These findings have implications for the development of novel therapeutic strategies targeting neurotrophic signaling pathways in various neurological disorders, including neurodegenerative diseases and psychiatric conditions (Lu, Nagappan, & Lu, 2014).

Another area of research that has benefited from the use of organotypic hippocampal slice cultures is the study of glial cells and their interactions with neurons. Glial cells, including astrocytes, microglia, and oligodendrocytes, play

essential roles in maintaining the homeostasis of the nervous system and are implicated in various neurological disorders (Verkhratsky & Parpura, 2016). Organotypic hippocampal slice cultures provide an excellent model system for investigating the roles of glial cells in neuronal function, plasticity, and neuroprotection (Humpel, 2015).

In addition, organotypic hippocampal slice cultures have been used to study the effects of neuromodulators, such as cannabinoids and opioids, on neuronal activity, synaptic transmission, and plasticity (Hofmann, Nahir, & Frazier, 2008; Svízenská, Dubový, & Sulcová, 2008). These findings have implications for understanding the physiological roles of endogenous neuromodulatory systems and for the development of novel pharmacological treatments targeting these systems in various neurological and psychiatric disorders.

Finally, the organotypic hippocampal slice culture model has been used to study the mechanisms of neurogenesis, the process by which new neurons are generated in the adult brain (Raineteau & Schwab, 2001). Understanding the factors regulating adult neurogenesis and its functional significance may provide new insights into the processes underlying learning, memory, and brain repair following injury or disease.

4. ORGANOTYPIC BRAIN SLICE CULTURE: TECHNIQUES AND APPLICATIONS

Organotypic brain slice cultures encompass not only hippocampal slice cultures but also other brain regions, such as the cortex, cerebellum, and striatum. These cultures maintain the native cytoarchitecture and cellular organization of the respective brain regions, making them a valuable model system for various neuroscience research areas. In this section, we will discuss the most commonly used techniques for preparing and maintaining organotypic brain slice cultures and highlight some of their key applications in neurobiology research.

4.1 Techniques for Preparing and Maintaining Organotypic Brain Slice Cultures

Similar to organotypic hippocampal slice cultures, various methods have been developed for preparing and maintaining organotypic brain slice cultures,

including the roller tube method, the membrane interface method, and the air-liquid interface method (Gähwiler, 1981; Noraberg et al., 2005; Stoppini et al., 1991). Additionally, the organotypic explant culture technique is another method for culturing brain slices, especially for smaller and more delicate brain regions like the olfactory bulb or the hypothalamus (Baker et al., 2001; Platel, Dave, & Bordey, 2008).

Each of these methods has its advantages and limitations, depending on the brain region of interest and the specific research questions being addressed. However, all methods aim to provide an optimal environment for preserving the structure and function of the cultured tissue (Humpel, 2015).

4.2 Applications of Organotypic Brain Slice Cultures in Neurobiology Research

Organotypic brain slice cultures have been employed in a wide range of studies, investigating various aspects of neurobiology, including synaptic function, neuronal plasticity, glial cell function, neurodevelopment, and the mechanisms underlying various neurological disorders (Gogolla et al., 2006a; Humpel, 2015; Noraberg et al., 2005).

One key application of organotypic brain slice cultures is the study of synaptic transmission and plasticity in different brain regions. For example, cerebellar slice cultures have been used to investigate the mechanisms underlying long-term depression (LTD) at parallel fiber-Purkinje cell synapses, which is essential for cerebellar motor learning (De Zeeuw & Yeo, 2005).

Organotypic brain slice cultures have also been utilized to model and investigate neurological disorders, such as multiple sclerosis, Huntington's disease, and traumatic brain injury (TBI). For instance, organotypic cerebellar slice cultures have been employed to study the effects of demyelination and remyelination in multiple sclerosis (Almeida & Lyons, 2014; Zhang, Jarjour, Boyd, & Williams, 2011). Organotypic striatal slice cultures have been used to investigate the mechanisms underlying neuronal dysfunction and degeneration in Huntington's disease (Cepeda et al., 2004). Organotypic cortical slice cultures have been employed to model TBI and to study the molecular and cellular mechanisms underlying injury-induced synaptic plasticity and repair (Tang, Wang, Feng, Kyin, & Tsien, 2001).

Furthermore, organotypic brain slice cultures have been used to study the development and maturation of neuronal circuits and the role of various growth factors, such as BDNF and NGF, in regulating neuronal survival, differentiation, and synaptic function (Bamji et al., 2006; Dijkhuizen & Ghosh, 2005). Additionally, these cultures have been employed to investigate the effects of environmental factors, such as oxidative stress, inflammation, and neurotoxins, on neuronal function and survival (Noraberg et al., 2005; Xiong et al., 2012).

Moreover, organotypic brain slice cultures have been used to study the role of glial cells, including astrocytes, microglia, and oligodendrocytes, in the maintenance of the nervous system's homeostasis and their involvement in various neurological disorders (Humpel, 2015; Verkhratsky & Parpura, 2016).

Also, organotypic brain slice cultures have been employed to assess the efficacy and mechanisms of action of novel therapeutic agents for various neurological and psychiatric disorders, such as Alzheimer's disease, Parkinson's disease, epilepsy, and schizophrenia (Cho, Wood, & Bowlby, 2007; Humpel, 2015; Lévesque & Avoli, 2013).

In recent years, new techniques and approaches have been developed to enhance the versatility and applicability of organotypic brain slice cultures. For example, the integration of these cultures with microfluidic platforms and three-dimensional (3D) culture systems allows for the creation of more complex and physiologically relevant in vitro models of the nervous system (Lancaster & Huch, 2019; Millet & Gillette, 2012). These advances hold great promise for improving our understanding of the cellular and molecular mechanisms underlying brain function and dysfunction and for accelerating the discovery and development of novel therapeutics for a wide range of neurological and psychiatric disorders.

Furthermore, combining organotypic brain slice cultures with cutting-edge technologies such as optogenetics, chemogenetics, and CRISPR/Cas9-mediated gene editing enables researchers to manipulate neuronal activity, signaling pathways, and gene expression with unprecedented precision, further expanding the range of questions that can be addressed using this model system (Incontro, Asensio, Edwards, & Nicoll, 2014; Kateriya, Jha, & Möglich, 2022; Jan Tønnesen, Sørensen, Deisseroth, Lundberg, & Kokaia, 2009).

In conclusion, organotypic brain slice cultures represent a powerful and versatile model system for studying various aspects of neurobiology across different brain regions. As the methods for preparing and maintaining these cultures continue to advance, along with the integration of complementary technologies, it is anticipated that the range of applications and insights that can be gained from this experimental model will continue to grow, further contributing to our understanding of the complex and fascinating world of the nervous system.

5. ADVANTAGES AND LIMITATIONS OF ORGANOTYPIC SLICE CULTURES

Organotypic slice cultures have numerous advantages that make them a valuable tool in neuroscience research. However, they also come with certain limitations that should be taken into account when interpreting the results obtained using this model system.

5.1 Advantages:

Preservation of tissue architecture: Organotypic slice cultures preserve the three-dimensional (3D) tissue architecture and cellular organization of the brain region being studied (Gähwiler, 1981). This allows for more physiologically relevant and meaningful observations compared to dissociated cell cultures, which lack the complex interactions between different cell types and the extracellular matrix.

Accessibility and experimental manipulations: The exposed surface of organotypic slice cultures allows for direct access to the tissue and easy manipulation of the experimental conditions, such as the application of pharmacological agents, viral vectors, or electrical stimulation (Gähwiler et al., 1997; Stoppini et al., 1991). This enables researchers to investigate the effects of various interventions on neuronal function and plasticity in a controlled manner.

Species and age versatility: Organotypic slice cultures can be prepared from a wide range of species, including rodents, primates, and humans, and at different developmental stages (Gähwiler et al., 1997; Sá, Ruela, & Madeira, 2007). This allows researchers to study species-specific differences in brain function, as well as developmental and aging-related changes.

Long-term maintenance: Organotypic slice cultures can be maintained in vitro for several weeks or even months, depending on the brain region and culture conditions (Gähwiler et al., 1997; Humpel, 2015). This enables the investigation of long-term processes, such as synaptic plasticity, neuronal survival, and regeneration.

5.2 Limitations:

Slice thickness and diffusion limitations: The thickness of organotypic slice cultures, typically ranging from 200 to 400 μm, can result in limited diffusion of nutrients and oxygen to the deeper layers of the tissue (Humpel, 2015; Noraberg et al., 2005). This may lead to cell death or altered cellular function, particularly in the center of the slice.

Loss of long-range connections: The process of preparing organotypic slice cultures inevitably severs long-range axonal connections between different brain regions (Gähwiler, 1981). While this allows for the study of local circuitry, it limits the ability to investigate interactions between distant brain areas.

Limited representation of in vivo conditions: While organotypic slice cultures preserve many aspects of in vivo brain tissue, they lack the full complement of cell types and extracellular components found in the intact brain (Humpel, 2015). Additionally, the absence of blood flow and immune cells may result in an incomplete representation of physiological and pathological processes.

Variability and reproducibility: The preparation of organotypic slice cultures can be subject to variability due to differences in tissue handling, slice thickness, and culture conditions (Humpel, 2015). This may affect the reproducibility of results across different laboratories or even within the same laboratory.

Despite these limitations, organotypic slice cultures remain a valuable and versatile model system for studying various aspects of neurobiology. By carefully considering and addressing the inherent limitations, researchers can continue to make significant contributions to our understanding of the nervous system using this powerful experimental tool.

In summary, organotypic slice cultures provide a versatile and valuable model system for studying various aspects of neurobiology. By preserving the

tissue architecture, allowing for experimental manipulations, and accommodating a range of species and developmental stages, organotypic slice cultures have contributed to our understanding of neuronal function, plasticity, and survival. However, researchers should be mindful of the limitations associated with slice thickness, diffusion limitations, loss of long-range connections, and limited representation of in vivo conditions. By addressing these limitations and applying appropriate controls, organotypic slice cultures can continue to provide valuable insights into the complex workings of the nervous system.

6. EMERGING TECHNOLOGIES AND METHODOLOGIES IN ORGANOTYPIC SLICE CULTURES

As the field of neuroscience advances, so does the development of new technologies and methodologies that can be applied to organotypic slice cultures. These emerging techniques hold the potential to overcome some of the limitations mentioned earlier, as well as to expand the range of possible applications for organotypic slice cultures.

Imaging techniques: Advances in imaging technologies, such as two-photon microscopy and light-sheet microscopy, have enabled researchers to visualize neuronal structures and activity at higher resolutions and depths within organotypic slice cultures (Holekamp, Turaga, & Holy, 2008; Silvestri, Bria, Sacconi, Iannello, & Pavone, 2012) These new imaging modalities can provide a better understanding of the spatiotemporal dynamics of neuronal networks and cellular processes in organotypic slices.

Microfluidic devices: The integration of organotypic slice cultures with microfluidic devices offers precise control over the culture environment, including nutrient supply, waste removal, and drug delivery (J. W. Park, Vahidi, Taylor, Rhee, & Jeon, 2006; Taylor et al., 2003). Microfluidic platforms can also enable the co-culture of different brain regions, allowing for the investigation of inter-regional communication and long-range connectivity (Rambani, Vukasinovic, Glezer, & Potter, 2009).

Optogenetics: Optogenetics, which involves the genetic manipulation of neurons to express light-sensitive proteins, enables researchers to selectively and non-invasively control neuronal activity using light (Deisseroth, 2011). By

incorporating optogenetic tools into organotypic slice cultures, researchers can investigate the causal relationships between neuronal activity and various cellular processes, such as synaptic plasticity, neuronal survival, and network function (J. Tønnesen et al., 2011; Jan Tønnesen et al., 2009).

CRISPR/Cas9 gene editing: The CRISPR/Cas9 system has revolutionized gene editing, allowing researchers to selectively manipulate genes with high precision and efficiency (Doudna & Charpentier, 2014). By employing CRISPR/Cas9 technology in organotypic slice cultures, researchers can study the effects of specific gene mutations or alterations on neuronal function and development (Swiech et al., 2015).

These emerging technologies and methodologies have the potential to greatly enhance our understanding of the nervous system and expand the range of applications for organotypic slice cultures. By integrating these advanced techniques with traditional organotypic slice culture methods, researchers can continue to make significant contributions to the field of neuroscience.

7. FUTURE DIRECTIONS AND POTENTIAL APPLICATIONS OF ORGANOTYPIC SLICE CULTURES

Organotypic slice cultures have already made significant contributions to our understanding of the nervous system, but their potential applications are far from exhausted. As new technologies and methodologies emerge, the scope of organotypic slice cultures will continue to expand, providing unique opportunities to address critical questions in neuroscience. Here, we discuss several future directions and potential applications for organotypic slice cultures.

Disease modeling: Organotypic slice cultures can serve as powerful tools for modeling neurological disorders, such as Alzheimer's disease, Parkinson's disease, and epilepsy. By incorporating disease-relevant mutations, researchers can use organotypic slice cultures to investigate the underlying cellular and molecular mechanisms contributing to these disorders and to screen for potential therapeutic interventions (Humpel, 2015; Noraberg et al., 2005).

Neurodevelopment: The ability to culture slices from different developmental stages provides a unique opportunity to investigate the processes involved in neurodevelopment, such as cell migration, synapse formation, and circuit maturation. Organotypic slice cultures can also be used

to study the role of specific genes in neurodevelopment by utilizing gene editing techniques like CRISPR/Cas9 (Swiech et al., 2015).

Neuronal plasticity: Organotypic slice cultures offer a suitable platform for studying various aspects of neuronal plasticity, including synaptic plasticity, homeostatic plasticity, and experience-dependent plasticity. By manipulating the culture environment and incorporating advanced techniques like optogenetics, researchers can investigate how different factors influence the capacity for plasticity in the nervous system (Jan Tønnesen et al., 2009).

Neuropharmacology: The ability to control the culture environment and apply precise drug treatments makes organotypic slice cultures an ideal model for studying the effects of pharmacological agents on neuronal function and network activity. This can be particularly useful for drug screening and the development of novel therapeutics targeting neurological disorders (Noraberg et al., 2005).

Brain-computer interfaces: With the growing interest in developing brain-computer interfaces (BCIs) for various applications, organotypic slice cultures can be used as a platform to investigate the feasibility and optimization of BCIs. Researchers can study the interactions between implanted devices and neuronal tissue, and assess the effects of various stimulation parameters on neuronal activity and network function.

Integration of advanced imaging techniques: Advanced imaging techniques, such as two-photon microscopy, light-sheet microscopy, and super-resolution microscopy, can be combined with organotypic slice cultures to provide detailed information about neuronal structure, function, and connectivity at the cellular and subcellular levels. These imaging techniques can also enable real-time, longitudinal monitoring of dynamic processes, such as neuronal growth, synapse formation, and plasticity, in living organotypic slice cultures (Silvestri et al., 2012).

Studying neuroimmune interactions: Organotypic slice cultures can be utilized to investigate the interactions between the nervous system and the immune system. By incorporating immune cells, such as microglia, into the cultures, researchers can study the role of these cells in various neurological processes, including neuroinflammation, neurodegeneration, and neuroprotection (Noraberg et al., 2005).

Investigation of the blood-brain barrier: The blood-brain barrier (BBB) plays a crucial role in maintaining the homeostasis of the brain, and its dysfunction has been implicated in various neurological disorders. Organotypic slice cultures can be used to study the properties of the BBB and the factors that regulate its permeability. Co-culturing brain slices with endothelial cells can help to develop more accurate models of the BBB, which can be used to investigate drug transport and delivery to the brain (Taylor et al., 2003).

Study of glioblastoma and other brain tumors: Organotypic slice cultures can serve as models for studying the biology and behavior of brain tumors, such as glioblastoma. Researchers can investigate the interactions between tumor cells and the surrounding brain tissue, as well as the mechanisms of tumor invasion and growth. Organotypic slice cultures can also be used to test the efficacy of potential therapeutic agents for the treatment of brain tumors (Noraberg et al., 2005).

Integration of organ-on-chip technologies: The development of organ-on-chip technologies has the potential to revolutionize the way we study organotypic slice cultures. By incorporating microfluidic devices and bioengineered scaffolds, researchers can create more physiologically relevant models of the nervous system that better recapitulate the in vivo environment. These advanced systems can be used to investigate the interactions between different cell types, as well as the response of neuronal tissue to various stimuli and perturbations (J. Park, Koito, Li, & Han, 2009).

Personalized medicine and drug screening: Organotypic slice cultures derived from patient-specific induced pluripotent stem cells (iPSCs) can be used for personalized medicine approaches, allowing researchers to investigate the individual response of a patient's neuronal tissue to different therapeutic interventions. This approach can help to identify the most effective treatments for individual patients, thereby improving the overall success rate of clinical interventions in neurological disorders (Mertens et al., 2015).

Study of neurovascular interactions: The complex interactions between neurons, glial cells, and the vasculature play a crucial role in maintaining the health and function of the nervous system. Organotypic slice cultures can be used to study these neurovascular interactions, providing insights into the mechanisms that regulate blood flow, oxygen supply, and nutrient delivery to the brain (Carmignoto & Gómez-Gonzalo, 2010).

By exploring these future directions and potential applications, organotypic hippocampal and brain slice cultures will continue to provide valuable insights into the complex workings of the nervous system. As new technologies and methodologies are developed and integrated with organotypic slice culture techniques, researchers will be able to address critical questions in the field of neuroscience and contribute to the development of novel therapeutic interventions for a wide range of neurological disorders.

8. CONCLUSION

In conclusion, organotypic hippocampal and brain slice cultures have already proven to be invaluable tools in neuroscience research. As new technologies and methodologies emerge, their potential applications will continue to grow, providing unique opportunities to address critical questions in the field of neuroscience. By embracing these advancements and integrating them with traditional organotypic slice culture methods, researchers will be able to further enhance our understanding of the complex workings of the nervous system.

Organotypic hippocampal and brain slice cultures have become essential tools in neuroscience research, providing a more accurate representation of in vivo conditions compared to dissociated cell cultures. With a preserved tissue architecture, organotypic slice cultures allow researchers to investigate various aspects of neuronal function, development, and pathology. In this review, we have discussed the techniques and applications of organotypic hippocampal and brain slice cultures, as well as their advantages and limitations.

Emerging technologies and methodologies, such as advanced imaging techniques, microfluidic devices, optogenetics, and CRISPR/Cas9 gene editing, have the potential to further expand the range of applications for organotypic slice cultures and overcome some of their limitations. By integrating these advanced techniques with traditional organotypic slice culture methods, researchers can continue to make significant contributions to the field of neuroscience and enhance our understanding of the complex workings of the nervous system.

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CHAPTER 8

SURGICAL APPROACH TO VESICOURETERAL REFLUX IN CHILDREN

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INTRODUCTION

Vesicoureteral reflux (VUR) can be described as the retrograde flow of urine from bladder to ureter and kidney. It's prevalence is approximately %1 in newborns and increases to 20% for patients with antenatal hydronephrosis (ANH) (Godley, 2010 and Szymanski 2012)

It can be primary due to an anatomic pathology of vesicoureteral junction causing impaired antireflux mechanism or can be secondary to primary bladder diseases with high pressure.

Primary VUR is mostly seen in clinical practice and it usually develops due to inadequate compress of the congenitally short intravesical ureter during bladder contraction. Primary reflux can resolve in first year of life and there are some factors that can help to predict time of spontaneous resolution (Knudson, 2007)

Secondary VUR is usually seen in patients with anatomic problems like bladder outlet obstruction or in patients with functional bladder problems like neurogenic bladder (Willemsen, 2000). Treatment of secondary VUR depends on the treatment of the underlying primary disease.

VUR is an important pathology in children due to possible long-term effects. Timely diagnosis and optimal treatment are needed to protect the patients from reflux nephropathy which may progress to chronic renal failure. VUR is more commonly seen in girls and it is usually diagnosed with mild hydronephrosis and urinary tract infection (UTI). In boys VUR is usually high grade with renal dysplasia and mostly detected antenatally. For all patients, most important comorbid pathology is bladder-bowel dysfunction (BBD). Children with BBD and VUR are more prone to UTIs and more resistant to therapy than children with only VUR (Lackgren, 2021).

The main principles of treatment are preventing UTIs and thus reducing the risk of renal scar development, detecting the children with underlying BBD and planning the optimal treatment. Prompt surgical treatment for patients who do not response conservative observation or effective medical treatment positively affects the long-term morbidity and mortality. Conservative close follow-up is the most preferred approach today. However, some patients need

surgical treatment during this close follow-up. Being able to accurately identify surgical candidates and intervene in a timely manner will create positive results in terms of long-term results.

Indications for surgical treatment are vary in different guidelines. And also there are many different personal clinical practices for every patient. Individual decision of surgical treatment should be made for each patient. Although there are no definite limits for surgical indication, they can be listed mainly as;

- Patients having UTIs under prophylactic antibiotics
- Symptomatic children who have completed potty training and VUR is not resolved spontaneously
- Patients who are not compliant with close observation/medical treatment or who cannot tolerate it for social reasons
- Patients with underlying anatomic abnormalities and VUR

Endoscopic treatment, open surgical techniques or minimal invasive surgical techniques are options for surgical treatment of VUR.

1. ENDOSCOPIC TREATMENT

1.1 Subureteric injection of bulking agents

This technique was first described by Matouschek et al. in 1981 and it aims to prevent reflux by narrowing the ureteral orifice by injection o a bulking agent just below the orifice (Matouschek, 1981). Over the years, different bulking agents with different biological properties have been used for this technique.

Subureteral transurethral injection, hydrodistension technique (HIT) and double hydrodistension injection technique (Double HIT) are different options used for injection. HIT procedure was first described by Kirsch et al in 2009. Hydrodistention is controlled with jet saline flow to the ureteral orifice and the agent is injected to submucosa to narrow the intramural part of the ureter. Based on this technique, they defined a grading system for VUR and acknowledged that there may be minimal interobserver variations and reported a high

correlation between the degree of hydrodistension and the degree of reflux (Kirsch, 2009).

Double HIT technique aims to inject the material both in the middle and the distal portion of intramural ureter. The success rate of Double HIT technique was reported 92% in the literature (kirsch, 2014).

O'Donnell et al first described the subureteral transurethral injection (STING) technique which includes subureteric injection of Teflon® (polytetraflouroethylen). They used an endoscopic needle for submucosal injection of the material below the orifice at the level of 6 o'clock and narrowed the ureteral orifice (O'Donnell, 1984) Because of easily migration of Teflon in other tissues, the use of this agent had been discontinued. Some other agents like collagen or macroplastique had been used for injection but discontinued in a short time due to their potential side effects like immune reaction.

Poliacrylate polyalcohol copolymer (Vantris®) is another agent being used for injection for recent years. Due to it's nature , it has been reported that the risk of obstruction after the procedure may be relatively higher than other agents (Alizadeh, 2013). It's not the mostly used agent nowadays but surgeons who decided to use it should be cautious about this potential side effect.

The most preferred agent which is used for subureteric injection is dextranomer/hyaluronic acid (Deflux® , Dexell ®) It was first described by Stenberg et al in 1995 and has been popular with having minimum side effects and being highly biocompatible (Stenberg, 1995)). In 2001, Deflux had FDA approval and it has been used widely since then (Puri, 2006). There are also many studies reporting high success rates in the literature.

Main complications of subureteric injection are insufficient narrowing , ureterovesical junction obstruction and migration of the agent. Immune reaction and carcinogenic effect are less reported side effects of this technique. After endoscopic treatment the patient should be followed closely for symptoms.

If the endoscopic treatment fails there are different options for clinical practice of each institution or each surgeon. A second attempt can be tried or other treatment options can be discussed with the parents. There are studies reporting that the success rates are decreasing in repeated injections (Puri, 2006).

2. OPEN REIMPLANTATION FOR VUR

Open surgical techniques can be applied in patients who does not have response to endoscopic methods, in whom reflux continues, or in patients who are not suitable for endoscopic treatment due to underlying anatomical pathologies (such as paraureteral diverticulum, obstructive-refluxive megaureter, ectopic ureteral opening). It is known that the success of open reimplantation techniques in vesicoureteral reflux is up to 98% (Sung, 2012).

Short term postoperative complications are similar to every open surgical procedures including bleeding, hematoma, surgical site infection etc. If the procedure is performed stentless, there may be a temporary obstruction due to surgical site edema with reported rate of 2% in the literature (Seseke, 2006). It usually resolves in a few days without need for intervention. If the obstruction persists ureteral stenting may be needed. And impairment of the distal ureteral circulation during dissection may also result in fibrosis in the long term and cause ureterovesical junction stenosis.

Long-term complications are persistence of VUR, ureterovesical junction obstruction, development of contralateral de novo VUR after unilateral reimplantation.

There are intravesical and extravesical techniques for open surgical treatment of VUR.

3.1 INTRAVESICAL OPEN TECHNIQUES

3.1.1 Leadbetter Politano Ureteric Reimplantation

The main steps of the technique are dissecting the distal ureter intravesically, creating a new ureteral hiatus which is located more medially and superior with it's submucosal tunnel extending to the orifice and mucosal anastomosis of the ureter. According to the surgeon's preference the technique can be performed with a ureteric stent or stentless.

3.1.2 Cohen Transtrigonal Ureteric Reimplantation

The technique was first described in 1966 and reported with case series in 1979 by Cohen (Cohen, 1975). The operation starts with a pfannenstiel incision and vertical opening of the bladder. The ureter is dissected

intravesically. After the length of the transtrigonal submucosal tunnel is measured and planned, the ureter is sufficiently dissected, passed through the tunnel across the trigone and anastomosis is performed with healthy ureteral portion after excision of the distal part. It is aimed to prepare a tunnel approximately 5 times the ureter diameter. If bilateral reimplantation is needed both ureters are passed through one submucosal tunnel and anastomosed to the opposite side.

3.2 EXTRAVESICAL OPEN TECHNIQUES

3.2.1 Lich Gregoir Ureteric Reimplantation

This technique, which continues to be applied with various modifications today, was first introduced in 1962. Many modified versions have been used since it's first description (Gregoir, 1962) After the retroperitoneal extravesical release of the ureter, the tunnel location and length are calculated on the posterolateral side of the bladder, and the detrusor is opened without opening the mucosa and anastomosis of the ureter to the hiatus formed from the most distal. One of the advantages of this technique is that it does not require the use of stent. And less postoperative hematuria and bladder spasms are reported due to the fact that the bladder is not opened (Leblanc, 1995).

Postoperative urinary retention is reported in several studies in the literature (Barrieras, 1999).

Minimal invasive techniques are;

- Transvesicoscopic ureteric reimplantation
- Laparoscopic ureteric reimplantation
- Robotic assisted laparoscopic ureteric reimplantation

3.2.2 Transvesicosopic ureteric reimplantation (TVUR)

The technique is not widely used in current practice. It's performed only in some centers by experienced endoscopic surgeons.

The technique includes intravesical ureteric reimplantation with transvesicoscopic approach after the insufflation of bladder through the trocars placed through abdominal wall.

There are studies reporting longer operation time fort his technique in the literature (Canon, 2007).

3.2.3 Laparoscopic ureteric reimplantation (LVUR)

In this technique, extravesical ureteric reimplantation technique is performed with transabdominal laparoscopic approach. It requires minimal invasive surgical experience. Additional to the generally known possible complications of laparoscopic surgery bilateral laparoscopic extravesical approach is reported to carry high risk of postoperative urinary retention (Barrieras, 1999).

3.2.4 Robotic Assisted Laparoscopic Ureteric Reimplantation (RALUR)

One of the areas where robotic surgery is frequently used today is ureteral reimplantation. With the help of robot, the surgical field can be viewed very clearly, surgical manipulation is facilitated, and postoperative urinary retention is minimized with a nerve-sparing approach (Casale, 2008). The robotic technique is similar to laparoscopic approach in it's common nature of using extravesical techniques. The success rate is high, similar to other methods. Less postoperative pain and shorter hospital stay are advantages of this technique. High cost and difficulty in accessing the robot are among its disadvantages (Yeung, 2017).

Over the years, there have been several comparative studies on the methods used for surgical treatment of VUR. It is still widely accepted that open ureteric reimplantation is the gold standard method with the highest success rate. Depending on the technological developments over the years, minimal invasive ureteric reimplantation methods have been gaining popularity. The method to be applied should be determined according to the clinical characteristics of the patient, the degree of reflux, the underlying anatomical pathologies and the surgeon's experience.

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CHAPTER 9

HISTOPATHOLOGICAL ROLE OF PROPOLIS ON UROGENITAL SYSTEM DISEASES

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INTRODUCTION

For centuries, propolis, also known as bee glue, has been recognized and used for its medicinal properties by ancient civilizations such as the Greeks, Romans, and Egyptians, for its medicinal properties and has been used in traditional folk medicine for many years. It is made up of a resinous substance that honeybees collect from different plant sources and then modify with their secretions and beeswax (Kuropatnicki, Szliszka, and Krol, 2013; Almuhayawi, 2020). Propolis was first discovered by the Greeks and used as a natural antibiotic, and is formed by combining the Greek words 'pro' meaning to defend, and 'polis' meaning city, which means defense of the city (Ghisalberti, 1979; Tran, Ogbourne, Brooks, Sánchez-Cruz, and Medina-Franco, 2020). Propolis is a natural product sourced from plants, and its property varies depending on the geographic location and origin (Bankova, Popova, and Trusheva, 2014).

Propolis consists of around 150 chemical compounds, over 20 mineral substances, as well as beeswax, resin, and pollen (Hegazi and Hady, 2001). Propolis has been reported to have pharmacological properties such as anti-inflammatory, antioxidant, anti-tumor, and anti-diabetic as well as cardioprotective, antibacterial, antiviral, hepatoprotective, renoprotective, and activities (Oliveira, Mancini, Oliveira, Passos, and Quilty, 2014; Almuhayawi, 2020; Barud, de Araújo Júnior, Saska, Mestieri, and Campos, 2013; Chiu, Han, Shen, Golovinskaia, and Venkatakrishnan, 2020; Bouzahouane, Ayari, Guehria, and Riah, 2021). Besides, it is protective against various types of cancer including head, lung, liver, brain and kidney, and prostate (Chiu et al., 2020). It possesses high antioxidant activity determined by its phenolic compounds that are usually consumed as an extract (Kurek-Górecka, Keskin, Bobis, Felitti, and Górecki, 2020; Chen, Ye, Ting, and Yu, 2018), and its preparations have been step bye step investigated of their standardization (Dantas Silva, Machado, Barreto, Costa, and Andrade, 2017).

Propolis contains a many number of compounds that explicate a lot of biological effects that quicly the healing process and is widely used in folk remedies, also a functional food due to the availability of bioactive compounds in its extracts (Chen, et al.2018). This book chapter aims to condense the results

on urogenital system diseases of histopathological effects of propolis and its compounds.

Physical and Chemical properties of Propolis

Propolis is a sticky and resinous substance that can range in color from yellow-green to dark brown, depending on where it was sourced and how long it has been stored. It has a hard and brittle structure in cold, and a soft and sticky structure in heat (Ebiloma, Ichoron, Siheri, Watson, and Igoli, 2020, Silici and Güçlü, 2010). Propolis is hard, solid, and brittle below 10ºC, soft and very sticky at 15-25 °C, and has an elastic structure like wax. Generally, it has a melting point between 80-105 °C, which is usually dissolved in alcohol (ethanol, methanol). While it is slightly soluble in water and hydrocarbons, it can be completely dissolved in ether or chloroform (Schmidt, 1997; Kaškonienė, Kaškonas, Maruška, and Kubilienė, 2014).

The chemical makeup of propolis varies based on factors such as its geographic origin, the type of honey bees that collect it, and the time of year when it is harvested. It consists of a complex structure containing compounds with different chemical properties. It also contains approximately 50% resin (containing flavonoids and phenolic acids), 25-30% wax, 10% essential and aromatic oils, 5% pollen, 5% other substances and organic residues (Castaldo and Capasso, 2002; Miguel, Nunes, Dandlen, Cavaco, and Antunes, 2010).

Over 400 compounds have been identified in propolis, including aldehydes, organic acids, esters, hydrocarbons, terpenes, phenolic acids and esters, phenolic aldehydes, alcohols, and ketones. At the same time, the amount of these components in propolis depends on the extraction method (Kaškonienė, et al., 2014; Bracho, Rosado, Pino, 1996). The volatile components of propolis vary according to the geographical and botanical origins of the plants collected by the bees, due to the availability of water, environmental factors, and regional and climatic conditions (Moţ, Soponar, and Sârbu, 2010).

The presence of at least 300 components in the propolis complex, which has been known by chemical analyses, has been revealed so far. These compounds are shown in Table 1. Phenolic compounds and their esters, flavonoids (such as flavonols, flavones, flavanones, dihydroflavonols, and

chalcones), terpenes, beta-steroids, aromatic acids, aromatic aldehydes and alcohols, sesquiterpenes, stilbene terpenes, and caffeic acid phenyl ester (CAPE) are among the organic compounds found in propolis (Kumova, Korkmaz, and Ceyran, 2002; Cantarelli, Camina, Pettenati, Marchevsky, and Pellerano, 2011). The most important group of isolated components is flavonoids. The quality of propolis is evaluated depending on the flavonoid content it contains (Zhang, Huang, Wei, Ping, and Shen, 2014). Flavonoids are plant phytochemicals that cannot be synthesized by humans and have been shown to have antioxidant effects by scavenging free radicals, inhibiting lipid peroxidation, and forming chelates with metals (Lairon and Amiot, 1999; Guido, Jos, Ronald, and Bast, 1997).

Table 1. Chemical components of propolis

Groups	Number of Identified Compounds
Flavonoids	38
Hydroxylavones	27
Hydroxyflavononones	11
Benzoic acid and derivatives	12
Acids	8
Esters	4
Bezaldehyde derivatives	2
Cinnamyl alcohol, Cinnamic acid and derivatives	14
Sterols and steroid hydrocarbons	6
Minerals	22
Aliphatic hydrocarbons	6
Terpene and Sekuterpene alcohol and derivatives	7
Alcohols, Ketones, Phenols and Heteroaromatic compounds	12
Chalcones	2
Sekuterpene and triterpene hydrocarbons	11
Other acids and derivatives	8
Sugars	7
Amino acids	2

(Kumova, et al. 2002)

In addition, propolis contains many minerals such as aluminum, copper, mercury, zinc, iron, fluorine, phosphorus, tin, calcium, chlorine, cobalt, chromium, lead, magnesium, manganese, molybdenum, nickel, potassium, silicon, and sodium. It has been determined that manganese and zinc are more abundant than the other nine elements. The vitamin content of propolis; A, B1, B2, B3, B6, C, E, nicotinic and pantothenic acids are present in different amounts. At the same time, alanine, arginine, phenylalanine, glycol, glutamic acid, histidine, lysine, leucine, and proline. It is found in amino acids such as serine, cystine, and tryptophan (Huang, Zhang, Wang, Li, and Hu, 2014; Yıldırım, 2015; GomesCaravaca, Gomez-Romero, Arraez-Roman, Carretero, & Gutierrez, 2006).

Plant Sources of Propolis

The plant varieties in which propolis is collected intensively vary according to the region and season. In the studies shown that propolis generally, resins of pine (Pinus spp.), birch (Betula spp.), poplar (Populus spp), horse chestnut (Aesculus hippocastanum), willow (Salix spp), alder (Alnus spp), fir (Abies spp), plum (Prunus) spp), elm (Ulmus spp), oak (Quercus spp.) and ash (Fraxinus excelsior) and the composition of propolis may vary depending on the plant source (Table 2) Bankova, Marcucci, and Castro, 2000; Kumova, et al. 2002; Groot, 2013, Yıldırım, 2015).

Table 2. Propolis types by plant source

Propolis type	Plant source
Poplar	Populus Species especially Aigeiros Species and often P. nigra L.
Green Brazilian Propolis (alecrim)	Brazil Baccharis Species predominantly B.dracunculifolia DC
Birch Propolis	Betula verrucosa Ehrh
Red Propolis	D. ecastophyllum and other Dalbergia species
Mediterranean Region Propolis	Cupressaceae (possibly C. sempervirens) and Pinaceae
Clusia Propolis	C. major, C. minor containing Clusia species

(Groot, 2013)

Poplar species are the main source of propolis in non-tropical areas of Europe, North America, and Asia, but where poplar does not grow, bees look for other sources of propolis. For example, Birch trees (Betula verrucosa) in Russia and Baccharis species in Brazil can be sources of propolis. Being aware of the plant sources from which propolis can be harvested is crucial for establishing chemical standardization and advancing scientific understanding (Silici, 2008; Yıldırım, 2015).

The Urogenital System and The Histopathological Role of Propolis

Propolis contains flavonoids, phenolics, and aromatic compounds, as well as beeswax, terpenes, and volatile oils, and has many biological activities. Humans have been using propolis since ancient times to treat numerous diseases supported by in vivo and in vitro studies (Kumari, Jain, and Suman, 2017). The pharmacological activity of propolis can be divided into four main classes, namely, the binding tendency to biological polymers, acceleration of electron transfer, binding to heavy metal ions, and the ability to capture free radicals. Thanks to its unique properties, propolis displays a range of biological activities, including antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, cytotoxic, immunomodulatory, antiulcer, antihepatotoxic, local anesthetic, antitumor, anticancer, and immunostimulatory effects. It is also popularly used in folk medicine, apitherapy, biocosmetics, and the pharmaceutical industry for various purposes (Seven, Aksu, and Tatlı, Yıldırım, 2015; Şahinler, Kurt ve Kaftanoğlu, 2003). The study results in show that propolis reduces lipid peroxidation and apoptosis (Yuluğ, Türedi, Yıldırım, Yenilmez, E, and Aliyazıcıoğlu, 2019; Nieva Moreno, Isla, Sampietro, and Vattuone, 2010). Rizk et al. (2014) reported in a study that evaluated the effects of propolis on by doxorubicin male genital toxicity that propolis could restore testicular oxidative balance and increase sperm count, which could reduce the risk of infertility (Rizk, Zaki, and Mina, 2014). In another study, propolis was found to reduce apoptosis and increase testosterone levels in mitomycin C-induced testicular damage, but it did not have any restorative effect on MDA, SOD, CAT, and GSH levels (Kumari et al.,2017). Yousef et al. (2010) stated that propolis had a protective effect on the testicular toxicity of triphenyltin in terms of enzyme concentrations, and testicular and semen parameters (Yousef,

Awad, and Mohamed, 2010). In a study maked by Yuluğ et al. (2019), it was observed that 100 mg/kg of propolis significantly reduced MDA levels, while was no significant change with 50 mg/kg of propolis. It was also stated that both treatment groups could be beneficial in terms of sperm viability and abnormal sperm count (Yulug et al., 2019). Yıldırım (2015) conducted a study to investigate the dose-dependent preventive effects of propolis on histopathological damage induced by cisplatin (CP) in rat testicular tissue. They reported that 50 mg/day and 100 mg/day propolis administration significantly increased the seminiferous tubule diameter and germinal epithelium thickness was reduced by CP. Additionally, propolis preserved the Johnsen Tubular Biopsy Score by 9% (especially at 100 mg/day). The histopathological findings such as degeneration, edema, separation, and opening observed in the germinal epithelium induced by CP could also be significantly prevented depending on the dose of propolis used (Yıldırım, 2015).

The germinal epithelium of the testicular seminiferous tubule is susceptible to damage caused by cytotoxic drugs due to its high mitotic activity. In studies, it was reported that administration of chemotherapeutic drugs to rats caused degeneration causes a decrease in seminiferous tubule diameter and germinal epithelial cell thickness, as well as shedding of germ cells (Vardi, Parlakpinar, Ates, Cetin, and Otlu, 2009; Atessahin, Sahna, Turk, Ceribasi, and Yilmaz, 2006). Nouri et al. (2009) emphasized that along with these findings, there was a significant decrease in spermatozoa number, motility and viability, and serum testosterone concentration (Nouri, Azarmi, and Movahedin, 2009). In studies investigating the preventive/protective effects of propolis against testicular and epididymal damage caused by antitumor drugs; oral intake of propolis can help mitigate the toxic effects of Taxol on male reproductive organs and semen quality by scavenging free radicals and enhancing antioxidant activities. Additionally, propolis can improve sperm mobility and viability by boosting the overall mitochondrial respiratory effectiveness in spermatozoa (Abd-Elrazek, El-Dash, and Said, 2020). Tohamy et al. (2014) demonstrated that propolis administration can reverse the harmful histopathological changes induced by cisplatin in the testes, including a reduction in spermatogonia vacuolation and an improvement in the organization of seminiferous tubule cells (Tohamy, Abdella, Ahmed, and

Ahmed, 2014). Kamiya et al. (2012) showed that propolis suppressed the Cd-cytotoxicity of COS7 cells (Kamiya, Izumi, Hara, and Adachi, 2012).

Male sexual disorders have been traditionally treated with various natural remedies in folk medicine (Soliman, Sharif, and Moustafa,2012). Yousef and Salama (2009) reported that propolis, administered at a dosage of 50 mg/kg, increased fertility in rats due to the augmentation of sperm production, as well as the enhancement of 17-ketosteroid reductase activity and steroidogenesis (Yousef and Salama, 2009). The maturation process of sperm involves the modification of the sperm surface by different proteins secreted by the epididymal epithelium (Hermo and Robaire, 2002). Consequently, epididymal function abnormalities can directly lead to infertility, highlighting the importance of epididymal integrity to maintain this physiological process (Jarvi, 2012). In a study investigating the effects of green Brazilian propolis on rat epididymis, histomorphometric assessments proved useful in providing information on target cells and the degree of toxicity, indicating the potential for recovery (Morankinyo, Ola-Davies, Adeyemi, Oluwasegun, and Kayode, 2011). The study revealed that propolis altered the histomorphology of the epididymis, with a higher proportion of tubules observed at a dose of 10 mg/kg/day. Principal and basal cells were observed throughout the entire duct, while clear cells were present only in the region of the cauda epididymis. The eas cells synthesize essentially all proteins secreted into the epididymal lumen and are known to have intense endocytic activity. Many natural products are employed in folk medicine to treat male sexual disorders (Capucho, Sette, de Souza Predes, de Castro Monteiro, and Pigoso, 2012).

In the male reproductive system, cell death in the testis and epididymis can occur through various mechanisms, including necrosis, autophagy, entosis, and apoptosis. Necrosis can be initiated by infection, toxins, or physical damage, while apoptosis is a regulated process that does not result in cell lysis and does not induce inflammation. A study by administering 400 mg/kg of royal jelly for 4 weeks showed a significant reduction in caspase-3-positive cells in the testicular seminiferous tubule. This decrease in apoptosis supports the anti-apoptotic activity of propolis (Suleiman, Bakar, and Mohamed, 2021).

Many researchers have investigated pharmacological ideas that could provide "novel therapies" for ovarian disease. It has also been emphasized that

further in vivo studies are needed to confirm the beneficial effects of new treatments in vascular disease (Geyikoglu, Koc, K., Erol, Colak, and Ayer, 2019). The reperfusion of ischemic ovaries initiates the death of parenchymal cells due to a combination of apoptosis and necrosis. Geyikoglu et al. (2019) suggested a new therapeutic potential for Propolis in the down-regulation of caspase-3 expression. Oxidative stress and inflammation are utilized to explain the mechanisms involved in the formation of apoptosis. The final step in the apoptotic cascade is the activation of caspase-3. Therefore, a general interpretation of the consequences of the apoptotic process can only be made based on the levels of caspase-3 mRNA expression. Propolis plays an important role in preserving ovarian tissue, and their combination provides a significant reduction in necrotic cells. (Geyikoğlu et al., 2019). El-Sharkawy et al. (2014) showed that propolis caused partial improvement in ovarian morphology against damage such as granulosa cell degeneration, cumulus cell damage, and vacuolization in the oocyte area due to MXC exposure (El-Sharkawy, Kames, Sayed, Nisr, and Wahba, 2014).

A research study aimed to investigate the impact of propolis on rats with polycystic ovary syndrome (PCOS), a hormonal disorder characterized by the production of irregular amounts of androgens and the presence of numerous cysts in the ovaries of women of reproductive age, according to John Hopkins Medicine. The study focused on evaluating the effects of two different doses (50 and 150 mg/kg) of propolis on various parameters such as p53 expression, ovarian folliculogenesis, serum progesterone, and LH levels in PCOS rats. The results showed a significant reduction in the number of cystic follicles in the propolis-treated group after 21 days of treatment, and the LH level was found to be significantly higher. However, no p53 immunoreactivity was observed (Ali, Paramanya, Poojari, Arslan-Acaroz, Acaroz, 2023).

Although renal cells do not have a high rate of division, they are highly susceptible to toxic injury because they encounter high blood flow, can concentrate toxins in the medullary interstitium, and have specific transporters in the tubular epithelium (Boogaard, Nagelkerke, and Mulder, 1990). The antioxidant activity of propolis is its flavonoid content. One of the benefits of propolis is its ability to act as a scavenger of free radicals, which in turn provides protection against lipid peroxidation (20). In another study on mice

bearing Ehrlich ascites tumors, propolis, and polyphenolic compounds were reported to protect blood, liver, and kidney cells from irinotecan toxicity (Yousef and Salama, 2009; Ulusoy, Öztürk and Sönmez, 2016). Yuluğ et al. (2019) investigated the dose-dependent protective effects of propolis and reported that 50 mg/kg and 100 mg/kg dose administration significantly decreased degeneration in tubular epithelial cells and dilatation in the bowman cavity in rat kidney tissue and provided continuity in proximal tubule epithelial cells. In addition, it was observed that the apoptotic index could be reduced with Propolis 100mg/kg/day (Yuluğ et al., 2019). In another study examining the antioxidant effects of propolis on kidney damage in hypersensitive (HT) rats, it was observed that the increased TOS and OSI levels decreased significantly in the propolis-administered group, and TAS activity increased (Salmas, Gulhan, Durdagi, Sahna, and Abdullah, 2017).

Apoptosis, known as programmed cell death, maintains tissue homeostasis and plays an important role in normal development. However, apoptosis is triggered by external stimuli such as radiation, nutritional deficiency, temperature, and chemicals (Türedi, Kerimoğlu, Mercantepe, and Odacı, 2017). It has been stated in many studies that propolis has an antiapoptotic effect in many tissues. In a study examining the antioxidant, anti-inflammatory, and antiapoptotic effects of Romanian propolis, it was reported that it reduced the number of activated caspase-3 and TUNEL-positive cells (Bolfa, Vidrighinescu, Petruta, Dezmirean, and Stan, 2013). It was observed that the increased apoptosis in kidney tissue induced by cisplatin could be prevented by the dose-dependent apoptotic cell count of propolis (Yuluğ et al., 2019)

Acute kidney injury is a complex disease with various stages that can lead to organ failure. While its pathogenesis is multifaceted, apoptosis can be triggered by factors such as endothelial damage, inflammation, and reactive oxygen species (ROS) (Basile, Anderson, and Sutton, 2012). Da Costa et al. (2015) investigated the protective mechanisms provided via red propolis treatment (by eNOS and HO-1) in a renal ischemia/reperfusion kidney injury model. The histological evaluation of the renal cortex and medulla following renal ischemia showed the presence of tubular necrosis, tubular dilation, inflammatory cell infiltration, and cellular edema in the tubular interstitium of

the renal cortex and outer medulla. They showed that red propolis decreased the tubular necrosis index in this morphologic damage and showed a protective effect and that this protection was associated with a decrease in oxidative stress and upregulation of eNOS, heme-oxygenase (da Costa, Libório, Teles, Martins, and Soares, 2015).

It is emphasized that green propolis reduces macrophage infiltration and apoptosis in sepsis-induced renal injury, fully restores renal tubular function, reduces oxidative stress, and improves renal mitochondria morphology (Silveira, Capcha, Sanches, de Sousa Moreira, and Garnica, 2021). The use of natural products in the treatment of diseases is becoming popular due to their reported multi-mechanistic beneficial effects. In a study by Nna et al. (2021), they investigated the effects of Malaysian Propolis on kidney damage in an experimental diabetes model of rats. It was reported that it improved renal tubular and glomerular damage (Nna, Abu Bakar, Zakaria, Othman, and Jalil, 2021).

In conclusion, propolis is remarkable for the diversity of its biological activities and the variety of its chemical composition. It can be of great importance to humans. In addition, it can be said that it can have preventive/protective effects against many diseases, but in appropriate doses.

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CHAPTER 10

RECURRENT LARYNGEAL NERVE ANATOMY AND CLINICAL SIGNIFICANCE

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INTRODUCTION

Recurrent laryngeal nerve (RLN) was first described as a branch of the cranial nerve by Galen of Pergamon in the 2nd century (Galen, 1962). Vesalius described the cranial nerves and the RLN, which is one of the branches of the vagus nerve, in 1555 by drawing (Saunders, 1982). On the other hand, Willis detailed the vagus nerve and RLN anatomy as it was presented in 16th-century textbooks (Testut et al., 1951).

1. Anatomy of the recurrent laryngeal nerve

RLN is one of the branches of the vagus nerve, which is the 10th cranial nerve. This nerve is named 'recurrens' because it travels in the opposite direction after leaving the vagus nerve and goes to the larynx muscles (Ardito et al., 2004). RLN contains fibers of motor, sensory and parasympathetic character. The last somatomotor fibers leaving the vagus nerve course in this nerve. In fact, it is known that these fibers originate from the the accessory nerve's cranial part. The RLN is involved in the motor innervation of the laryngeal muscles except for the cricothyroid muscle. These muscles are oblique-transverse arytenoid muscles, , thyroarytenoid muscle, lateral cricoarytenoid muscle, posterior cricoarytenoid muscle and vocalis muscle (Maranillo et al., 2016). Sensitive fibers in the RLN are distributed in the mucosa located in the lower part of the vocal fold and transmit the tension sensation from this region to the relevant centers (Arıncı, 2006).

The place of separation of the left and right RLN from the vagus nerve and its course after separation show differences. The right RLN aries from the vagus nerve in front of to the first portion of the subclavian artery. It loops first from the lower portion of the subclavian artery and then from its posterior side and courses upward on the lateral side of the trachea and behind the common carotid artery. It usually courses in the tracheoesophageal sulcus and passes behind the right thyroid gland lobe and enters the larynx under and behind the thyroid cartilage. The left RLN leaving from the vagus nerve on the left side of the aortic arch and at the level of its lower margin. It passes under the arterial ligament and surrounds the aortic arch. It ascends again in the tracheoesophageal sulcus. It enters the larynx below and beyond the thyroid

cartilage after passing behind the left thyroid gland lobe (Ardito et al., 2004; Ling et al., 2016; Myssiorek, 2004).

2. Variations of the recurrent laryngeal nerve

During its path in the tracheo-esophageal sulcus, the RLN crosses behind (50.95%), anterior (20.95%), or between two branches (28.1%) of the inferior thyroid artery. The 'non-recurrent' laryngeal nerve is an uncommon anatomical variation (0.57%) of the RLN (Ling et al., 2016). First reported by Steadman, this variant is usually only found on the right side. It is called non-recurrent because it leaves the vagus nerve in the neck region and descends to the larynx (Cannon, 1999).

In the study of Külekçi et al., the relationship between the RLN and its branches with the inferior thyroid artery and its branches were defined in 6 different types. In type 1 (20.6%), the trunk of the RLN crosses the trunk of the inferior thyroid artery. Type 2 (26.3%) RLN passes between or in front of or behind the branches of the inferior thyroid artery. In the 3rd type (2.1%), the inferior thyroid artery passes between the RLN branches or in front of or behind these branches. In type 4 (14.9%), the branches of the inferior thyroid artery cross the body of the RLN or pass behind, in front of or between the RLN's branches. In the 5th type (32.9%), the inferior thyroid artery's branches and the branches of the RLN cross each other or pass between each other. In type 6 (3.1%), the RLN and its branches are unrelated to the inferior thyroid artery (Kulekci et al., 2012).

In the study of Yin et al., 6 different anatomical variations of the RLN were revealed. In type 1, the RLN runs as a single nerve in the tracheo-esophageal sulcus up to the entrance to the larynx. In type 2, there is duplication of the RLN in the tracheo-esophageal sulcus. Type 3, in the tracheo-esophageal sulcus, it has three separate branches of RLNs. In type 4, the downward branch of the vagus nerve and the upward part of the RLN join and enter the larynx. In the 5th type, these nerves, which merge in the 4th type, enter the larynx as two branches. In type 6, it enters the larynx as a non-recurrent (Yin et al., 2021).

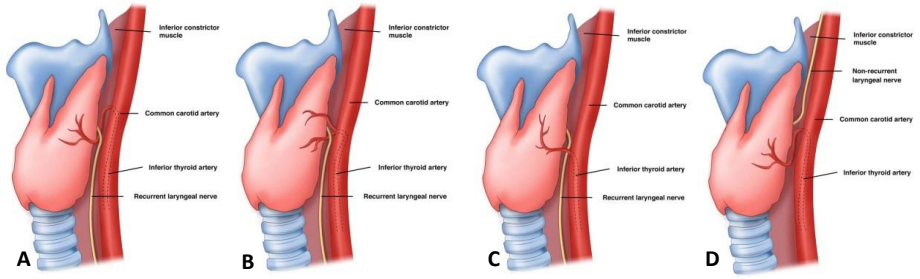

Figure 1. A. Type in which the RLN passes in front of the inferior thyroid artery. **B.** Type in which the RLN travels between the inferior thyroid artery's branches. **C.** Type where the RLN lies behind the inferior thyroid artery. **D.** Non-RLN.

Source: Ling et al. (2016)

3. Anastomoses of the recurrent laryngeal nerve

Laryngeal nerves can form various anastomoses among themselves. These connections between RLN, external laryngeal nerve and internal laryngeal nerve have been studied by many anatomists for centuries. Claudius Galen first described the connection between the RLN and the internal laryngeal nerve (Naidu et al., 2012; Sañudo et al., 1999).

The direct union of the recurrent latyngeal nerve with the posterior branches of the internal laryngeal nerve is known as Galen's anastomosis (Furlan et al., 2002). It has been stated that Galen's anastomosis can be in the form of a single body, as well as in the form of a double body and plexus. This anastomosis is located below the hypopharyngeal mucosa on the posterior cricoarytenoid muscle's posterior surface and transverse-oblique arytenoid muscles (Henry et al., 2017a; Naidu et al., 2014).

The arytenoid plexus connects the anterior branch of the RLN and the arytenoid branch from the posterior portion of the internal laryngeal nerve. This anastomosis is located superficially or deep to the arytenoid muscle. Studies have shown that this neurological association relates to the transverse arytenoid muscle's motor innervation, which takes part in closing the Rima glottidis, and the sensory innervation of the posterior commissure of the larynx (Henry et al., 2017a; Sañudo et al., 1999). According to Sanders et al., this happens when axons from the superior laryngeal nerve enter the RLN via the transverse

arytenoid muscle and descend to the posterior cricoarytenoid muscle and further intrinsic laryngeal muscles (Sanders et al., 1995).

The thyroarytenoid connection is the connection between the ascending branch of the RLN and the descendens branch emerging from the anterior branch of the internal laryngeal nerve. This connection is located on the thyroarytenoid muscle (Naidu et al., 2014; Sañudo et al., 1999). Maranillo et al. showed in their study that a branch goes to the thyroarytenoid muscle at a rate of 1%. (Maranillo et al., 2005).

The cricoid connection is the connection made by the upper branch from the deep part of the arytenoid plexus and the ascending branches of both RLNs. This junction located anterior to the lamina of the cricoid cartilage. The subglottic mucosa's posterior surface receives branches from this juncture (Henry et al., 2017a; Maranillo et al., 2016).

The cricothyroid anastomosis (communicating nerve, piriform nerve) has been defined as a submucosal connection between the RLN and the external laryngeal nerve (Dilworth, 1921). It enters between the pyriform sinus and the lateral cricoarytenoid muscle and reaches the lateral aspect of the thyroarytenoid muscle. At the entrance to this muscle, it divides into two branches and terminates in the subglottic mucosa (Henry et al., 2017a; Naidu et al., 2014). Studies have suggested that this connection does not play a role in muscle innervation and is solely in charge of sensory innervation (Naidu et al., 2014; Sakamoto, 2013; Vogel, 1952).

4. Clinical significance of the recurrent laryngeal nerve

The RLN can be impacted in a variety of circumstances due to its physical interaction with numerous significant structures. The RLN, frequently the left RLN, can be compressed or affected by thyroid masses, mediastinal and lung malignancies, and cardiovascular diseases (Engeseth et al., 2018).

Additionally, due to the thyroid gland's connection to the superior laryngeal nerve and the RLN, surgically removing the thyroid gland may directly or indirectly harm both nerves (Engeseth et al., 2018).

RLN damage is one of the serious complications commonly seen in surgical approaches to the thyroid gland.This is due to a complete or partial

incision on the nerve or due to incorrectly applied surgical techniques such as stretching/traction, ligature, transection, compression and thermal stress. In cases of idiopathic RLN, the presumed cause is usually ischemia (Liu et al., 2020). While hoarseness may occur as a result of unilateral paralysis of the RLN, vocal cord dysfunction that can be life-threatening may occur if it is bilaterally paralyzed.

Unilateral RLN injury causes significant changes in swallowing mechanism and voice in patients. The variation in voice ranges from a soft, whispering voice to a voice that sounds normal but doesn't get loud enough to shout. This difference is caused by the ability of the opposite vocal cord to cross the midline and adhere to the affected cord. If the cords cannot come together, the sound will be soft and not clear. If the cords can come together, the speaking voice will be normal but the ability to shout will be lost (Misiolek et al., 2001).

Bilateral total vocal fold paralysis can cause respiratory problems and obstructive sleep apnea (Dionigi et al., 2016; Liu et al., 2020). If both vocal folds remain in abduction, airway movement will continue, but the risk of aspiration due to inadequate coughing and associated lower respiratory tract infection increases (Dralle et al., 2008). RLN injuries can cause various complications of varying severity. Most of these injuries are temporary, not life-threatening, and usually heal within 6 months. However, the quality of life of the patient is substantially impacted by RLN damage (Dionigi et al., 2016; Yin et al., 2021).

Lahey's study in 1923 and Riddell's study in 1956 reported that During procedures performed on the thyroid gland, the RLN must be exposed. (Lahey, 1923; Riddell, 1956). It has been observed that in surgical applications where the RLN is not visible, the probability of paralysis of this nerve increases 3-4 times, and the probability of damage increases in cases where the dissection is not done well. If the nerve is made visible and dissected, the damage rate is 0-0.2%, while it is 4-6.6% if the nerve not clearly idintified (Zakaria et al., 2011).

RLN damage is the most frequent post-thyroidectomy complication, so routinely exposing and maintaining this crucial structure during thyroidectomy should become standard practice. As a result, best knowledge of the RLN anatomy and its anatomical anomalies or variations is of great importance in

surgical procedures involving the thyroid (Henry et al., 2017a; Ling et al., 2016).

In order to reveal the RLN without injury during the operation, the anatomical landmarks associated with this structure should be well evaluated. These structures are the tracheo-esophageal sulcus, inferior cornu of thyroid cartilage, inferior thyroid artery, Zuckerkandl's tubercle and Berry's ligament (Dvořák et al., 2021; Henry et al., 2017b; Mantalovas et al., 2022).

The inferior thyroid artery and the RLN have a close relationship. However, using this artery alone as an anatomical landmark is unreliable because there are many variations that may occur between the inferior thyroid artery and the RLN. Instead, it would be more reliable to use it as an anatomical landmark in the inferior cornu of thyroid cartilage (Dvořák et al., 2021; Henry et al., 2017b; Mantalovas et al., 2022).

The Berry's ligament, which joins the thyroid gland to the larynx and trachea, is a powerful and vascular structure. A branch from the inferior thyroid artery comes to the lower border of this ligament. Also, the RLN passes through this ligament. Therefore, locating this ligament is important to prevent possible damage to the inferior thyroid artery and the RLN (Dvořák et al., 2021; Rajabian et al., 2017).

Zuckerkandl's tubercle is the posterior-outer lobe of the thyroid gland located above the Berry's ligament. RLN has a varying relationship with this part. Because of this, it is challenging to dissect the distal portion of the RLN (Yin et al., 2021).

As a result, it is important to evaluate the anatomical landmarks together, not alone, in order to reduce the injuries and complications that may occur. Increased knowledge of the anatomical variations, anastomoses, and relations of the RLN with neighboring anatomical structures will minimize injury to this nerve in operations to be applied to the relevant area.

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CHAPTER 11

DEXPANTHENOL FROM YESTERDAY TO TODAY

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DEXPANTHENOL FROM YESTERDAY TO TODAY

Dexpanthenol, also known as provitamin B5, is an alcohol derivative of pantothenic acid (PA). Pantothenic acid gets its name from pantos, meaning "everywhere", and is found in every living cell, including plant and animal tissues. Dexpanthenol whose chemical structure is (R)-2,4-Dihydroxy-N- (3-hydroxypropyl)-3,3-Dimethylbutyramide, is important for the biosynthesis of Coenzyme A (Biro, Thaçi, Ochsendorf, Kaufmann and Boehncke, 2003; Slyshenkov Slyshenkov, Omelyanchik, Moiseenok, Trebukhina and Wojtczak, 1998). It is enzymatically oxidized by coenzyme A and converted to pantothenic acid, which is mainly distributed in tissues. While it is freely soluble in water and alcohol, it is almost insoluble in oils and appears to be the most stable form of pantothenic acid in liquids (Moiseenok, Komar, Khomich, Kanunnikova, and Slyshenkov, 2000).

Dexpanthenol was first identified as a yeast growth factor by Roger Williams in 1933. It is an enzymatic cofactor involved in the oxidation of coenzyme A, carbohydrates, fatty acids, pyruvate, ketone bodies, lactate, and amino acids, and in many synthetic reactions. It is known that pantothenic acid and its derivatives increment the synthesis level of reduced glutathione (GSH), mitochondrial coenzyme A (CoA), and adenosine-5'-triphosphate (ATP) in cells. With these effects, it is stated that it plays an important role in cellular defense, inflammation, and oxidative stress repair systems. It does this with its epithelialization, rapid anti-inflammatory effect, and cellular antioxidant effects against physiopathological conditions caused by free oxygen species (Karadag et al., 2015; Ucar, Aydogan, Vardı and Parlakpınar, 2018).

Vitamin B5 is a molecule that plays a role in essential oils, cholesterol, and steroid hormones for life (Bayrak et al., 2012) In addition, vitamin B5 has an important role in processes such as the production of blood cells and obtaining energy from food. Pantothenic acid aids in the synthesis and metabolism of growth hormones, vitamin D, and some neurotransmission mechanisms. In addition, pantothenic acid conduces to the reduction of fatigue and weakness (Ersungur & Ecem, 2019). Vitamin B5 deficiency is not very common. Vitamin B5 deficiency is seen only in individuals who are malnourished and unbalanced. Vitamin B5 deficiency causes problems such as headache, fatigue, impaired muscle coordination, digestive problems, nausea,

depression, burning feet, and upper respiratory tract infections. These symptoms disappear when vitamin B5 supplementation is provided (Ozdemir, Demirtas., Parlakpinar, Polat, and Tanbag, 2016).

The most effective way of taking vitamin B5, which is necessary for the body, is to have an adequate and balanced diet. Vitamin B5 is found in many foods and can be easily obtained in adequate amounts in the daily diet. It is also produced by normal bacteria living in the colon. Healthy pantothenic acid sources include whole grains, nuts, nutritional yeast, sweet potatoes, legumes, broccoli, red meat, poultry, dairy products, eggs, tomatoes, and mushrooms (Moiseenok, Komar, Khomich, Kanunnikova and Slyshenkov, 2000).

Supplementary preparations obtained from dexpanthenol (vitamin B5) and its derivatives and drugs containing vitamin B5 are generally used to help relieve symptoms such as acne, hyperactivity due to distraction (ADHD), alcohol addiction, allergies, baldness, burning feet syndrome, asthma, carpel tunnel syndrome, chronic fatigue, celiac, conjunctivitis, colitis, dandruff, cysts, depression, diabetes-induced pain, enlarged prostate, dizziness, headaches, insomnia, heart failure, low blood pressure, low blood sugar, multiple sclerosis (MS), osteoarthritis, leg cramps, obesity, Parkinson's disease, premenstrual syndrome (PMS), respiratory problems, tongue infections, rheumatoid arthritis, wound healing, and fungal infections (Proksch, de Bony, Trapp and Boudon, 2017).

Nowadays there are different forms of topical dexpanthenol (cream, moisturizing, gel, drops, lotion, oil, balm, solution, and spray) adapted for pediatric to adult employ. Dexpanthenol can also be used as a pastil for mouth and throat diseases. Topical dexpanthenol has been shown to act as a humectant with barrier-healing properties and additionally to have wound-healing effects. Dexpanthenol plays an important role in numerous skin conditions such as impaired skin barrier, dry skin, sensitive skin, atopic dermatitis, seborrheic dermatitis, or contact dermatitis. One of the important features of skin care is the moisturizing and repair of the stratum corneum skin barrier of the epidermis. Dexpanthenol improves skin hydration in topical applications. In an experimental study, it was observed that dexpanthenol increased the molecular motility of various lipid and protein sections of the stratum corneum. Dexpanthenol has been demonstrated to improve skin hydration by enhancing

the skin's natural moisture barrier. It accomplishes this by interacting with the lipid components of the skin barrier, specifically the extracellular lamellae, and proteins present in the stratum corneum, resulting in an increase in skin hydration and the maintenance or enhancement of skin barrier function (Björklund et al., 2016).

The process of wound healing is typically divided into three phases: inflammation, proliferation, and remodeling. Dexpanthenol has been suggested to promote wound healing by increasing fibroblast proliferation and accelerating epithelialization (Oguz, Uslukaya., Alabalik, Turkoglu, Kapan and Bozdag, 2015). Both conditions are important for both deep and superficial wounds. Ointments or sprays based on dexpanthenol can be used to restore damaged nasal mucosa. Dexpanthenol can be used for the general maintenance of mucous membranes during postoperative wound healing, infections, or allergies (Mösges, Shah-Hosseini, Hucke and Joisten, 2017). In a study by Behr et al. (2023) on the effects of dexpanthenol and formulations containing dexpanthenol on the ciliary beat frequency (CBF) of nasal epithelial cells, it was shown that dexpanthenol reduced CBF at clinically appropriate concentrations (1.67% and 3.33%).

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by itching and repeated inflammation (Abramovits et al., 2008). Preclusive skin hygiene, such as stabilizing the skin barrier function, is critical in the treatment of patients with AD (Girolomoni et al., 2021). Due to its highly hygroscopic properties, dexpanthenol provides high moisturizing properties and increases skin hydration, and decreases transepidermal water loss (TEWL). Thus, it maintains the smoothness and elasticity of the skin (Ebner, Heller, Rippke and Tausch, 2002; Gehring & Gloor, 2000). One study reported that dexpanthenol minimized epidermal disruption during acute and atopic dermatitis exacerbations. Thanks to this feature, it is thought that dexpanthenol can be used as an influential and well-tolerated agent for the control of exacerbations and the treatment of AD (Cho, Kim, Woo and Lee, 2022).

In the pathophysiology of traumatic brain injury, the primary injury caused by trauma is followed by the secondary injury that exacerbates this damage (Kaur & Sharma, 2017). Secondary injury mostly contains deterioration of energy metabolism, lipid peroxidation, oxidative stress,

inflammation, and apoptosis (Lozano et al., 2015). Dexpanthenol, which is related in the synthesis of various neurotransmitters, has an important role in brain health and function. Bektaşoğlu et al. (2023) investigated the antioxidant and neuroprotective effects of dexpanthenol in rats induced by traumatic brain injury. In this study, it has been shown that dexpanthenol stimulates antioxidant systems, reduces oxidative damage and neuroinflammation, suppresses apoptosis, and alleviates brain damage.

One of the most common neurodegenerative disorders associated with aging is Alzheimer's disease (AD). AD leads to a progressive buildup of cognitive impairments, such as significant memory loss, alterations in personality and behavior, and eventually, disability and mortality. (Ortman, Velkoff and Hogan, 2014). The oldest and most prevalent indication of AD is learning and memory impairment (Grand, Caspar and MacDonald, 2011). Since current treatments are only symptomatic, more effective methods for the prevention and treatment of AD were deemed necessary (Lee et al., 2012). The streptozotocin (STZ) compound is a diabetogenic factor that has toxic effects especially on B cells in the pancreas and on insulin receptors in the mammalian brain (Clark et al., 2012). Intracerebroventricular Streptozotocin (ICV-STZ) administration is one of the methods used in animal models of infrequent AD (Ravelli, Rosário, Camarini, Hernandes and Britto, 2017). In their study, Alper Erdogan, Yigitturk, Erbas and Taskıran (2022), investigated the potential neuroprotective benefits of dexpanthenol in rats with streptozotocin-induced neuronal damage. In this study, it was observed that dexpanthenol treatment effectively reduced memory loss. According to the study findings, it was shown that dexpanthenol treatment considerably obstructed the memory loss caused by ICV-STZ by decreasing neuronal injury, cholinergic insufficiency, and neuroinflammation in the hippocampus in rats.

The spine not only supports the transfer of body weight to the lower extremities, promoting proper body posture and balance, but it also safeguards the spinal cord, which acts as a conduit for neural signals between the brain and the peripheral nervous system and vice versa (Bogduk, 2016; Ebraheim, Hassan, Lee and Xu, 2004; Gatterman, 2012). Infection, trauma, neurodegenerative conditions, tumors, and thoracoabdominal aortic surgery are all potential causes of spinal cord damage. Spinal cord damage may impair

motor, sensorial, and autonomic functions momentarily and for good (Cassada et al., 2001; Fan et al., 2011). The spinal cord's limited anaerobic metabolic capacity and glycogen reserves make it highly vulnerable to ischemia. When blood flux to the tissue reduces, cellular energy stores are rapidly consumed, activating ischemic gradually that cause cellular death (Gürer et al., 2017). Reperfusion refers to the restoration of blood flow following a period of reduced or interrupted blood supply, such as during ischemia. However, despite re-establishing blood flow, the process can contribute to tissue damage due to oxygenation, biochemical and molecular alterations that occurred during ischemia, and the formation of free radicals, a phenomenon known as ischemia/reperfusion (IR) injury (Lin, Wang and Yu, 2016). The re-establishment of blood flow following ischemia can lead to an increase in cell death, resulting in oxidative stress, enhanced oxidant mechanisms, inflammation, and an upsurge in apoptotic activity (Gürer et al., 2015; Yilmaz et al., 2012). Gulmez et al. (2022) investigated the neuroprotective effects of dexpanthenol on the ischemia/reperfusion injury model in rabbit spinal cord tissue. In the study, biochemical, histopathological, and functional findings showed that dexpanthenol has neuroprotective effects on spinal cord ischemia/reperfusion injury thanks to its anti-inflammatory, antioxidant, and anti-apoptotic features.

Peripheral nerve damage can occur as a result of congenital, thermal, mechanical, chemical or other causes. Nerve damage can cause loss of sensation and muscle function, as well as neuropathy if not treated in a timely manner or if appropriate techniques are not used. Nowadays, non-steroidal anti-inflammatory agents, nerve growth factors, steroids, thyroid hormone, erythropoietin, adrenocorticotropic hormone, growth hormone, and insulin-like peptides are used in the treatment of experimentally induced peripheral nerve injury (Erbayraktar et al., 2003; Korkmaz et al., 2004; Varejão, Meek, Ferreira, Patrício and Cabritae, 2001). Fatih Korkmaz et al. (2020) investigated the therapeutic effect of dexpanthenol on sciatic nerve damage in a rat model. While myelin degeneration, vacuolization, and edema occurred in rats that were not treated with dexpanthenol, the sciatic nerve damage was observed to be improved in the dexpanthenol group. After histopathological examinations and

motor coordination tests, it was stated that dexpanthenol treatment had a significant curative effect.

Sepsis is a potentially life-threatening condition that occurs as a result of the body's immune response to an infection, often leading to dysfunction of multiple organs (David & Brunkhorst, 2017). The increase in the number of apoptotic cells during sepsis causes microvascular dysfunction and organ failure (Cao, Yu and Chai, 2019). The development of sepsis is closely linked to the dysregulation of inflammatory cytokines and increased apoptotic activity, both of which contribute significantly to its pathogenesis. Zhao, Zhang and Shao (2022), investigated the efficacy of dexpanthenol on the kidney and liver tissues of septic mice in their sepsis animal model by cecal ligation and puncture (CLP). As a result, it has been reported that dexpanthenol reduces inflammatory injury and apoptosis in kidney and liver tissues caused by sepsis, and can also serve as an influential therapeutic drug.

Diabetes mellitus (DM) is a metabolic disease that improves when insulin secretion from pancreatic β-cells is insufficient (Liu, Liu, Han and Sun, 2007). It is thought that hyperglycemia increases oxidative stress both enzymatically and non-enzymatically. The accumulation of reactive oxygen species (ROS) as a result of oxidative stress is known to be cytotoxic, particularly to cell membranes, and is thought to contribute significantly to the pathogenesis of diabetes (Coskun, Ocakci, Bayraktaroglu and Kanter, 2004). In a study investigating the impact of dexpanthenol on kidney tissues of diabetic rats, it was found that dexpanthenol exerted an antioxidant effect by increasing tissue GSH levels, which is critical for protecting against oxidative stress and plays a role in the inflammatory response. In light of the findings, it has been reported that dexpanthenol acts as a free radical sweeper and decreases diabetic nephropathy and renal oxidative stress in rats (Tutun et al., 2018).

Type 1 diabetes mellitus (T1DM) is an autoimmune-mediated metabolic endocrine disorder that results in the destruction of insulin-producing pancreatic β-cells within the islets of Langerhans, leading to insufficient insulin production, which causes a drastic increase in blood glucose levels, resulting in various local and systemic pathological effects (Eizirik & Mandrup-Poulsen, 2001). The study conducted by Gulle, Ceri, Akpolat, Arasli and Demirci (2014), aimed to investigate the impact of dexpanthenol on the histology of the

liver and pancreas, as well as cytokine levels in rats with streptozotocin-induced diabetes. As a result of histopathological examination of liver and pancreas tissue taken from diabetic rats, it has been reported that dexpanthenol reduces hepatic glycogen levels. In addition, dexpanthenol has been shown to have many beneficial features, comprising the normalization of cytokine levels and the protection of pancreatic β-cells and hepatocytes.

Acute myocardial infarction (AMI), defined as the death of the heart muscle due to ischemia, is among the most common diagnoses in patients. A widely used experimental model to investigate the possible protective effects of drugs on myocardial damage from AMI is the induction of infarction in rats by administering isoproterenol (ISO). It is known that ISO causes myocardial necrosis in rats, similar to the damage observed as a result of AMI in humans (Tanriverdi et al., 2017). In a study by Kalkan et al. (2018), the protective and therapeutic effects of dexpanthenol were investigated in ISO-induced cardiac injury in rats. According to the findings of the study, it was shown that there was a important increase in heart weight in the ISO-administered groups compared to the control groups, and dexpanthenol administration significantly reduced these effects. Another important finding is that the application of ISO significantly reduces oxygen saturation. In addition to these findings, it has been reported that the oxidative stress caused by ISO and the morphological damage to the heart tissue can be alleviated and prevented by the application of dexpanthenol.

Bronchopulmonary dysplasia (BPD) is a debilitating respiratory condition that commonly affects premature infants and is associated with a high risk of morbidity and mortality (Alphonse & Thébaud, 2011; Gien & Kinsella, 2011). Recent studies have suggested a strong link between inflammation and hyperoxia, as hyperoxia is a potent proinflammatory agent and its involvement in the development of BPD has been reported (Ambalavanan et al., 2009; Wright & Kirpalani, 2011). Exposure to hyperoxia can result in the overproduction of reactive oxygen species, which may trigger inflammation in the lungs via various pathways such as the activation of transcription factors, signal transduction, and the upregulation of proinflammatory mediators (James, Catharine Ross, Nicola, Steele and Ambalavanan, 2013; Saugstad, 2005). In the studies of Ozdemir, Demirtas, Parlakpinar, Polat and Tanbag (2016) , it was

shown that dexpanthenol provides a protective effect against hyperoxic lung injury in newborn rats. This study is significant because it is the first to demonstrate the beneficial effects of dexpanthenol on alveolarization, oxidative stress, and inflammation in the lungs of neonatal rats with hyperoxic lung injury. These findings highlight the potential of dexpanthenol as an antioxidant and anti-inflammatory agent for the treatment of BPD in premature infants.

Necrotizing enterocolitis (NEC) is a serious gastrointestinal disease that affects primarily premature infants with a birth weight of less than 1500 grams and can result in significant morbidity and mortality. Recent data show that 7% of these infants develop NEC and 20-30% do not survive (Neu & Walker, 2011). The pathogenesis of NEC is multifactorial, and although the cause is not fully understood, local intestinal inflammation initiated by perinatal stress appears to be among these factors in the literature (De Plaen, 2013). While it has been stated that reactive oxygen species play an important role in the pathogenesis of NEC (Clark et al., 1988), it has been stated that the use of various anti-inflammatories and antioxidants reduces the severity of NEC (Guven et al., 2011; Travadi et al., 2006). Karadağ et al. (2015) investigated the protective efficacies of dexpanthenol in the NEC model, assuming that the inhibition of the formation of free radicals and inflammatory mediators or the neutralization of these factors has a decreasing effect on the severity of NEC. This study showed that dexpanthenol treatment alleviates intestinal damage by reducing inflammation, decreases lipid peroxidation and oxidation, and increases antioxidant levels.

Testicular torsion is a urologically urgent and serious condition that can result in testicular atrophy, testicular dysfunction, and infertility (Zhao, Lautz, Meeks and Maizels, 2011). Hypoxia due to torsion is one of the main causes of testicular injury (Makgür, Kilinç, Cahit Tanyel, Büyükpamukcu and Hicsönmez, 1994). Reactive oxygen species (ROS) released by re-establishing blood flow to the testis due to reperfusion provided by detorsion during surgery may cause secondary damage called ischemia-reperfusion injury (Erol et al., 2009; Karbalay-Doust, Noorafshan, Ardekani, Mirkhani and Baker, 2007). In a study by Aydın et al. (2021), the effect of intratesticular dexpanthenol on experimentally induced testicular ischemia/reperfusion injury was investigated. All the results of the study showed that histological and hormonal results in the

dexpanthenol-applied groups differed positively in terms of testicular functions compared to the torsion group, except for interstitial edema. According to the results, it has been shown that intratesticular dexpanthenol application after detorsion preserves the functional capacity of the testis and reduces ischemia-reperfusion injury.

The incidence of ovarian torsion has been increasing in women of reproductive age, making it an important gynecological concern (Yurtcu et al., 2015). In cases of ovarian torsion, early laparoscopic detorsion is the preferred approach for women who wish to preserve their fertility by minimizing the risk of ovarian damage. Various antioxidants can be used to prevent oxidative damage and inflammation in ovaries exposed to ischemia-reperfusion damage (Li & Jackson, 2002). In the study of Soylu Karapınar et al. (2017), in which they examined the effects of dexpanthenol on experimentally induced ovarian ischemia/reperfusion injury, it was shown that dexpanthenol treatment applied before reperfusion improved tissue damage scores, parameters indicative of oxidative stress decreased, and MDA levels decreased significantly.

The small intestines are the organs most susceptible to ischemia/reperfusion injury among other internal organs (Sasaki & Joh, 2007; Takeyoshi et al., 1996). Acute impairments in mesenteric blood flow for any reason can cause ischemic lesions if early diagnosis and treatment is not possible and may result in death (Schneider, Longo, Ure and Vernava, 1994). Many antioxidants and anti-inflammatory agents have been tested in mesenteric ischemia/reperfusion studies in hopes of a new perspective. In the study of Cagin et al. (2016), in which they examined the utility effects of dexpanthenol on mesenteric ischemia/reperfusion injury in an experimental rat model, it was reported that dexpanthenol reduces oxidation, improves intestinal motility and antioxidant system.

Dexpanthenol is considered necessary in epithelial reconstruction processes due to its regenerative and anti-inflammatory properties. Raczyńska, Iwaszkiewicz-Bilikiewicz, Stozkowska and Sadlak-Nowicka (2003), in their study, performed the clinical evaluation of dexpanthenol drops and gel for the postoperative treatment of corneal and conjunctival injuries. This study stated that the difference started on the second day of the operation and that the congestion and edema in the conjunctiva receded in patients who were

administered dexpanthenol. According to the results of the study, it has been shown that dexpanthenol effectively accelerates the healing process of conjunctival and corneal wounds.

The hair growth cycle is a dynamic process that involves three main stages: anagen, catagen, and telogen. Anagen is the active growth phase, during which hair follicle cells rapidly divide and differentiate to produce the hair shaft (Schneider, Schmidt-Ullrich and Paus, 2009). During catagen and telogen, loss of the hair shaft occurs with shrinkage of the hair follicle and apoptosis, respectively, in the cells of the lower part of the hair follicle (Whiting, 2001). Abnormal termination of the anagen phase in human hair growth disorders can lead to gradual hair thinning, highlighting the clinical significance of the anagen-to-catagen conversion. In a study, the in vitro hair growth-promoting activities of dexpanthenol were investigated. According to the study findings, dexpanthenol has been shown to stimulate cell proliferation, prevent apoptosis and reduce the expression of cell aging markers in cultured human hair follicle cells (Shin, Kim, Choi, Kang and Lee, 2021).

Androgenetic alopecia, commonly known as male or female pattern baldness, is a genetic disorder that results in the gradual transformation of thick, terminal hairs into thin, vellus hairs (Tanaçan, Karaosmanoğlu, Kutlu and Ekşioğlu, 2018). Androgenetic alopecia is a prevalent disorder that involves a progressive reduction in the size of hair follicles due to genetic factors (Trüeb, 2002). The pathogenesis of androgenetic alopecia is attributed to the activity and metabolism of androgens, as well as increased levels of reactive oxygen species and nitric oxide (Kaufman, 1996). In a study investigating the possible effects of dexpanthenol for male androgenetic alopecia, it was reported that follicular inflammation and increased radical oxygen species, which are two extensions of the pathogenesis of androgenetic alopecia, can be inhibited by dexpanthenol. While hair growth was observed in patients treated with dexpanthenol, it was concluded that the transformation of hair density and anagen cycle phase could be increased by dexpanthenol (Kutlu, 2020).

As a result, dexpanthenol plays an important role in the protection of human health. Dexpanthenol has been shown to have possible protective effects in many diseases, histopathological, biochemical, and physiological conditions.

However, we think that with further studies, the role of dexpanthenol in all these damage markers can be clarified and its usage area can be expanded.

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CHAPTER 12

NEUROTRANSMITTER-RELATED DISORDERS

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Neurotransmitters, defined as the body's chemical messengers, are molecules secreted by neurons that are used to transmit signals between neurons or at the neuromotor junction. The transmission of messages between neurons is electrochemical in nature and is carried out by electric current until it is transmitted to the axons within the nerve cell, and by chemical means through neurotransmitters in the transmission between neurons at the synaptic gap. Neurotransmitters carry out message transmission by binding to receptors in the target cell. Every neurotransmitter has a specific receptor. Neurotransmitters that carry their messages are recycled by being degraded.

Neurotransmitters can affect the neuron in 3 different ways; excitatory, inhibitory or neuromodulators. Excitatory neurotransmitters provoke activation of the target cell by supporting the generation of action potentials. Inhibitory neurotransmitters reduce the likelihood of target cell activation and have a relaxing effect. Neuromodulatory neurotransmitters are not present in the synaptic cleft and have the potential to affect many neurons.

There are more than 100 neurotransmitters, and more are being discovered. The best known and clinically important neurotransmitters in the body are acetylcholine, dopamine, gamma-aminobutyric acid (GABA), glutamate, endorphin, epinephrine, serotonin, and glycine. This article discusses the first 4 norotransmitters mentioned above and the clinical disorders associated with them.

1. ACETYLCHOLINE

Acetylcholine is a neurotransmitter used to increase the activity of the cholinergic system at the neuromuscular junction. Acetylcholine is an ester of acetic acid and choline. Acetylcholine, the primary neurotransmitter of the parasympathetic system, is secreted by motor neurons for muscle activation. Acetylcholine is also a neurotransmitter that belongs to the group of neuromodulators and plays an important role in attention, memory and motivation.

The amount of acetylcholine in the body is very sensitive, and disturbing this balance in either direction seriously damages the mechanism of the nervous system. Chemicals such as sarin gas irreversibly inhibit the enzyme acetylcholine asterisk, stopping the breakdown of acetylcholine and causing the

muscles to contract continuously, leading to spastic paralysis. Low doses of atropine, used in the treatment of heart disease, destroy muscarinic acetylcholine receptors in high doses.

In the central nervous system, acetylcholine supports cognitive activities by stimulating the cholinergic system in the cerebral cortex and hippocampus, while in the peripheral nervous system it is involved in muscle activation at the neuromuscular junction.

It contains two main receptors, muscarinic and nicotinic. When acetylcholine binds to nicotinic receptors, it opens calcium, potassium, and sodium channels, allowing ions to pass through. There are two main types of nicotinic acetylcholine receptors, the muscle type and the neuronal type. The receptors of the muscular type are found on muscle cells, while the receptors of the neuronal type are found in the autonomic ganglia and in the central nervous system.

The mechanism is more complex for muscarinic acetylcholine receptors. There are 5 subtypes (M1-M5). They all function as G protein-coupled receptors. Found in the central nervous system, peripheral nervous system and organs (heart, lungs, sweat glands), this receptor is inhibitory.

1.1. Myasthenia Gravis

Myasthenia gravis (MG), characterised by defective conduction at the neuromuscular junction, is a neurological disorder with an autoimmune mechanism. Patients with MG have autoantibodies to acetylcholine receptors. These autoantibodies cause blockage and destruction of acetylcholine receptors, disrupting the cholinergic rhythm between nerve terminals and muscle fibres, resulting in a muscle disease that causes fatigue of the ocular, bulbar, respiratory and limb muscles (Dresser et al., 2021).

2. DOPAMINE

Dopamine, a neuromodulatory molecule synthesised in plants, animals and humans, is a chemical belonging to the catecholamine and phenethylamine families. Its precursor chemical is levadopa (L-dopa), which is synthesised in the kidney and brain. Dopamine, an important component of the brain's reward system, is responsible for communication between nerve cells in the brain.

Because an increase in dopamine levels activates the brain's reward mechanism, the main mechanism of addictive drugs is to increase dopamine levels or inhibit dopamine reuptake. In addition, dopamine plays a role in the control mechanism of motor and hormone secretion. Pharmacologically, dopamine is important for motivational stability.

The relationship of dopamine to norepinephrine, another neurotransmitter secreted from the ventral tegmental area and locus coeruleus (blue dot), is clinically very important. Both dopamine and norepinephrine are neuromodulators that control alertness, reward and learning (Ranjbar-Slamloo & Fazlali, 2020). By combining these two neuromodulators, norepinephrine dopamine reuptake inhibitors are used clinically to treat Parkinson's disease, attention deficit hyperactivity disorder and clinical depression (Huot et al., 2016). Dopamine also causes vasodilation by inhibiting the release of noradrenaline in the blood vessels.

Dopamine, which plays important roles outside the central nervous system, increases sodium excretion and urine output in the kidney. In experimental animal models of genetic hypertension, the renal paracrine function of dopamine in increasing extracellular fluid volume is lost (Jose et al., 2000).

Dopamine produced locally in the pancreas signals via adrenergic and dopaminergic receptors with different affinitiesIt controls the release of glucagon and insulin. The interaction of dopamine and norepinephrine signalling is a novel form of regulation to modulate pancreatic hormone secretion. Studies have shown that insulin and glucagon secretion is significantly increased by pharmacological blockade of dopamine D2-like receptors in human pancreatic islet cells with antipsychotic drugs (Aslanoglou et al., 2021).

Dopamine is also an intestinal neurotransmitter involved in digestive secretive and motile functions. Molecular abnormalities of these receptors are thought to play an important role in the pathogenesis of gastrointestinal disorders, as studies have shown the presence of specific dopamine in human gastric and duodenal mucosa (Hernandez et al., 1987).

Disruptions in the dopamine mechanism can cause conditions such as Parkinson's disease, attention deficit hyperactivity disorder, Tourette's syndrome, schizophrenia and bipolar disorder.

2.1. Parkinson's Disease

Parkinson's disease, the most common neurodegenerative movement disorder, is characterised by age-, sex-, genetic- and environmental-related loss of dopaminergic neurons in the substantia nigra pars compacta. The prevalence of Parkinson's disease in Europe is estimated to be 108-257/100,000 and the incidence 11-19/100,000 (Balestrino & Schapira, 2020). In Parkinson's disease, neurons in the substantia nigra degenerate in the frontal lobe, while the amount of dopamine available for neurotransmission in the corpus striatum decreases as the disease progresses. Dopamine deficiency leads to classic Parkinson's symptoms such as rest tremor, rigidity, bradykinesia, loss of postural reflexes and mask-face (DeMaagd & Philip, 2015).

Reduced expression of the dopamine type 3 receptor (D3R) leads to a tragic decrease in dopamine levels, which in turn increases the severity of Parkinson's disease. The D3R receptor is therefore a litmus test for identifying Parkinson's disease (Emamzadeh & Surguchov, 2018).

2.2. Attention deficit hyperactivity disorder (ADHD)

ADHD, the most common childhood neurodevelopmental disorder, is usually diagnosed in childhood and persists into adulthood. ADHD is characterised by difficulties in focusing attention, controlling impulsive behaviour and hyperactivity in children with ADHD (Mandali et al., 2021).

While hyperactivity and attention deficit used to be two separate diagnoses, the American Psychiatric Association has combined them into a single diagnosis with three sub-dimensions; predominantly inattentive type, predominantly hyperactive type and combined type. It has symptoms such as inattention, difficulty concentrating, difficulty completing tasks, forgetfulness and losing things, and these symptoms must be present before the age of 12 and last for 6 months for ADHD to be diagnosed. Due to the presence of functional impairments in the executive functions of the frontal lobe, individuals have

problems with decision-making and emotional regulation in addition to the main symptoms (Schoorl et al.,2016).

Recent studies have highlighted the role of dopamine deficiency in the pathophysiology of children with ADHD. With 5 different receptors (D1-D5), pathological changes in dopamine levels, which play a role in the dopaminergic system, are becoming increasingly important in the pathophysiology of neuropsychological disorders. Studies have shown that the dopamine receptor genes DRD4.7 and DRD5 are associated with ADHD (Wu et al., 2012).

2.3. Tourette's syndrome

Tourette's syndrome, a neurological disorder of childhood onset, is characterised by persistent motor and vocal tics. While the underlying pathophysiology of these disorders remains a mystery, disinhibition of corticostriatal-thalamocortical circuits has been the focus of research. It is known that the pathogenesis shares similarities with other neuropsychiatric disorders such as autism, ADHD and OCD. Tourette's syndrome affects boys more than girls. Patients with this disorder of inhibition have been found to have abnormalities in dopamine function and GABA levels (Hallett, 2015).

Dopaminergic blockers have been reported to be effective in reducing tics in patients with Tourette syndrome. Studies have shown that the use of tetrabenazine, which inhibits the accumulation of dopamine in presynaptic storage vesicles, and α-methylpaatirozine, which blocks dopamine synthesis, prevents the development of tics symptomatically (Lin et al., 2022). Although one study reported an association between Tourette syndrome and a length polymorphism with the restriction fragment of the DRD_2 locus, which is a dopamine receptor (Yuan et al., 2015), another study reported that there was no significant difference in DRD_2 A_1 allele frequency between patient subgroups (Lin et al., 2022).

Although there is some disagreement, it is ultimately thought that excess dopamine in the striatum stimulates cortical circuits and produces tics, and that stimulating these pathways produces more dopamine (Leisman & Sheldon, 2022).

2.4. Schizophrenia

Schizophrenia, derived from the Greek words schizo (split) and fren (mind) by Eugen Bleuler in 1908, is a psychotic disorder that includes delusional beliefs, hallucinations and perceptual disturbances. The symptoms of schizophrenia are divided into two main categories; positive and negative. Delusions, hallucinations and formal emotional disturbances are positive symptoms, while poor speech and lack of motivation are negative symptoms (Hany et al., 2023).

In schizophrenia, there is no major brain pathology. However, there are pathological signals in neuronal populations and in cell-cell communication (Kahn et al., 2015).

Risk factors for schizophrenia include congenital complications, maternal nutritional deficiency, maternal influenza, family history, cannabis use, etc. Neurotransmitters such as dopamine, glutamate and GABA have also been implicated in post-mortem brain studies (Kahn et al., 2015).

Post-mortem studies have found that the brains of schizophrenic patients have a higher than normal density of dopamine receptors and high concentrations of dopamine in the subcortical region. On the other hand, abnormally low dopamine activity in the prefrontal cortex is thought to lead to positive syndromes. The overlap of these high and low dopamine activities in schizophrenia is thought to influence the course of the disease (Davis et al., 2015).

2.5. Bipolar Disorder

Bipolar disorder, a neuropsychiatric disorder characterised by successive periods of abnormally high euphoria and depression, used to be defined as manic-depressive. When the euphoria is severe and associated with psychosis, it is called mania; when it is mild, it is called hypomania. During mania, people are outgoing, sociable, talkative and energetic. In the period of depression that follows mania, symptoms such as asociality, negative outlook on life, crying and avoidance of eye contact are observed (Anderson et al., 2012). The risk of suicide is quite high during the depressive phase and it has been found that 6%

of manic-depressive patients die by suicide within 20 years and 30-40% of them harm themselves (Yatham et al., 2018).

In bipolar disorder, dopamine plays a key role in both the patient's manic and depressive phases. In a hyperdopaminergic state, the number of D2 and D3 receptors rapidly increases, leading to hyperactivation of the brain's reward system and supporting the manic phase. Increased striatal D2 and D3 receptors lead to mania and increased striatal dopamine transporters lead to decreased dopaminergic function and depression. Dysfunction of dopamine receptors and transporters is evident in the pathology of bipolar disorder (Ashok et al., 2017).

3. GAMMA-AMINOBUTYRIC ACID (GABA)

Gamma-aminobutyric acid (GABA), which is the main developmental inhibitor in the central nervous system, reduces neuronal excitability in the nervous system. While 25-40% of synapses in the central nervous system use GABA as a neurotransmitter, this rate is very low in the peripheral nervous system. The neurotransmitter GABA has 3 main types of receptors, $GABA_A$, $GABA_B$ and $GABA_C$ (Li & Xu, 2008).

$GABA_A$ receptors are chloride-permeable, GABA-gated ion channels that are responsible for the vast majority of fast, inhibitory neurotransmission (Phulera et al., 2018). Both in early life and in adulthood, $GABA_A$ receptors are sensitive to small changes in the environment. These neurochemical responses to stress in adulthood depend on the individual's sex. Because of the short- and long-term sensitivity of the GABAergic system to stress, $GABA_A$ receptors have been implicated in the non-genetic aetiology of psychiatric disorders such as depression and schizophrenia, in which stress may be an important factor (Skilbeck et al., 2010). Mutations in the gene expression of this receptor are directly linked to epilepsy syndromes. Schizophrenia, autism, alcoholism, manic depression and eating disorder syndromes are also thought to be associated with abnormalities in this receptor (Hirose, 2014).

$GABA_B$ receptors are G protein-coupled receptors for GABA. They are metatropic receptors that communicate with potassium channels by binding to G proteins. They hyperpolarise the cell by changing the potassium concentration. $GABA_B$ receptors are generally found in the central nervous system, but also in the autonomic nervous system in the periphery (Hyland

2010). $GABA_B$ receptors stimulate potassium (K+) ions to open G protein-coupled potassium channels, which bring the potassium (K+) ions closer to the equilibrium potential. $GABA_B$ receptors also reduce the activity of calcium (Ca+2) ions by stimulating G-proteins. $GABA_B$ receptors are involved in the behavioural effects of ethanol, pain and gamma-hydroxybutyric acid (Ariwodola & Weiner, 2004).

$GABA_C$ receptors, also known as $GABA_A$ rho, have recently been identified and form ligand-gated calcium channels like the $GABA_A$ type. To date, 5 different $GABA_C$ sub-receptors have been identified in different species. Academic studies conducted in the early 2000s revealed associations between the sequence of subunit genes and disease (Enz & Cutting, 1998).

Alcohol dependence, epilepsy, anxiety and idiopathic hypersomnia are GABA-related disorders.

3.1. Alcohol use disorder

Alcohol use disorder can be defined as alcohol dependence that causes problems for both society and the user. Drugs used clinically for alcohol dependence have not been clearly successful. Although benzodiazepines reduce alcohol withdrawal, they cause cross-tolerance and cross-dependence. Acamprosate has been successful in European trials, but has failed in American trials. Naltrexone has had considerable success in preventing dependence but has not eliminated the withdrawal syndrome (Liang & Olsen, 2014).

3.2. Epilepsy

Epilepsy is a non-contagious neurological disorder characterised by recurrent epileptic seizures. An epileptic seizure is caused by an abnormal, excessive and synchronised electrical discharge in nerve cells. Epileptic seizures can range from brief and almost undetectable periods to prolonged, severe convulsions due to abnormal electrical activity in the brain. GABA is the main inhibitory neurotransmitter in the brain and should ideally be in balance with glutamate. Excess glutamate and/or insufficient GABA can lead to over-excitation of the central nervous system, resulting in seizures. The role of GABA in the mechanism of epilepsy has been demonstrated by experimental and clinical studies with 5 lines of evidence. These are;

- GABAergic dysfunction in genetic and animal models of epilepsy
- Decarboxylase activity, binding to $GABA_A$/benzodiazepine receptors, GABA in CSF and brain tissue, and GABA reduction detected in microdialysis studies.
- GABA agonists suppress seizures and GABA antagonists cause seizures to occur.
- Seizures caused by drugs that inhibit GABA synthesis
- Benzodiazepines and barbiturates act by increasing GABA-mediated inhibition (Treiman, 2001).

3.3. Idiopathic Hypersomnia

It is defined as the inability to resist daytime sleep during the day when it is necessary to stay awake, and falling asleep involuntarily for a long time that is not restorative. Idiopathic hypersomnia usually begins before the age of 25 (Sowa, 2016).

In a study analysing the cerebrospinal fluid (CSF) of 32 patients with excessive sleepiness, it was reported that there were more $GABA_A$ receptors in the patients than in the control group, suggesting that $GABA_A$ receptors play an important role in the pathogenesis of hypersomnia (Kaplan et al., 2023).

4. GLUTAMATE

One of the major excitatory neurotransmitters in the brain is glutamate, which in neuroscience refers to glutamic acid anion. It is the most abundant excitatory neurotransmitter in the vertebrate nervous system. Our knowledge of the glutaminergic synapse has advanced rapidly since the 1990s, thanks to the application of molecular biology methods. It plays a role as the primary neurotransmitter in more than 90% of the synaptic connections of major excitatory functions in the human brain and in granule cells of the cerebellum. There are 3 families of ionotropic receptors that are intrinsically cation permeable:

1- N-methyl-D-aspartate (NMDA): This is an ionotropic receptor and is sensitive to calcium ions.

2-A-amino-3-hydroxy-5-methyl-4-isox-azolepropionic acid (AMPA): an ionotropic receptor specialised in rapid excitability, it produces an electrical response to its target in less than 1 millisecond.

3- Metabotropic glutamate receptors: activated by a second messenger system to produce slow but long-lasting effects on their targets (Meldrum 2000).

Because of glutamate's effects on plasticity, they play an active role in brain functions such as learning and memory. All 3 glutamate receptors are involved in learning and memory. NMDA receptors are the conductors in the learning process and are particularly active in brain coding. Although the role of AMPA receptors alone is not known, when these receptors are blocked, neuronal transmission is blocked and the learning process is negatively affected. Metabotropic glutamate receptors are involved in learning functions such as memory formation, consolidation and modulation of (Riedel et al., 2003).

Excess glutamate is produced in diseases such as amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Alzheimer's, Parkinson's, stroke, fibromyalgia and chronic fatigue syndrome.

4.1. Amyotrophic Lateral Sclerosis (ALS)

ALS, also known as Lou Gehrig's disease, is a neurological disease that causes neurodegeneration of motor neurons. An analysis of the etiology of ALS shows that both hereditary and environmental factors play a role. In ALS, lower motor neurons in the proximal limbs degenerate first and symptoms appear. As the disease progresses, both upper and lower motor neurons are affected. In the final stages of the disease, the respiratory muscles are also affected and death is inevitable. Although there is no definitive treatment for the disease, life expectancy can be increased to 10 years or more and symptoms can be relieved with pharmacological treatment (Brotman et al., 2022). In ALS patients, glutamate levels are higher than normal. Abnormally high levels of glutamate in the synapse lead to overactivation of glutamate receptors, which means an excessive influx of calcium into the cell. This excess calcium causes mitochondrial dysfunction, an increase in reactive oxygen species and

ultimately cell death. The glial synapses surrounding these destroyed neurons stimulate the production of glutamate, allowing more calcium to enter the cell and the mitochondria. This is the vicious cycle of neuronal degeneration (Collins et al., 2022). An antioxidant diet is recommended to reduce the effects of oxidative stress in ALS. Foods rich in curcumin, creatine, coenzyme Q10, vitamin E, vitamin A and vitamin C should be included in the patient's diet (D'Antona et al., 2021).

4.2. Multiple Sclerosis (MS)

The disease destroys the myelin sheathing in the central nerve system and causes plaque build-up in the brain. MS affects young adults and is the most common non-traumatic disability. Although the underlying cause of MS and the mechanisms behind the increasing incidence of MS are not fully understood, genetic and environmental factors are known to play a role. It is thought that low serum vitamin D levels, smoking habits, childhood obesity and Epstein-Barr virus infection may play a critical role in the development of MS epidemiology (Dobson & Giovannoni, 2019).

Studies since the early 2000s have shown that overactivity of glutamate receptors leads to cell death. In MS, it has been suggested that this mechanism may lead to inflammation and cell death. Glutamate levels are known to be higher in people with MS than in healthy people (Kostic et al., 2013).

4.3. Alzheimer's Disease

Alzheimer's disease, a chronic progressive neurodegenerative disorder, usually has a slow onset and a gradually deteriorating course. Alzheimer's disease accounts for between 60 and 70 per cent of all cases of dementia. Alzheimer's symptoms start with difficulty remembering recent events and progress to language and communication problems, disorientation, mood changes, lack of motivation and behavioural problems.

One of the factors underlying impaired learning and memory in Alzheimer's disease is a dysfunction in glutamate neurotransmission. Glutamate is an agonist of the N-methyl-d-aspartate receptor (NMDAR). Synaptic NMDAR activity initiates plasticity and promotes cell survival, whereas extrasynaptic NMDAR activity promotes cell death. In addition,

excessive NMDAR activity causes excitotoxicity. In patients with Alzheimer's disease, glutamate levels are elevated, triggering NMDAR overactivity (Chang et al., 2020; Wang & Reddy, 2017).

4.4. Chronic Fatigue Syndrome

Chronic fatigue syndrome, also known as myalgic encephalomyelitis, is characterised by debilitating fatigue associated with physical symptoms that do not improve with rest. According to the Centres for Disease Control and Prevention criteria, it must include at least 4 of the following syndromes.

Post-exertional fatigue

- Revitalising sleep
- Impaired memory or concentration
- Muscle pain
- Polyarthralgia
- Sore throat
- Tender lymph nodes
- New headaches

However, before a diagnosis of chronic fatigue syndrome can be made, other conditions need to be ruled out. Depression, pain and sleep disorders should be assessed in people with chronic fatigue syndrome (Yancey & Thomas, 2012).

Glutamate neuroexcitotoxicity mechanisms may contribute to neuropathology and neuroinflammation in the GWI subgroup who do not develop post-exertional tachycardia in an academic study of patients with chronic fatigue syndrome. Glutamate is increased in the GWI STOPP phenotype, suggesting that neuroexcitotoxicity is a component of post-exertional weakness and fatigue in these individuals (Baraniuk et al., 2021).

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CHAPTER 13

LIMBIC SYSTEM

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INTRODUCTION

The common name of the regions that support functions such as smell, long-term memory, emotion and behavior, formed by the structures surrounding the hypothalamus, is the limbic system. In the limbic system, which derives from the Latin word "limbus", gyrus means "edge". Paul Broca named it the "large limbic lobe" for the first time in 1878. In 1949, the "limbic system" was defined by Paul Donald MacLean.

This system is also called the "visceral system". Since it is closely related to the olfactory structures in the limbic system, a part of this system is located in the rhinencephalon. While the sense of smell is the only sense that connects directly with the limbic system, the other senses connect with the limbic system after the thalamus. The limbic system also has an effect on the autonomic nervous system and the endocrine system through the hypothalamus. The limbic system is particularly concentrated in the temporal lobe region.

When the limbic system is functionally examined:

- It takes part in senses such as sleep, hunger, thirst, stress, excitement and instinct.
- Plays a role in the level of libido (sexual desire).
- It takes part in functions such as learning new information, keeping this learned information in our memory, and remembering this information when we need it.
- It takes part in creating an emission and bodily response with the instinct of survival against the stimuli coming from the external and internal environment.

Structures that make up the limbic system:

A. Limbic lobe
    1. Cingulate gyrus
    2. Parahippocampal gyrus
    3. Septal area (Septum pellucidum, Paraterminal gyrus, Subcallosal area)

B. Other structures found in the limbic system
    4. Rhinencephalon
    5. Thalamus
    6. Hypothalamus
    7. Epithalamus
    8. Amygdala
    9. Hippocampal formation
    10. Prefrontal cortex
C. Connective structures in the limbic system
    11. Cingulum
    12. Fornix
    13. Papez circuit
    14. Medullary stria
    15. Terminal stria
    16. Diagonal band of Broca
    17. Medial forebrain bundle

Limbic lobe:

It consists of structures surrounding the corpus callosum. It consists of the cingulate gyrus, the parahippocampal gyrus, and the septal area.

1. Cingulate gyrus: This part, whose anterior part belongs to the frontal and posterior part belongs to the parietal lobe, is located on the corpus callosum on the inner surface of the brain. The association pathways in it are called cingulum. These pathways connect with the hippocampus. It has functions related to memory, emotion and neuropathic pain.
2. Parahippocampal gyrus: It is the gyrus located on the lower-inner side of the temporal lobe, between the collateral sulcus on the outside and the hippocampal sulcus on the inside. The anterior part of the uncinate gyrus, known as the olfactory center, is called the entorhinal cortex (Brodmann 28).
3. Septal area: It is an important part of the limbic system, located in front of the lamina terminalis and anterior commissure. If the region is stimulated, there is a sense of satisfaction because the reward center is located in this region.

Septal area; It is formed by the paraterminal gyrus, the septum pellucidum, and the subcallosal area. It is located deep to the septal nuclei and is attached to the amygdala by the diagonal band of Broca.

When the structures forming the septal area are examined:

Paraterminal gyrus: It is the part located behind the posterior parolfactory sulcus and in front of the lamina terminalis.

Septum pellucidum: It is the structure between the two hemispheres of the brain, located between the fornix and the corpus callosum.

Subcallosal area: It is located in front of the posterior parolfactory sulcus, behind the parolfactory sulcus and in the lower part of the rostrum of the corpus callosum.

Other structures found in the limbic system

Rhinencephalon: Smell in the air is taken by the receptors and comes to olfactory nerve, olfactory tract, lateral olfactory stria and uncus. Entorhinal cortex, uncus, anterior perforated substance are collectively called “olfactory area”.

The entorhinal cortex is known as Brodmann field 28 and is located anterior to the parahippocampal gyrus and deep to the uncus. It functions in coding, cognitive function, spatial memory, memory and navigation functions. In the early stages of Alzheimer's disease, this area is damaged. The object smelled in the lesion cannot be visualized and the smell is not remembered.

Thalamus: It receives afferent fibers from the amygdala, hypothalamus, and accumbens nucleus via the dorsomedial and anterior thalamic nuclei. It is involved in memory formation by connecting to the prefrontal cortex, limbic lobe and temporal association cortex.

Hypothalamus: It sends impulses from the limbic system to the thalamus and activates the endocrine system and autonomic nervous system with the emotional stimuli it receives from the limbic system, since it is the center of the autonomic nervous system. In the hypothalamus, the lateral nucleus, mammillary body, and ventromedial nucleus are nuclei related to the nutrition reflex and the limbic system.

Epithalamus: Another name for the epithalamus is the habenula, and it is a small area associated with the limbic system. Its medial part receives fibers from the septal area and attaches to the pineal gland. Its lateral part receives afferent fibers from the hypothalamus and globus pallidus and attaches to the mesencephalon. The nuclei of the epithalamus are related to the sleep cycle, nutrition, reproduction, stress and learning.

Amygdala: Almond-shaped and is the nucleus located below the caudate nucleus and uncus. It takes part in the expression of emotions such as anger, crying, excitement, happiness, and fear and is the place where visceral-somatic sensations are stored.

Hippocampal formation: It plays a role in memory and learning. It consists of three parts.

Hippocampus: The region resembling a seahorse is a 6-8 cm long gray matter structure located in the inferior horn of lateral ventricle (Figure 1).

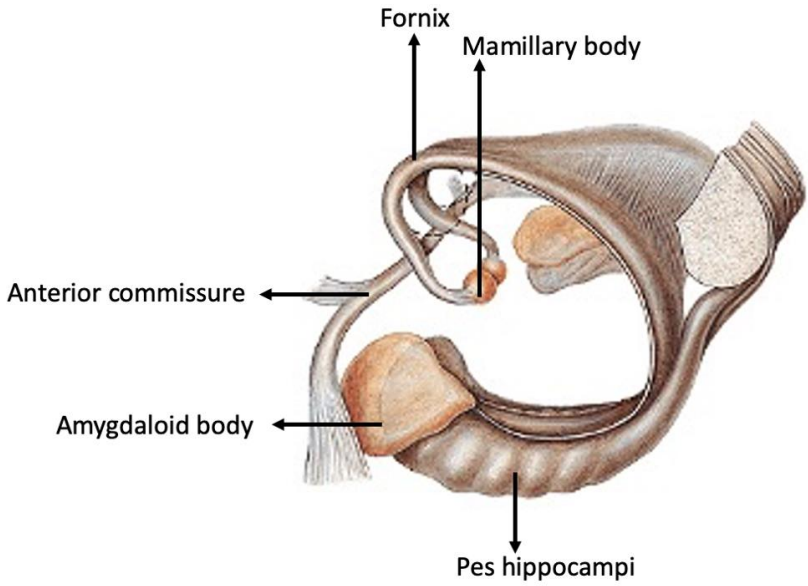

Figure 1. Fornix, Pes hippocampi, Commissura anterior

It is one of the oldest parts of the phylogenetic brain, the memory center, also called ammonis cornua. The upper part of the hippocampus is covered with the alveus of hippocampus and the claw-shaped structures extending forward

from the hippocampus are called pes hippocampi. The toothed parts of this structure are called hippocampal digitations (Figure 2).

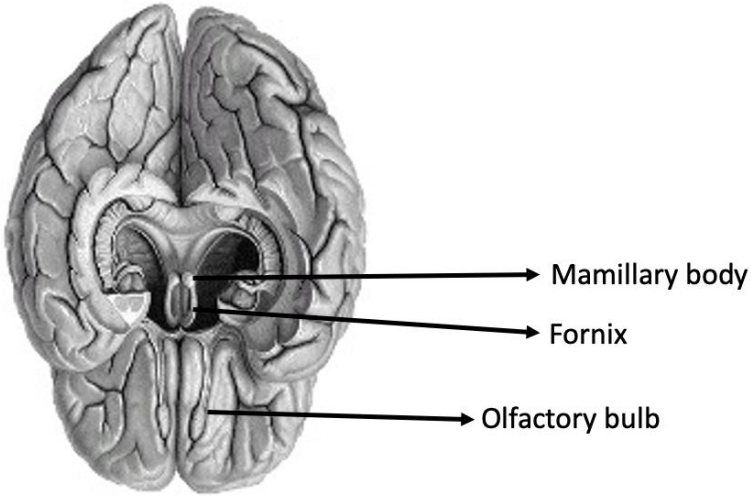

Figure 2. Hippocampus

The hippocampus is cellularly divided into 4 different regions. The CA1 region is the most sensitive part located between the hippocampus and the subiculum. CA2 v CA3 regions are in the hippocampus. The CA4 region is located between the hippocampus and the dentate gyrus.

The hippocampus is involved in direction finding, spatial memory and transformation of movements into behavior. When stimulated, behaviors such as sex drive and anger occur, while epilepsy attacks are observed if over-stimulated.

Subiculum: It is the transition place between the parahippocampal gyrus and the hippocampus. The subiculum functions to complete memory components. It has sections as presubiculum, parasubiculum, postsubiculum and prosubiculum.

Gyrus dentatus: It is the reddish colored area between the fimbria of the hippocampus and the subiculum. Between the parahippocampal gyrus and the dentate gyrus is the hippocampal sulcus. It is located in the input region of the hippocampus.

Prefrontal cortex: The area in the frontal lobe known as the personality center and working memory.

CLINICAL RELATIONS

When the limbic system diseases are examined, we encounter schizophrenia, memory disorders, hallucinations, swallowing-chewing tics, epileptic seizures, hypersexuality, emotional and memory disorders.

Schizophrenia: It is generally a chronic mental illness that is reflected as thought, behavior and emotion disorders in the cortical and subcortical limbic system. It consists of the Greek words "frenos" meaning mind and "schizo" meaning divided.

Obsessive-compulsive: It is a mental disorder known as perfectionist, over-organized, obsessive preoccupation with details.

Kluver-Bucy syndrome: It is a condition that occurs in bilateral destruction of the hippocampus and amygdala as quick forgetting, excessive sex drive, putting everything in the mouth and extreme fearlessness.

Memory disorders: If there is a lesion in the hippocampus, visual memory is affected if the lesion is on the right, and verbal memory is affected on the left. Emotional disorders such as calmness and anger are seen and short-term memory cannot be converted into long-term memory.

Hyperosmia: The condition known as excessive olfactory perception is seen in cocaine and hysteria addicts. Parosmia is the condition of changing the meaning of smell, it is seen in limbic system disorders and schizophrenia.

Kleptomania: It is the state of not being able to prevent the person from stealing things even if his financial situation is good.

Unipolar depression: It is a condition that is seen as not being interested in enjoyable activities and lack of self-confidence.

Bipolar disorder: It is a condition that affects the performance of daily activities, known as manic-depressive. It is a disorder that can develop as a result of chemical imbalances in the brain, whose genetic history is important. It is divided into three periods.

In the manic period, symptoms such as attracting attention, excessive joy, excessive increase in sexual impulses, use of pleasurable substances, impatience, excessive spending and sleeping less are observed.

In the depressive period, there are situations such as boredom, fatigue, hopelessness, loss of appetite, incompatibility, and suicidal ideation.

In the mixed period, the findings of both periods are seen in the patient.

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CHAPTER 14

ROLE OF INFLAMMASOMES IN DISEASES

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INTRODUCTION

Inflammasomes are multimeric protein structures that develop in response to various physiological and pathological stressors. Inflammasome activation, which is one of the essential components of the innate immune response, has an important function to clear pathogens or damaged cells. With the activation of the inflammasome, which is the trigger of autoimmune and metabolic disorders, the physiological and pathological relationships in the process can be better understood (Sharma and Kanneganti, 2016).,

In terms of their structural features, we can classify inflammasome sensors as AIM 2 (absent in melanoma 2)-like receptors (ALRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and pyrin. Its structure includes a NLR, ALR, or pyrin sensor, and an enzymatic component (caspase-1),as well as an adapter molecule, usually ASC (CARD-containing apoptosis-associated speck-like protein). Inflammasomes can be put together by these receptors, and the cysteine protease caspase-1 can be activated. The activation of the sensor by detecting certain stimuli causes the ASC to nucleate to form a focus or granule in the activated cell. As a result, caspase-1 is successively attached to the nucleated ASC after undergoing autocatalytic cleavage to create active subunits like p10 and p20.Pyroptosis, or inflammatory cell death, is induced by the cytokines IL-1 and IL-18, which are proteolytically processed by these active caspase-1 subunits (Sagoo et al., 2016).

The host cell's defensive mechanisms are provided by activated caspase-1, which causes the production of mature cytokines and the elimination of affected or harmed cells. Defense mechanisms for the host cell, such as mature cytokine production and the destruction of infected or damaged cells are provided by activated caspase-1. Thus, the inflammasome assembly is a component of the so-called coordinated signaling, which entails mounting a suitable immune response in the wake of pathogenic or sterile attacts.In this mechanism, a variety of chemical and cellular signals are involved. The balance between the inflammatory response and resolution is maintained in this system by a number of molecular and cellular signals (Sagoo et al., 2016; Sharma and Kanneganti, 2016).

2.1.Types of Inflammasome

2.1.1.NLR Family

While NLR family members also possess a central nucleotide-binding domain (NBD), the majority of members of the NLR family with an NBD have a C- terminus leucine-rich repeat (LRR) domain and a changeable N- terminus domain. The process of cleavage of the NLR family into NLRP or NLRC depends on whether there is a pyrin or caspase activating and collector domain (CARD) at the N terminus, respectively (Sharma and Kanneganti, 2016).

2.1.1.1. NLRP1

Together with the canonical NBD and LRR domains, the pyrin domain (PYD), function finding, and C-terminal CARD domain form Human NLRP1. It is encoded by the NLRP1 (a-c) mouse genome, consisting of three paralogs, without PYD. The anthrax-killing toxin produced by Bacillus anthrax activates NLRP1b.The formation of pores in the protective antigen and host cell membrane by this deadly toxin, which has protective antigen and lethal factor, causes the lethal factor to enter the cell. Thus, NLRP1 cleaves from an N-terminal region, activating inflammasomes (Figure 1) (Chavarría-Smith and Vance, 2013; Sharma and Kanneganti, 2016).

The NLRP1b function-finding domain must be proteolytically cleaved before an inflammasome can operate. With different bacterial toxins, NLRP1b can act as a protease activity sensor. Parasite clearance is promoted by NLRP1b activation, and mice are protected against death by response to *Toxoplasma gondii* infection (Chavarría-Smith and Vance, 2013; Gorfu et al., 2014; Sharma and Kanneganti, 2016).

NLRP1 activation is important in determining parasite control of infected macrophages as well as its induction by *Toxoplasma* in susceptiblerat strains. *Toxoplasma* infectivity is increased with NLRP1 gene single nucleotide polymorphisms and congenital toxoplasmosis associated with NLRP1 damage in human monocytic cell lines. Its clinical significance is known to be hidden in the connection between the immunological response to *Toxoplasma* infection and the NLRP1 inflammasome. (Cirelli et al., 2014; Cavailles et al., 2014; Ewald et al., 2014; Sharma and Kanneganti, 2016) (Figure 1).

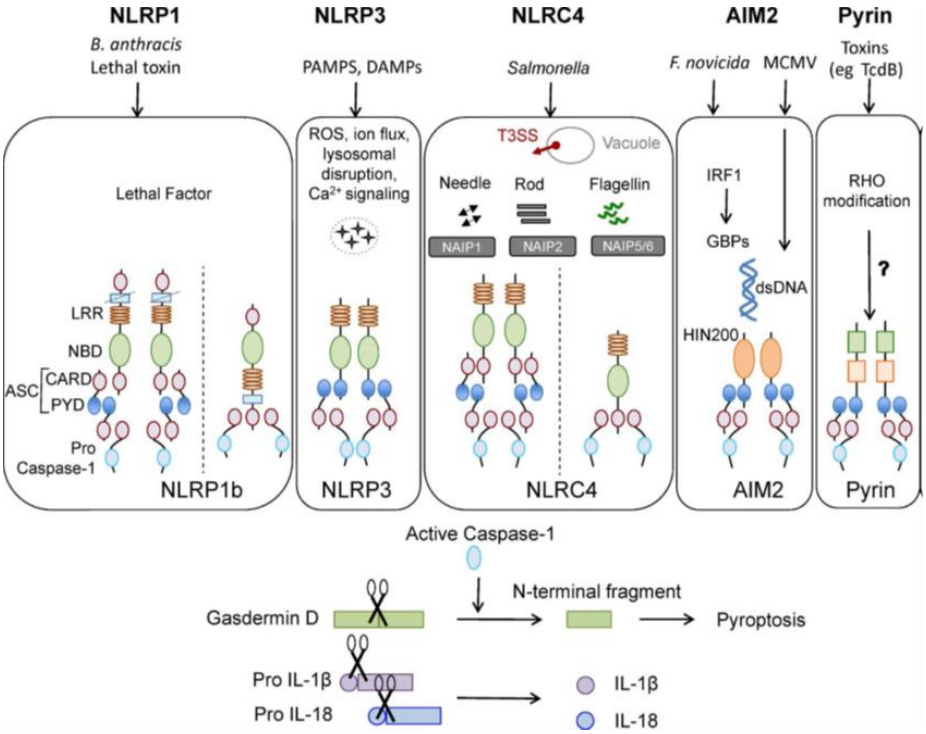

Figure 1. Canonical NLRP1, NLRP3, NLRC4, AIM2 and pyrin inflammasomes (Sharma and Kanneganti, 2016)

2.1.1.2. NLRP3

NLRP3 is linked to inherited autoinflammatory disorders known as cryopyrin-associated periodic syndromes, which are characterized by episodes of skin rashes and fever. Known to respond to many different infectious and endogenous ligands, NLRP3 is an inflammasome that that plays a role in the development of numerous autoinflammatory disorders such as arthritis, gout, diabetes, obesity, and Alzheimer's disease (Sharma and Kanneganti, 2016).

Among the factors that cause NLRP3 activation are components of microbial cell walls, nucleic acids, pathogen-derived ligands like pore-forming toxins, environmental crystal pollutants like silica, asbestos, and aluminum, endogenous danger signals like ATP, serum amyloid A, and uric acid crystals.It is not possible to interact directly with each of these activators. The NLRP3 inflammasome is therefore thought to recognize a common secondary activator

downstream of inputs or react to cellular stress brought on by infection or physiologicalinjury. The upstream signals necessary for the activation of the NLRP3 inflammasome include the production of reactive oxygen species (ROS), potassium flux, changes in cell volume, calcium signaling, and lysosome degradation (Muñoz-Planillo et al., 2013; Man and Kanneganti, 2015; Sharma and Kanneganti, 2016).

2.1.1.3. NLRC4

Procaspase-1 is activated by NLRC4 and the CARD domain to induce cell death in response to *Salmonella* infection. Host defense against different pathogens is provided by the NLRC4 inflammasome, which is activated by bacterial flagellin and multiple components of bacterial type III secretion systems (Yang et al., 2013; Sharma and Kanneganti, 2016).

NLRC4 does not interact with its activators directly, however NAIPs(NLR family, apoptosis-inhibiting proteins) that have their NLRC4 inflammasomes activated by the ligand-recognition route exhibit sensory function.Flagellin is identified by NAIP5 and NAIP6, whereas bacterial needle and inner rod proteins are recognized by NAIP1 and NAIP2, respectively. With the human genome detectable by flagellin and needle proteins, only one NAIP is encoded, which is similar to the two constitutively linked ligand recognition modes (Fig. 1) (Rayamajhi et al., 2013; Yang et al., 2013; Kortmann et al., 2015; Sharma and Kanneganti, 2016).

2.1.1.4. NLRP6

The lack of NLRP6 characteristic is due to the recognition and observation of downstream effects by the abnormal ligand. It is well known that NLRP6 acts as a negative regulator of the NF-kB and mitogen-activated protein kinase pathways in macrophages that have already been exposed to pathogens including *Salmonella*, *Escherichia coli*, and *Listeria*monocytogenes. Additionally, it has been established that NLRP6 plays a role in the activation of caspase-1 to maintain the mucosal defense against infections and chemical damage. The intestinal barrier integrity is weakened due to the absence of NLRP6, which combats microbial dysbiosis by producing mucus, and the

animal becomes vulnerable to pathogenic and chemical attack (Wlodarska et al., 2014; Nowarski et al., 2015; Sharma and Kanneganti, 2016).

2.1.1.5. NLRP12

In response to infections with *Yersinia pestis* and *Plasmodium*, caspase-1 is activated and NLRP12, an inhibitor of non-canonical NF-κB signaling, is involved. It controls the immunological response following a *Salmonella* infection and in cases of colorectal cancer because NLRP12 participates in NF-κB-mediated signaling.At the same time, their colithogenic and encephalitogenic potentials are affected by intrinsic modulation of NF-κB signaling in the activation of T cells (Ataide et al., 2014; Lukens et al., 2015; Sharma and Kanneganti, 2016).

2.1.2. ALR Family

Mammalian species have been found to express ALRs with an N-terminus PYD and a 200 amino acid repeat nuclear protein domain activated by a C-terminal hematopoietic interferon. AIM2 has a particular cytosolic localization since other members of the family lack a nuclear localization signal. Once more, the PYD of AIM2 is functionally distinct from other ALRs in that it can communicate with the PYD of ASC (Sharma and Kanneganti, 2016).

2.1.2.1. AIM2

Specifically in the double-stranded DNA (dsDNA) response, it has been determined that AIM2, which has been identified as a protein inducible by IFN-γ(interferon-gamma)on the tumor suppressor surface, is a nucleic acid sensor that can change into an inflammasome. AIM2 has differential importance for immune responses against different viral and bacterial infectious infections such as vaccinia virus, mouse cytomegalovirus, *Francisella tularensis* and *Listeria* (Figure 1) (Sharma and Kanneganti, 2016).

It is possible for AIM2 to interact with PYD in the absence of ligand. Autoinhibition is alleviated by DNA binding to the HIN200 domain and homotypic interaction of AIM2's PYD with the adapter ASC. Many filamentous star shapes radiating from a central core were seen in the AIM2 –

ASC – caspase-1 inflammasome imaged by electron microscopy (EM). AIM2 and ASC in this image represent the center, while caspase-1 represents filaments (Jin et al., 2013b; Li et al., 2014; Sharma and Kanneganti, 2016).

dsDNA secretion by *Mycobacterium* and *Legionella-like bacteria* is reduced and virulence factors that get rid of AIM2 can be encoded. However, AIM2 is given the capacity to react to host DNA released during the cellular damage response through identification of nucleic acids.It serves as the basis for several autoinflammatory disorders, including psoriasis, systemic lupus erythematosus, and abdominal aortic aneurysm, as a result of inflammasome activation after identification. The likelihood of a therapeutic effect on autoimmune disorders can be increased with AIM2 (Dihlmann et al., 2014; Sharma and Kanneganti, 2016).

2.1.3. Pyrin

Pyrin is an inflammasome-forming protein associated with familial mediterranean fever (FMF), which is known to be an autoinflammatory disorder. Pyrin was expressed for the first time in a mouse model with FMF mutation, and its participation in inflammasome activation was realized. These mice displayed an autoinflammatory disease that was mediated by IL-1 and ASC. In addition, pyrin inflammasome aggregated in response to Rho-modifying toxins produced by different bacteria such as *Clostridium difficile* (TcdB), *Vibrio parahemolyticus* (VopS), *Histophilus somni* (IbpA), *Clostridium botulinum* (C3) and *Burkholderia cenocepacia* (Figure 1)(Xu et al., 2014; Sharma and Kanneganti, 2016).

2.2. Activation and Regulation Mechanisms of Inflammasomes

2.2.1. ASC oligomerization and ASC granule

Homotypic PYD-PYD or CARD-CARD interactions are interactions between major inflammasome components themselves. The nucleation capacity of inflammasome assemblies, whose structural basis is formed by oligomerization induction, depends on the PYD and CARD domains. Complex collection of inflammasome sensors with or without ASC adapter is due to fields. Pirin couples NLRP3, AIM2, PYD, and ASC for inflammasome assembly, whereas NLRP1 and NLRC4, which span CARD, are caspase-1

coupled (Jin et al., 2013a; Ponomareva et al., 2013; Cai et al., 2014; Lu et al., 2014; Sborgi e al., 2015; Sharma and Kanneganti, 2016).

NLRP1 and NLRC4 can trigger pyroptosis independently of ASC, but effective cytokine processing requires ASC-mediated supramolecular complex assembly.Homotypic interactions are necessary for dominant-negative regulators, pyrin-only proteins, and only CARD proteins to stop the inflammasome signal from being transmitted (Guey et al., 2014; Van Opdenbosch et al., 2014; Matusiak et al., 2015; Sharma and Kanneganti, 2016).

Two consecutive nucleation takes place in ASC-dependent NLRP3 and AIM2 activations. The sensor initiates the formation of oligomeric filaments from the ASC. Caspase-1 is then nucleated by high concentrations of ASC and signal amplification of the sensor, adapter and enzyme complex occurs (Lu et al., 2014).

By directing pyroptosis and maturation of cytokines, the release of ASC particles phagocytosed by adjacent cells is mediated by inflammasome activation. With ASC nucleation, signals that can maintain the inflammasome of the particles in the recipient cells occur (Baroja-Mazo et al., 2014; Franklin et al., 2014).

By initially including a prionic mechanism by nucleating soluble ASC monomers, the PYD with CARD domains coupled with filamentous structures and the ASC particle can be offered as a scaffold. Consequently, by boosting the inflammasome response via a variety of mechanisms, the ASC particle canexacerbate inflammation. (Cai et al., 2014; Franklin et al., 2014; Sharma and Kanneganti, 2016) (Figure2).

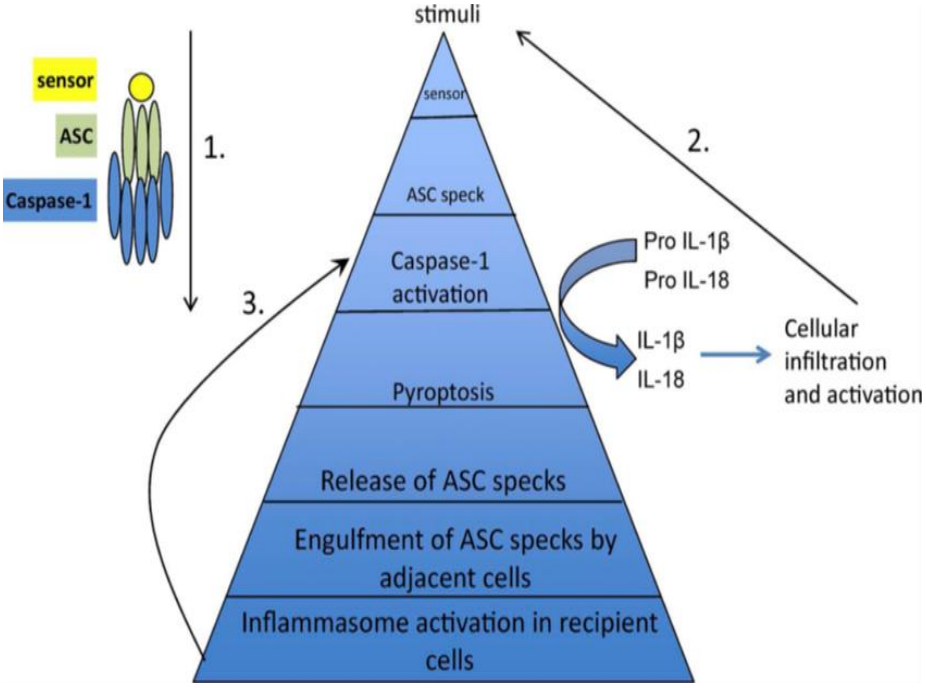

Figure 2.ASC-mediated amplification of inflammasome activation (Sharma and Kanneganti, 2016)

2.2.2. Caspase-11 and NLRP3 inflammasome

Recent research has revealed the significance of the inflammatory caspase, caspase-11, whose function has long been unknown because of its close genetic relationship to caspase-1. Thus, caspase-11, which is defined as a pyroptosis inducer separate from caspase-1, plays a role in the activation of NLRP3 inflammasome with the CARD domain in the response of pathogens such as gram-negative bacteria (Shi et al., 2014; Sharma and Kanneganti, 2016).

Caspase-11, the sensor and inducer of LPS-induced pyroptotic reactions, activates the NLRP3 inflammatory assembly. Thus, two characteristics of inflammasome activation emerge. The processing of cytokines is induced by NLRP3-mediated activation of caspase-1, while caspase-11 activation promotes the progression of pyroptosis. Although caspase-11 may be implicated in caspase-1, it has little effect on the normal NLRP3-ASC complex

assembly, indicating that it is not a necessary adaptor. (Sharma and Kanneganti, 2016) (Figure 3).

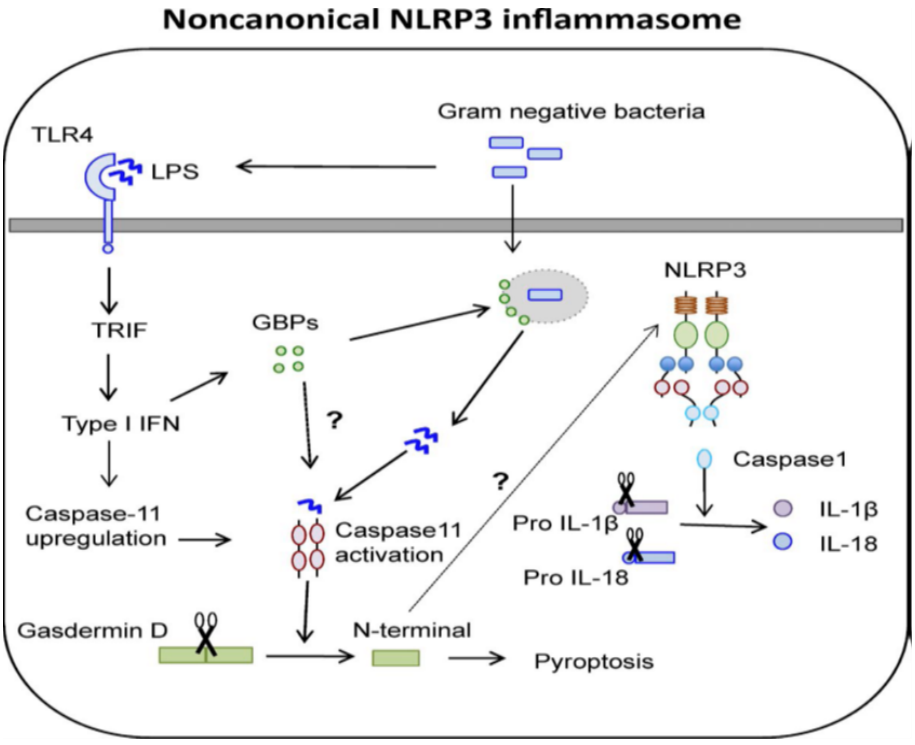

Figure 3. Caspase-11-regulated NLRP3 inflammasome
(Sharma and Kanneganti, 2016)

2.2.3. Pyroptosis and Gasdermin D

Pyroptosis, an inflammation-induced cell death process in which cellular lysis, the synthesis of intracellular components, and an inflammatory reaction take place, is only possible with inlamasome activation. So far, it has been stated that macrophages, dendritic cells, enterocytes and hematopoietic progenitors can undergo caspase-induced pyroptosis. However, it is now known that neutrophils and monocytes do not undergo pyroptosis after activation of the inflammasome (Chen et al., 2014; Sellin et al., 2014; Gaidt et al., 2016; Vincent et al., 2016; Sharma and Kanneganti, 2016).

Pyroptosis, a different type of cell death, has common aspects similar to apoptosis and necrosis. Just like necrosis, pores between 1 and 2 nm are created in the cell membrane by pyroptosis. However, cytoplasmic enlargement, osmotic lysis, and intracellular content creation keep the process going. It is similar to apoptosis with its nuclear condensation and DNA damage properties. Even without the presence of the executioners' caspases, DNA fragmentation during pyroptosis can still happen due to membrane permeability (Sharma and Kanneganti, 2016).

The non-linkage between caspase-1 activation and pyroptosis has led to the emergence of gasdermin D (GSDMD). Caspase-1 is required for GSDMD, pyroptosis process and IL-1β release, which is a direct target of caspase-4 and caspase-11. The recognition and separation of GSDMD in the same region is performed by caspase-1, caspase-4, caspase-5, and caspase-11. GSDMD is cleaved to show activity. The N-terminus induces pyroptosis by acting as an auto-inhibitor for the GSDMD C-terminal (He et al., 2015; Kayagaki et al., 2015; Shi et al., 2015; Sharma and Kanneganti, 2016).

As a result, the functions of NLR, ALR and pyrin family inflammasome types in physiological and pathogenic processes, inflammasome activation and regulation mechanisms, pyroptosis and gasdermin D, which are inflammatory cell death caused by the activation of caspase species in canonical and noncanonical inflammasome mechanisms, are associated with the physiological and pathology of diseases.

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CHAPTER 15

CURRENT TRAUMATIC PATHOLOGIES OF CRANIAL NERVES

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Anatomically, the nervous system is divided into the central nervous system (CNS) and the peripheral nervous system. Physiologically, it is divided into somatic and autonomic system. The CNS consists of the brain and the spinal cord. Peripheral nervous system includes spinal and cranial nerves. The somatic nervous system contains structures that work voluntarily, whereas the CNS contains structures that work against our will. Spinal nerves originate from the spinal cord and have 31 pairs. These nerves go to structures by forming plexuses in certain regions. Cranial nerves have 12 pairs and all but the first two originate from the brain stem. The first two are developmentally an extension of the brain (Elhan, 2020; Arifoğlu, 2022; Özbağ, 2019).

I. Olfactory nerve (nervus olfactorius)
II. Optic nerve (nervus opticus)
III. Oculomotor nerve (nervus oculomotorius)
IV. Trochlear nerve (nervus trochlearis)
V. Trigeminal nerve (nervus trigeminus)
VI. Abducens nerve (nervus abducens)
VII. Facial nerve (nervus facialis)
VIII. Vestibulocochlear nerve (nervus vestibulocochlearis)
IX. Glossopharyngeal nerve (nervus glossopharyngeus)
X. Vagus nerve (nervus vagus)
XI. Accessory nerve (nervus accessories)
XII. Hypoglossal nerve (nervus hypoglossus)

Traumatic head injury affects millions of people and causes neurological consequences leading to high morbidity and mortality in severe cases. Recent favourable developments in intensive care have potentially reduced mortality in severe cases of traumatic head injury. However, survivors may remain with impaired consciousness resulting in vegetative or minimally conscious states. Adults and children with traumatic brain injury are unlikely to regain consciousness after 12 months, and this is even less likely to occur if there is an anoxic component to the injury (Shi, 2013).

1. OLFACTORY NERVE (I.)

The first cranial nerve is the olfactory nerve. It transmits our sense of smell to the brain and consists only of sensory (afferent) fibres. It is the shortest cranial nerve and is developmentally an extension of the brain. Therefore, it is known as a structure belonging to the central nervous system (CNS). Together with optic nerve, it is one of the two cranial nerves that are not associated with the brain stem (Elhan, 2020; Arifoğlu, 2022).

The olfactory region is located in the uppermost part of the nasal cavity and the middle part of the nose. The lower part of the nasal cavity is the region of the airway. In humans, each nostril contains between 6 and 10 million olfactory neurons distributed over a surface area of 2.5 cm^2. The cell bodies of bipolar olfactory neurons (receptor cells), a specialised epithelial tissue located in the olfactory region, detect odour. The extensions of these structures form the so-called fila olfactoria. The sense of smell, which is transformed into an electrical impulse, passes through the holes in the cribriform plate in the ethmoid bone with these extensions and reaches the anterior fossa of cranium and synapses with mitral cells in the bulge called olfactory bulb. The central extensions, i.e. axons, of the mitral cells lie in the olfactory tract, which is the posterior continuation of the olfactory bulb. The olfactory bulb divides into 2 main parts, stria lateral and medial olfactory stria, after a short distance on the lower surface of the frontal lobe. The medial branch connects with the olfactory bulb of the opposite side, allowing the odour to be felt in both hemispheres. The lateral branch connects with the primary olfactory region on the same side and enables us to perceive odour (Elhan, 2020; Özbağ, 2019).

1.1. Traumatic pathologies

Anosmia is the loss of the sense of smell. Traumatic anosmia is caused by damage to the olfactory epithelium of the nasal mucosa and/or mechanical stretching or cutting of the nerve roots. In addition, damage to the cortical structures of the olfactory tract may also cause traumatic anosmia (Aziz, 2015).

Anosmia is the inability to smell and dysosmia is olfactory dysfunction. Head trauma is one of the most common causes of anosmia and is seen in 17% of head traumas. Due to the location of the olfactory bulb, damage to the

olfactory region occurs as a result of damage to the frontal bone, especially in frontal blows. The Computed Tomography (CT) images may diagnose a fracture of the cribriform plate as a result of trauma and anosmia may occur due to rupture of the fibres passing through it. In some cases, imaging techniques cannot determine the cause of anosmia. Therefore, trauma-related pathologies such as cutting of the fila olfactoria or coup and contrecoup movement should be considered (Ottaiano, 2022; Gaillard, 2023).

Coup injury develops just below the area of the object hitting the skull, while contrecoup injury occurs on the opposite side of the impacted area. Coup and contrecoup injuries can occur separately or together. Coup injuries are typical when a moving object strikes a stationary head, while contrecoup injuries occur when a moving head strikes a stationary object. In such cases, olfactory dysfunction may develop with damage to the olfactory bulb or olfactory region. In addition, cerebral contusion, and haemorrhages in the anterior fossa of cranium caused by impact may lead to olfactory dysfunction (Miao, 2015; Poirier, 2003).

The trauma risk of olfactory nerve increases with age. Only 40% of patients with dysfunction are aware of the deficits. Most olfactory dysfunction occurs following trauma. However, delayed dysfunction may be seen due to excessive fibrous tissue in the cribriform plate. The severity of dysfunction may vary depending on the severity of the head injury, and in addition to anosmia, rhinorrhea (cerebrospinal fluid from the nasal cavity) and epistaxis (nose bleeding) may also be seen in head trauma. Recent animal studies show that the recovery of the olfactory system varies according to the severity of the injury and drug treatment may improve recovery by reducing injury-related oedema (Aziz, 2015).

2. OPTIC NERVE (II.)

Optic nerve is the second cranial nerve and provides our sense of vision. It consists only of sensory fibres. Like olfactory nerve, it is an extension of the brain in terms of embryological development and structure (Elhan, 2020).

The central extensions of the multipolar ganglion cells in the retina, the retinal layer of the eye, form the optic nerve. The optic nerve arises from the

posterior aspect of the eye bulb (eyeball) and extends posteriorly in the center of the orbit (orbital part) and enters the optic canal (intracanalicular part). The right and left optic nerve, which approach each other at this level, merge with each other after the optic canal and form the optic chiasm (intracranial part) on the sphenoid bone. Fibres from the optic chiasm extend posteriorly and outwards as optic tract to the metathalamus and terminate in the nucleus there. The axons of the cells here connect to the primary visual region (field 17) in the occipital lobe as optic radiation (Elhan, 2020; Özbağ, 2019).

Optic nerve is surrounded by dura mater, arachnoidea mater and pia mater in the orbit. The central retinal artery, a branch of the ophthalmic artery, enters the optic nerve just behind the eyeball and supplies the eyeball and retina. The ophthalmic artery passes together with the optic nerve in the optic canal. The optic nerve and the sphenoid sinus are close structures and the bony partition between them is very thin. Sometimes air can accumulate in this bone, and it is called Onodi cell (Elhan, 2020; Arifoğlu, 2020).

2.1. Traumatic pathologies

Traumatic optic nerve injuries (traumatic optic neuropathy) account for between 2% and 5% of head injuries. It can be classified according to the site of injury or the mode of injury. The severity of optic nerve injury can range from simple contusion to complete avulsion of the optic nerve. Primary (direct) damage to the optic nerve is caused by shearing, contusion, and axon damage with mechanical impact at the time of impact. Secondarily (indirectly), damage occurs with apoptotic cell death, compartment syndrome and oedema due to ischemia. Mechanical damage occurs with sharps penetration or gunshot injury to the orbit. The prognosis is poor if the optic nerve in the orbit is cut. In addition, hematoma accumulates in the orbit with vascular injury in the optic nerve. Vascular injury also causes swelling of the optic disc (blind spot) in the eyeball, thus obstructing the vascular circulation. With the accumulation of hematoma in the orbit, intraorbital compartment syndrome develops. In addition, damage to the optic nerve and inhibition of drainage increases intraocular pressure and glaucoma develops (Aziz, 2015; Yu-Wai-Man, 2015).

Optic canal injury and optic chiasm injury are classified as indirect injuries. Due to the close adherence of the dural sheath to the periosteum, the

intracanalicular segment of the optic nerve is particularly susceptible to this type of injury. The intracranial part of the optic nerve, close to the dural fold, is the next most likely to get injured. Especially blows to the frontal bone may be transmitted to the optic canal and therefore optic nerve damage may occur in the canal. In addition, some studies have reported traumatic damage to the optic nerve during transnasal endoscopic sinus surgery. Diagnosis of optic nerve injury can be difficult. Especially conditions such as craniofacial fractures and traumatic brain injury may cause damage to the optic nerve, which has a direct connection with the brain. Since optic atrophy does not become apparent for 3 to 4 weeks, retinal examination is usually not helpful in the diagnosis of optic nerve damage. Retinal examination is important in the diagnosis of orbital hemorrhage and other ocular injuries (Aziz, 2015; Wang, 2008).

Patients with traumatic optic neuropathy may have decreased central visual acuity and color vision, afferent pupillary defects and/or visual field deficits. The first symptom of traumatic optic neuropathy is an afferent pupillary deficit. Patients with traumatic optic neuropathy may have decreased central visual acuity and colour vision, afferent pupillary defects and/or visual field deficits. To detect this, a normal eye is lighted, and a constriction of the ipsilateral pupil and an indirect constriction of the contralateral pupil are expected. If both optic nerves are normal, the pupillomotor stimulation is the same. The pupil constricts equally in both eyes. However, the pupillomotor response of the injured eye is less and both pupils dilate when light is passed from the normal eye to the injured eye. Bilateral damage is difficult to detect (Yu-Wai-Man, 2015). This test measures the time it takes for a visual stimulus to travel from the eye to the primary visual region. In unilateral optic nerve damage, using the VEP ratio between the patient's normal eye and the damaged eye helps to estimate the extent of nerve damage (Yu-Wai-Man, 2015).

There is little or no improvement in patients with direct optic nerve damage. Indirect damage to the optic nerve can be treated and prevented. Treatment includes observation, medical therapy and decompressing the optic canal or opening the neural sheath. Surgical intervention should be considered if the nerve becomes compressed or if visual loss persists despite medical treatment (Aziz, 2015).

Optic canal fractures can be identified by CT. An optic canal fracture may result in nerve compression or hematoma formation in the optic nerve. Optic nerve decompression is performed through various approaches, notably transnasal, transsphenoidal and transethmoidal. Potential side effects of decompression include injury to the extraocular muscles, orbital hematoma, arterial injury, rhinorrhoea, and infection (Aziz, 2015).

Anopsia (an=negative prefix + opsia=to see) is the loss of vision. Types of anopsia vary according to the location where the optic nerve is exposed to trauma.

Haemianopsia is loss of vision or blindness in half of the visual field. Stroke, brain tumour and trauma are the most common causes of this damage. There are different types:

Loss of half the visual field on the same side in both eyes is called homonymous hemianopsia. Damage to the right side of the back of the brain or to the right side of the optic canal can lead to a loss of the left side of the visual field in both eyes. Loss of half the visual field on different sides in each eye is called heteronymous hemianopsia. It is divided into binasal (loss of the central part of the field) and bitemporal (loss of the outer parts of the field) (Elhan, 2020).

3. OCULOMOTOR NERVE (III.)

Oculomotor nerve is the 3rd cranial nerve. It contains somatomotor and parasympathetic fibres. Somatomotor fibres innervate the external eye muscles (except the superior oblique, lateral rectus, and superior levator palpebral muscles). Parasympathetic fibres innervate ciliary muscle and sphincter muscle of pupil. The motor and parasympathetic nuclei are located in the mesencephalon. Fibres from these nuclei leave the CNS in front of the brain stem. After passing between posterior cerebral artery and superior cerebellar artery, it enters the cavernous sinus. As soon as it leaves here, it reaches the orbit through the superior orbital fissure (Elhan, 2020; Taner, 2019).

Ciliary ganglion is a parasympathetic ganglion, 1-2 mm in diameter, located on the outer side of optic nerve. Preganglionic parasympathetic fibres coming to this ganglion via inferior branch of oculomotor nerve synapse in the

ciliary ganglion and then enter the eyeball as postganglionic parasympathetic fibres. These fibres provide the pupillary light reflex (myosis) (Elhan, 2020; Arifoğlu, 2020).

3.1. Traumatic pathologies

Head injuries are caused by significant kinetic forces acting on the brain parenchyma and skull bones. Oculomotor nerve is among the cranial nerves most commonly affected by these traumas. Severe head trauma with skull fracture, orbital injuries and intracranial hemorrhage can damage the nerve. Isolated oculomotor nerve palsy may occur with minor trauma without any symptoms (Kim, 2013).

In isolated oculomotor nerve palsy, different clinical cases occur depending on the affected branch or the affected muscle. In case of complete paralysis, pathologies such as ptosis (eyelid drooping) due to paralysis of the levator palpebrae superioris muscle, mydriasis (dilation of the pupil) due to damage to the parasympathetic fibres, and downward-outward gaze of the eye due to the activity of the superior oblique muscle. Partial paralysis can be detected by assessing the traumatic situation and following the path of the nerve. Paralysis or damage of the oculomotorius nerve alone is not common in cranial trauma. Damage to the oculomotorius nerve alone can usually be caused by damage to the ipsilateral oculomotorius nerve by the posterior petroclinoid ligament (Ülker, 2022; Kim, 2013).

In intraorbital unilateral injuries, limitation of eye movements, ptosis and mydriasis are observed in the ipsilateral nerve. Clinical and neurological examination should be performed for the control of the nerve in head traumas and the situation requires urgent intervention. The prognosis of traumatic oculomotorius nerve palsy is unpredictable, it is a slow process and requires a long follow-up and complete recovery is rare. Heinz classified oculomotorius nerve injuries as avulsion of the nerve roots, focal stretching of the parasellar segment and intraneural hemorrhage in the superior orbital fissure (Costello, 2022; Ülker, 2022; Kim, 2013).

4. TROCHLEAR NERVE (IV.)

Trochlear nerve is the 4th cranial nerve and contains only somatomotor fibres. Its somatomotor fibres innervate superior oblique muscle, one of the external eye muscles of the eye. Its motor nucleus is located in the mesencephalon. It is the thinnest of the cranial pairs (0.75 - 1 mm) and the longest cranial nerve within the skull (60 mm). It is the only cranial nerve originating from the back of the brain stem. It has central, cisternal, cavernous and orbital segments. The nerve, which is displaced anteriorly, pierces the dura mater of brain. It enters the orbit through the superior orbital fissure. It is located above the other nerves in the orbit. It extends medially from here and enters the superior oblique muscle from its medial side (Elhan, 2020; Özbağ, 2019; Arifoğlu, 2022).

4.1. Traumatic pathologies

Although traumatic paralysis of the cranial nerves is relatively common, traumatic pathologies of the trochlear nerve are rare. Because it innervates the superior oblique muscle, extraocular movement disorders facilitate the determination of nerve damage. However, since trochlear nerve is the thinnest cranial nerve, it is difficult to determine trauma or cutting of the nerve radiologically (Ko, 2018; Dhaliwal, 2006).

Knowing the course of the nerve is important in terms of knowing the extent of trauma. In closed head traumas, subarachnoid hemorrhage in the ambiens cistern may affect the nerve because it exits behind the brain stem. In addition, because of its proximity to the tentorial notch and its passage between the posterior cerebral artery and superior cerebellar artery, trauma of the nerve in these regions is possible (Ko, 2018).

The primary action of the superior oblique is intorsion (turning the eye inwards), the secondary action is depressing (mainly in adduction), and the tertiary action is abducting (turning the eye outwards). The effect of the superior oblique on the depression movement is most evident when the eye is in the adducted position. This is because as the eye abducts, the superior oblique's contribution to depressing the eye decreases. On neurological examination of

the muscle, the patient is asked to look inwards and downwards. Vertical diplopia (double vision) is seen with nerve damage (Aclands, 2003; Ko, 2018).

5. TRIGEMINAL NERVE (V.)

The fifth cranial nerve, trigeminal nerve, is the thickest cranial nerve. It is called trigeminus, meaning triplets, because it is distributed in three main branches. It arises from the anterolateral face of the pons. It consists mostly of sensory fibres and a small part of somatomotor fibres. The sensory root consists of many fine fibres and is formed by the central extensions of the trigeminal ganglion. The peripheral extensions receive sensation from the skin of the face and mucous membranes of the head. The motor root innervates the masticatory muscles, mylohyoideus muscle, anterior belly of digastric muscle, tensor tympani muscle and tensor veli palatini muscle in the mandibular nerve (Elhan, 2020; Özbağ, 2019).

Trigeminal ganglion (ggl. semilunare , Gasser's ganglion) sits in the trigeminal impression at the top of the pyramis part on the temporal bone. It is a sensory ganglion, and its central extensions go to the CNS together with the trigeminal nerve, while its peripheral extensions are distributed together with the ophthalmic nerve, maxillary nerve, and mandibular nerve (Elhan, 2020).

The ophtalmic nerve is the first branch of the trigeminal nerve and it innervates lacrimal gland, paranasal sinuses, and part of the nasal mucosa, superior palpebra, nasal skin and anterior part of the forehead and scalp. When it enters the orbit, it divides into 3 main branches as lacrimal nerve, frontal nerve and nasociliary nerve (Elhan, 2020; Taner, 2019).

The maxillary nerve arises from the middle part of the trigeminal ganglion and consists only of sensory fibres. It receives sensation from the middle part of the face, inferior palpebra, sides of the nose, skin of the upper lip and nasopharynx, maxillary sinus, palatina tonsil, hard palate and soft palate, maxillary teeth, and gingiva. It passes through the outer wall of the cavernous sinus and reaches foramen rotundum. From here, it leaves the cranial cavity and reaches the pterygopalatine fossa. After giving its branches here, it passes into the orbit via the inferior orbital fissure and from here it continues under the name of infraorbital nerve. It gives many branches, but infraorbital

nerve, zygomatic nerve and superior alveolar nerves are the most important branches (Elhan, 2020; Taner, 2019; Arifoğlu, 2022).

Mandibular nerve is the thickest branch of trigeminal nerve. It consists of somatomotor and sensory fibres. Sensitive fibres receive sensation from the lower jaw teeth and gingiva, lower lip and lower skin of the face, skin of the temporal region, anterior 2/3 of the tongue, cheek, jaw joint, dura mater of brain, auricle, and eardrum. The masticatory muscles, the mylohyoid muscle, the anterior belly of the digastric muscle, the tensor tympani, and the tensor veli palatini are innervated by the somatomotor division. It leaves the cranial cavity through the foramen ovale and enters the infratemporal fossa after leaving the trigeminal ganglion. The auriculotemporal, lingual and inferior alveolar nerves are its most important branches (Elhan, 2020; Arifoğlu, 2022).

5.1. Traumatic pathologies

Trauma and injuries can affect the trigeminal nerve. Accidents, tumors, and dental operations can damage the nerve, or damage from facial surgery, such as cosmetic surgery, can injure or cut the nerve or its branches. The damage caused by a trigeminal nerve injury depends on where the nerve damage occurs. Injury to the maxillary or mandibular nerve can result in loss of sensation in the teeth and gums. In addition, problems with chewing and speech can occur as a result of mandibular nerve injury. The damaged nerve usually regains its function over time. Sometimes it is necessary to reattach the severed nerves (Shankar Kikkeri, 2023; Gao, 2019).

Trigeminal neuralgia (tic douloureux) is a trigeminal neuropathy caused by nerve damage. With this condition, sudden and severe pain develops on the ipsilateral side of the face. Although the pain is typical, it can feel like an electric shock. Trigeminal neuralgia can be of two types: primary and secondary. Primary trigeminal neuralgia occurs when a blood vessel surrounds the nerve and causes compression. Secondary trigeminal neuralgia develops as a result of a tumor, cyst, or facial injury. Nerve damage can also be caused by a disease such as multiple sclerosis. The pain usually comes in short episodes that can last from a few seconds to 2 minutes. The episodes stop as suddenly as they start. Attacks can recur and occur every few months. Trigeminal neuralgia

can have a significant negative effect on a person's quality of life and can lead to problems such as depression (Ferreini, 2021; Gao, 2019).

Trauma is the most common cause of trigeminal neuropathy. Especially nerve injuries are commonly seen during lower molar tooth extraction. As a result, sensory defects are seen in the lower jaw teeth. Similarly, due to the anatomical location of the lingual nerve close to the third molar tooth, it may be damaged during applications to extract the molar tooth (Shankar Kikkeri, 2023).

Microvascular decompression for pain relief involves surgical exposure of the trigeminal nerve root by a neurosurgeon, decompression of the nerve and removal of the blood vessel from the point of compression. This can result in relief of painful attacks and return of the nerve to normal function (Gao, 2019).

6. ABDUCENS NERVE (VI.)

The abducens nerve innervating the external eye muscle lateral rectus muscle is the 6th cranial nerve. It consists of somatomotor fibres only. It arises from the bulbopontine groove between the pons and bulbus. It travels inside the cavernous sinus and passes through the superior orbital fissure and reaches the orbit. It proceeds lateral to the orbit and gives branches to the muscle it innervates (Elhan, 2020).

6.1. Traumatic pathologies

Abducens nerve palsy is the most common ocular motor paralysis in adults (second in children). In nerve damage, the eye turns inwards. Damage to the nerve anywhere along its long intracranial pathway can cause paralysis. The symptoms of abducens nerve paralysis depend on the anatomical location of the damage (Geressu, 2021).

Congenital paralyses are rare, and birth trauma and hydrocephalus are associated with neurological conditions. Children may suffer from abducens nerve paralysis due to trauma, infection, and idiopathic causes. The abducens nerve is very sensitive to head injuries because it passes over the temporal bone into the cavernous sinus. In addition, traumatic abducens nerve palsy may occur secondary to intracranial hemorrhage. Closed head trauma may cause high intracranial pressure, resulting in non-localised nerve palsy. (Graham, 2023).

Blunt trauma to the outer edge of the orbit is a common cause of both orbital fractures and damage to soft tissues (Elder, 2016; Azarmina, 2013).

Diplopia (double vision) is the most common symptom of nerve paralysis. Patients have non-transverse double vision, which is more intense at close range. In cases due to high intracranial pressure, patients may experience symptoms such as headache, pain around the eyes, nausea, vomiting or tinnitus (Hofer, 2015).

7. FACIAL NERVE (VII.)

The seventh cranial nerve, facial nerve, consists of somatomotor, sensory and parasympathetic fibres. Somatomotor fibres are thicker. The parasympathetic and sensory fibres run together with the branch called intermediate nerve. Somatomotor fibres innervate the mimic muscles, platysma, stapedius muscle, posterior belly of digastric muscle. Parasympathetic fibres innervate submandibular gland, sublingual gland, lacrimal gland, nasal glands, and palate glands. Sensitive fibres receive taste sensation from the anterior 2/3 of the tongue, external auditory canal, soft palate, and upper part of the pharynx (Elhan, 2020; Özbağ, 2019).

Both roots arising from the bulbopontine groove enter the internal acoustic meatus together with the vestibulocochlear nerve. At the bottom of this meatus, they pass into the canal of facial nerve. This canal passes through the inner wall of the middle ear and opens out through the stylomastoid foramen. Facial nerve gives 3 important branches in the canal: greater petrosal nerve, chorda tympani and stapedius nerve. After exiting the canal, it gives motor branches (Elhan, 2020 Arifoğlu, 2022).

Geniculate ganglion is a sensory ganglion located in the canal of facial nerve. The central extensions of this ganglion travelling in the intermediate nerve, while most of its peripheral extensions go to the tongue. Pterygopalatine ganglion is a parasympathetic ganglion located in the pterygopalatine fossa. The preganglionic parasympathetic fibres coming to this ganglion come in the facial nerve and innervate the lacrimal gland as postganglionic parasympathetic fibres after synapsing. The submandibular gland is a parasympathetic ganglion located in the oral cavity. The preganglionic parasympathetic fibres coming

here with the chorda tympani branch of the facial nerve synapse and go to the submandibular gland and sublingual gland as postganglionic parasympathetic fibres (Elhan, 2020; Arifoğlu, 2022).

7.1. Traumatic pathologies

Nerve trauma is one of the most common causes of facial paralysis. The nerve can be injured anywhere along its course. Common causes of facial nerve damage include skull base fractures, gunshot wounds, sharp penetrating injuries, and injuries sustained during surgical procedures. The location and mechanism of the injury plays an important role in determining the treatment. Blunt trauma to the head and neck in traffic accidents and physical blows can lead to facial nerve injury and hearing loss. Nerve injury can also occur during parotid gland surgery, mastoidectomy, cosmetic surgery and tympanic membrane reconstruction (Houston, 2023; Pamuk, 2018).

Trauma accounts for 6-27% of all facial nerve palsies and therefore the effect of trauma is important in the differential diagnosis of facial paralysis. Facial nerve palsy significantly affects quality of life due to psychological and aesthetic damages (Pamuk, 2013; Houston, 2023).

The most common causes of n. facialis damage:

- Skull base fracture
- Penetrating trauma facial nerve
- Birth trauma
- Iatrogenic (mostly during excision)
- Barotrauma (usually due to scuba diving or airplane travel)
- Lightning strike

8. VESTIBULOCOCHLEAR NERVE (VIII.)

Vestibulocochlear nerve consists of two parts, vestibular nerve, and cochlear nerve. It is the 8th cranial nerve and contains only sensory fibres. The vestibular nerve part is related to the sense of balance and the cochlear nerve part to the sense of hearing. These two parts are connected to the brain as a single root in the groove between the bulbus and the pons, behind the facial nerve (Elhan, 2020; Taner, 2019).

Vestibular nerve carries the sense of balance. Its cell body, the vestibular ganglion (Scarpa ganglion), is located at the base of the internal acoustic meatus. Its peripheral extensions go to the balance structures in the inner ear and its central extensions continue as vestibular nerve. Cochlear nerve carries the sense of hearing. The cell body cochlear ganglion (spirale ganglion) is located in the cochlea in the inner ear. Its peripheral extensions go to the organ of Corti (organum cochleare) in the modiolus. Its central extensions pass at the base of the cochlea and continue as cochlear nerve through the internal acoustic meatus (Elhan, 2020; Özbağ, 2019).

8.1. Traumatic pathologies

Traumatic temporal bone fractures are classically classified as longitudinal, transverse or mixed according to the long axis of the petrosal part of the bone. Sudden onset facial paralysis and total hearing loss are the hallmarks of fracture (Pamuk, 2018).

The vestibulocochlear nerve provides hearing and balance. Damage to the nerve often causes vertigo, tinnitus, and hearing disorder. Hearing loss is usually a sensory loss. Patients often complain that the room revolves around them. Nystagmus may also be seen. Injury to this nerve occurs in 1.51% of cases and vestibular therapy can help treat nerve paralysis (Cleveland, 2023; Pamuk, 2018).

Vestibular neuritis, which develops due to inflammation in the inner ear, is a disease that affects the vestibular nerve in the inner ear and symptoms similar to loss of balance occur as a result of nerve damage. (Cleveland, 2023).

Damage to the vestibulocochlear nerve can cause the following symptoms:

- Hear dysfunction
- Vertigo
- Loss of equilibrium (in the absence of light)
- Nystagmus
- Movement dysfunction
- Tinnitus (Cleveland, 2023).

9. GLOSSOPHARYNGEAL NERVE (IX.)

It is distributed on the tongue and pharynx. It is the 9th cranial nerve and has somatomotor, parasympathetic and sensory fibres. Somatomotor fibres belong to the nucleus ambiguus in the bulbus and innervate the pharyngeal muscles and stylopharyngeus muscle. The parasympathetic fibres belong to the inferior salivatory nucleus in the bulbus and are separated from the preganglionic parasympathetic fibres by the tympanic nerve. The parasympathetic fibres are secreted to the glands in the parotid gland, the posterior part of the tongue and the adjacent glands in the mucous membrane of the pharynx. The sensitive part receives taste sensation from the 1/3 posterior part of the tongue and sensation from the oropharynx, tonsilla palatina, larynx and 1/3 posterior part of the tongue (Elhan, 2020; Arifoğlu, 2022).

Fiber roots to the glossopharyngeal nerve originate from the retroolivary groove together with the vagus nerve. After exiting, the nerve travelling downwards comes to the jugular foramen. It passes through this opening together with vagus nerve and accessory nerve. It then passes between internal carotid artery and internal jugular vein. The nerve travelling downwards is distributed in the pharynx and tongue. Glossopharyngeal nerve has two sensory ganglia named ganglion superius and ganglion inferius at the level of jugular foramen (Elhan, 2020; Taner, 2019).

Otic ganglion is a parasympathetic ganglion located in the infratemporal fossa just below the foramen ovale. Preganglionic parasympathetic fibres coming together with glossopharyngeal nerve synapse here and then go to the parotid gland as postganglionic parasympathetic fibres (Elhan, 2020).

9.1. Traumatic pathologies

Glossopharyngeal neuralgia develops as a result of trauma to the glossopharyngeal nerve. Compression of the nerve by the vessel is a typical cause. It is characterised by extreme pain in the throat, tongue, or ear. It can also occur in people with throat or neck cancer. Some people describe the sensation of a sharp object lodged in their throat. Attacks of intense, electric shock-like pain can occur with swallowing. Medication can initially relieve the

pain, but surgery is often required for long-term relief (Urculo, 1996; Alberio, 2005).

When the glossopharyngeal nerve is damaged, an attack of severe pain, similar to an electric shock, is felt in the back of the throat, tongue, tonsils, or ear. Initially, there may be short, mild attacks with periodic relief. Swallowing, chewing, talking, coughing, yawning, or laughing can trigger an attack. However, neuralgia may progress and cause longer, frequent and burning pain attacks (Urculo, 1996; Alberio, 2005).

10. VAGUS NERVE (X.)

The vagus nerve, composed of somatomotor, parasympathetic and sensory fibres, is the longest head pair and has an extensive network distributed in the neck, chest, and abdomen. Somatomotor fibres innervate the muscles of the larynx, pharynx, proximal part of the esophagus and the soft palate except for the tensor veli palatini muscle. This innervation originates from the accessory nerve. Parasympathetic fibres innervate the organs of the thoracic cavity and the structures of the digestive tract from the esophagus to the left colic flexure. Sensitive fibres receive sensation from the external acoustic meatus, posterior part of the auricle, pharynx, larynx, bronchi, lungs, heart, esophagus, stomach, intestines, and kidneys (Elhan, 2020; Arifoğlu, 2022).

Vagus nerve arise as roots from the retroolivary groove in the bulbus. The fibres leave the cranial cavity through the jugular foramen. Here it has two sensory ganglia, ganglion superius and ganglion inferius. After leaving the skull, it travels downwards in the carotid sheath together with internal carotid artery and internal jugular vein. It enters the thoracic cavity through the superior thoracic aperture and travels downwards on the esophagus. After passing through the aortic hiatus in the diaphragm, it reaches the abdominal cavity. Here, it branches around the veins and travels to the organs (Elhan, 2020; Arifoğlu, 2022; Taner, 2019).

10.1. Traumatic pathologies

Vagus nerve palsy may be idiopathic or may develop due to trauma, surgery, tumour, internal carotid artery dissection, infection. Trauma-induced NVP is often seen in addition to other cranial nerve trauma and is also

associated with cranial or facial fractures. Many studies in the literature have reported a case of glossopharyngeal nerve and vagus nerve paralysis after isolated or non-isolated occipital condyle fracture due to trauma. In addition, isolated NVP is seen due to non-traumatic causes such as lesions (Urculo, 1996; Aygün, 2013; Alberio, 2005).

Possible mechanisms of traumatic injury of the vagus nerve include excessive rotation and/or lateral flexion of the neck which may cause stretching of the vagus nerve and direct compression of the vagus nerve trunk in the upper cervical region due to trauma-related muscle spasm or direct trauma to the neck, causing fascicular damage (Aygün, 2013).

NVP caused by herpes simplex virus (HSV) infection is rare. In this case, acute onset otalgia, sore throat, dysphonia, dysphagia, and some vesicles in the deep soft palate may be observed (Tang SC). Cranial nerve palsies at the base of the skull after head trauma are rare and if they occur, a thorough examination should be performed to search for posterior cranial base and cranio-cervical lesions. NVP is seen due to bone fracture, especially in blows to the jugular foramen. NVP results in dysphagia and dysphonia. However, most of the signs and symptoms of vagus nerve dysfunction are vague and non-specific, except dysphonia. Paralysis of the superior and inferior laryngeal nerves may result in ipsilateral vocal cord paralysis (Alberio, 2005).

11. ACCESSORY NERVE (XI.)

The 11th cranial nerve, accessory nerve, is exclusively a somatomotor nerve. It begins in two parts, the cranial root, and the spinal root. The spinal root (part) originates from the anterolateral groove of the first 5 cervical segments of the spinal cord. These fibres, also called external branch, pass through foramen magnum, and enter the cranial cavity. Here it bends outwards and travels towards jugular foramen. Cranial root (cranial part, internal branch) arises from the lateral of bulbus as 4-5 fibres. At the level of the jugular foramen, they merge with the spinal part to form the accessory nerve trunk. After passing through the jugular foramen, the spinal part and cranial part separate again. The cranial part (vagal part) merges with the vagus nerve. After exiting, the spinal part turns to the posterior-external side and travels

downwards and innervates the sternocleidomastoid muscle and trapezius muscle (Elhan, 2020; Arifoğlu, 2022).

11.1. Traumatic pathologies

The pathophysiology of spinal part injury of the accessory nerve depends on the etiology and mechanism of injury. Following axonal injury, Wallerian degeneration occurs. Also, the spinal part can be damaged when passing through the posterior cervical trigone. In some cases, the branches of the nerve innervating the upper part of the trapezius muscle may be damaged if not clearly identified during neck dissection surgery. With damage, segmental demyelination occurs due to local ischemia, leading to reduced or complete dysfunction of the nerve (AlShareef, 2023; Popovski, 2017).

Stretching of the nerve disrupts intraneural microvascular flow, causing ischemia and thus axonal degeneration. Generally, when the stress on the accessory nerve due to traction is spread over a longer period of time, it is better tolerated than sudden and large strains, as encountered in sudden acceleration-deceleration injuries (AlShareef, 2023).

Diagnosis of accessory nerve spinal part injury requires assessment of patients, clinical examination, and evaluation of electrophysiological studies of nerves and muscles. The most common symptoms following injury are shoulder pain and weakness. The radiation of pain is to the upper back, neck, and ipsilateral arm. The intensity of the pain may increase with the strain of the rhomboid muscles that try to compensate for the nerve damage (AlShareef, 2023).

12. HYPOGLOSSAL NERVE (XII.)

The 12th cranial nerve, consisting only of somatomotor fibres, innervates all tongue muscles except palatoglossus muscle. It arises as a series of fibres from the anterolateral groove between the oliva and pyramis in the bulbus. It passes through the canal of hypoglossal nerve in the occipital bone, passes behind the cervical vascular nerve bundle and comes to the submandibular trigone. It passes under the submandibular gland and enters the tongue (Arifoğlu, 2022).

12.1. Traumatic pathologies

Hypoglossal nerve injuries are rare. Most reported cases are the result of malignancy and traumatic causes are less common. Fractures of the occipital-cervical junction resulting from penetrating trauma, iatrogenic causes and motor vehicle accidents account for the majority of traumatic cases. Dysphagia and dysarthria are the main signs of nerve trauma. In skull base fractures that may occur in wrestling matches and motor vehicle accidents, nerve paralysis is seen in occipital condyle fractures. Since occipital condyle fractures are not always evident on plain radiographs of the cervical spine, they may sometimes go unrecognized or underdiagnosed. The nerve travelling in the canal of hypoglossal nerve may be cut or avulsed with the fracture (Chugh, 2006).

The prognosis of isolated unilateral hypoglossal nerve paralyses is good and most of them recover within 6 months. The movements of the tongue are restricted with damage to the nerve (Chugh, 2006).

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CHAPTER 16

PSYCHODERMATOLOGY

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INTRODUCTION

Our body's largest and most visible organ, the skin serves as our window to the outside world (Marshall, Taylor and Bewley, 2016). As a result, the skin significantly affects one's perceptions and psychological health (Gupta and Gupta, 2014). More than a third of patients seeking treatment for skin diseases have a psychiatric or psychological problem that complicates the skin disease (Jafferany and Franca, 2016). Psychiatric disorders occurred more frequently in dermatology inpatients compared to general medical care, and psychiatric symptoms occurred more frequently in dermatology outpatients than in the general population (Marshall, Taylor and Bewley, 2016). Both the skin and the nervous system share a common origin, the ectoderm (Jafferany and Franca, 2016; Shenefelt, 2011).

Through afferent and efferent neurons as well as neurotransmitters, there is a complex interaction between the nervous system and the skin (Jafferany et al., 2010).

Psychodermatology is an emerging collaborative subspecialty which focuses on the relationship between psychiatry and dermatology (Altunay and Mercan, 2005; Jafferany and Franca, 2016).

The three main categories of psychodermatologic disorders are as follows (Yadav, Narang and Kumaran, 2013).

- Psychophysiological Disorders
- Primary Psychiatric Disorders
- Secondary Psychiatric Disorders

Psychophysiological Disorders

Psychophysiological disorders are those in which the mental state of the patient affects the progression of a particular skin disease. These disorders are often caused or exacerbated by emotional stress and/or anxiety (Leon, Levin and Koo 2013). Skin disorders that are inflammatory, immune-mediated, or behavioral are significantly influenced by psychosomatic illnesses (Yadav, Narang and Kumaran, 2013).

Diseases exacerbated by emotional stress and anxiety include psoriasis, lichen planus, acne, alopecia, rosacea, urticaria, pompholyx, vitiligo vulgaris, atopic dermatitis, seborrheic dermatitis, mucosal herpes simplex, hyperhidrosis, neurotic excoriations, lichen simplex chronicus, trichotillomania, and chronic telogen effluvium (Yadav, Narang and Kumaran, 2013). The precise mechanisms of how stress and emotion affect the skin are being further worked out (Shenefelt, 2011).

Dermatologists must always try to determine the extent of the role of psychosocial and occupational stress in a particular case to avoid aggravation of the stress disorder and ultimately worsening of the underlying disease (Yadav, Narang and Kumaran, 2013).

Besides, with appropriate treatments for the skin disorders, reducing stress with anxiolytic drugs or antidepressants as such as a selective serotonin reuptake inhibitor (SSRI) and benzodiazepines and non-pharmacological methods of stress reduction such as relaxation, meditation, and physical exercise or yoga can aid in the healing process (Shenefelt, 2010; Leon, Levin and Koo 2013). For patients who have not responded to these measurements, a psychiatric referral is required and the patient should be informed of the intended purpose and benefits of the referral (Yadav, Narang and Kumaran, 2013). As stress is unavoidable in daily life, we need to educate patients how to control their stress to prevent their skin problems from getting worse (Leon, Levin and Koo 2013).

Primary Psychiatric Disorders

PPD's skin signs and symptoms are self-induced and secondary. Mostly, they are easily recognized by dermatologists, but in some instances, they may act as cutaneous disorders. Underlying psychopathology should be identified and managed properly. The underlying psychological functional problem can be delusion, obsessive-compulsive disorder (OCD), anxiety, depression, impulse control disorder, or personality disorder, and resultantly, trichotillomania, delusions of parasitosis, and neurotic excoriations may occur (Yadav, Narang and Kumaran, 2013; Leon, Levin and Koo 2013).

Secondary Psychiatric Disorders

Dermatological disorders have a great impact on quality of life. Nearly 30–60% of patients with skin diseases have concurrent psychological disorders. Skin disorders, especially those that affect visible parts of the body and are chronic in duration, may result in embarrassment, depression, anxiety, poor self-image, low self-esteem, and suicidal ideation. Moreover, patients face social isolation and stigmatization. It was found that patients with psoriasis were more associated with alcohol abuse and that there was a correlation with the severity of psoriasis. The most common psychiatric disorders in vitiligo and psoriasis patients, were adjustment disorders, depression, and dysthymia. A dermatologist should investigate the signs of psychological effects on patients and should refer the patient to psychiatry (Yadav, Narang and Kumaran, 2013; Leon, Levin and Koo 2013).

Effect of primary psychiatric disorders on skin diseases

1. Primary anxiety

Many skin diseases are worsened by acute or chronic anxiety. On the contrary patients with dermatological diseases may have anxiety. 13% of patients visited dermatology clinic have been found to have associated anxiety disorder (Shenefelt, 2011; Leon, Levin and Koo 2013).

2. Psychogenic pruritus

After other causes of pruritus have all been excluded psychogenic pruritus should be thought in patients with pruritus (Shenefelt, 2011). Pruritic episodes have an abrupt onset and terminates with relaxation (Yadav, Narang and Kumaran, 2013).

3. Delusions of parasitosis

Parasitic delusions occur when the patient insists that he has insects growing in or on his skin when objectively they do not (Shenefelt, 2010; Shenefelt, 2011). A middle-aged or older female who presents in an anxious, ruminative, and overwhelmed condition after seeing many doctors with no relief fits this character. Typically, patients present with skin crust, clothes lint, or other debris that may be mistaken for parasite pieces, larvae, eggs, or the complete organism. Dermatologists should pay special attention to the material

provided by the patient, listen to them empathetically, and pay attention to what they have to say (Yadav, Narang and Kumaran, 2013). These patients generally will respond to antipsychotics (Shenefelt, 2011).

4. Primary depression

Patients with depression may harm their skin by scratching, picking, digging, burning, cutting, pulling, tearing. On the contrary, 32 % of the patients visited dermatology clinics reported to have depression. Depression with somatization usually accompany patients with neurotic or psychogenic excoriations. Appropriate management of depression may aid in treatment process of dermatological diseases (Shenefelt, 2011).

5. Impulse control

Acne excoriée, neurodermatitis, trichotillomania can be named as impulse control disorder. Cognitive–behavioral methods or hypnosis and self-hypnosis may be beneficial to control the symptoms (Shenefelt, 2010; Shenefelt, 2011).

5. 1. Trichotileomania can be described as recurrent pulling out of one's own hair and result in traumatic alopecia. The patient expresses tension before pulling hair and pleasure after pulling out the hair. TM is most commonly seen in children and is usually a habitual disorder without significant psychiatric morbidity. In adults, female predominance exists and is associated with depression, anxiety, and OCD (Yadav, Narang and Kumaran, 2013).

5.2. Acnee excoriee is more commonly seen in females and associated psychiatric comorbidities includes depression, anxiety, OCD, body dysmorphic disorder, delusional disorder, personality disorder, and social phobias (Yadav, Narang and Kumaran, 2013).

5.3. Neurotic excoriation is commonly seen in females aged 30 and 50 years. The lesions are characterized with erosions, crusts and excoriations and heal with depigmentation and hyperpigmentation. Prurigo nodularis is a variant of severe this disorder. There usually is precipitating emotional stress in majority of the patients and depression is the most commonly seen psychiatric comorbidity (Yadav, Narang and Kumaran, 2013).

6. Obsessive–compulsive disorder

OCD may primarily induce a cutaneous disorder or may exacerbate preexisting skin diseases including, eczema, psoriasis and acne (Shenefelt, 2010; Shenefelt, 2011). 5 in 100 patients visited dermatology clinic reported to have accompanying OCD (Shenefelt, 2011). The patient with OCD has awareness of the inappropriateness of their obsessions and compulsions. A separate diagnosis, such as delusional illness or psychosis, should be taken into consideration if the patient completely lacks this understanding (Leon, Levin and Koo 2013). Acne excoriée, onychotillomania and neurodermatitis are the examplaes of OCD induced skin disorders. Cognitive-behavioral methods and SSRI may help to manage the OCD (Shenefelt, 2011).

7. Somatization with dissociation

Common somatization with dissociation syndromes in dermatology may be seen in pruritus, urticaria, or angioedema, self-induced dermatitis artifacta and trichotillomania and body dysmorphic disorder. Body dysmorphic disorder was reported in 14-21 % of acne patients (Shenefelt, 2011).

8. Neurogenic cutaneous sensory dysesthesias

8.1. Dysesthesias arising in the central nervous system

General pruritus after exclusion of possible underlying diseases and neurotic excoriations are examples of dysesthesias arising in the central nervous system and may be treated with sedating antihistamines or doxepin (Shenefelt, 2011).

8.2. Dysesthesias arising in the peripheral nervous system

In brachioradial pruritus, glossodynia, notalgia paresthetica, postherpetic neuralgia and pruritus ani, pruritus scroti, and pruritus vulvae are examples of these categorie (Shenefelt, 2011).

9. Dermatitis artefacta

Dermatitis artefacta or factitious dermatitis is defined as the intended and purposeful production of self-imposed skin, hair, nail and mucosal lesions to get an unconscious psychological or emotional need, capture attention or avoid

responsibility (Gupta and Gupta, 2014; Chandran and Kurien, 2023). DA usually begins during puberty and there is a female predominance. The most common presentation bizarre non-healing ulcers and wounds. Patient can't tell the evolution and reason of the lesion (Yadav, Narang and Kumaran, 2013).

10. Body Dysmorphic Disorder (BDD)

Body dysmorphic disorder (BDD) is a psychiatric disorder characterized by a preoccupation with nonexistent or slight defects in appearance that are either not noticeable or only slightly observable by others (Gupta and Gupta, 2014; (Yadav, Narang and Kumaran, 2013; Hardardottir, Hauksdottir and Bjornsson, 2019). Patients have repetitive behaviors as mirror checking or mental acts as comparing one's appearance others (Gupta and Gupta, 2014).

Although the prevalence of BDD is around 0.75-12 % (Yadav, Narang and Kumaran, 2013). Physicians working in cosmetic and dermatology clinics more commonly encounter it (Hardardottir, Hauksdottir and Bjornsson, 2019). BDD results in significant distress and/or impairment at work or school. Major psychiatric comorbidities are depression, alcohol or substance use disorder, social anxiety disorder, OCD and suicide idea. Cognitive behavioral therapy (CBT) and SSRI medication are the treatment of choice for BDD (Hardardottir, Hauksdottir and Bjornsson, 2019).

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CHAPTER 17

STEREOLOGY AND ITS IMPORTANCE IN BIOLOGICAL SCIENCES

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1. Stereology

The term stereology comes from the Greek word "stereo" meaning solid. Stereology is generally defined as the scientific study of examining solids. In simple terms, it is the study of calculating geometric quantities. In other words, it is the science of obtaining quantitative data related to a structure of interest, such as number, volume, surface area, and length (Braendgaard and Gundersen, 1986; Sterio, 1994). Within the discipline of stereology, there are many methods have been developed based entirely on mathematical proofs to obtain these data (Mayhew and Gundersen, 1996). Stereological methods are not only used in the field of health sciences, but can also be used in many other fields such as engineering, agriculture, aquatic sciences, and earth sciences. What is important here is to select the appropriate stereological method to obtain the quantitative data of interest (Howard and Reed, 1998).

As we know, biological structures such as tissue, cells, and organs are three-dimensional (3D) and their structural features can be examined in two-dimensional (2D) sections. However, if the correct methods are not used to obtain data on the 3D properties of the structure based on the images obtained from 2D sections, accurate results cannot be obtained. This is because the images obtained from 2D sections are actually the reflections of 3D structures on a 2D plane. These reflections do not reflect the true properties of the examined structure. For example, the boundaries of a cell that is actually spherical are seen as a ring shape on the 2D section surface (Canan, Şahin, Ünal, and Aslan, 2002; Odacı et al., 2004). Therefore, obtaining data about the 3D properties of a structure based solely on the data obtained from 2D sections can be misleading if the correct methods are not used (Gundersen and Jensen, 1987; Gundersen, Jensen, Kieu, and Nielsen, 1999).

Using stereological methods, it is possible to obtain information about the 3D properties of structures from 2D sections. The most important points to note here are that the structures must be made visible using histological methods, the correct method must be selected, and the basic principles of stereological methods must be applied correctly. In short, by using stereological methods, it is possible to obtain meaningful quantitative data about the geometry or structure of 3D structures based on measurements made

on 2D images or sections (West, 2012). It is important to apply the principles of objectivity and efficiency in stereological studies to obtain reliable data. Objectivity involves eliminating any subjective effects that may arise from personal or working conditions during the study, while efficiency can be summarized as obtaining reliable data in a short time (Gundersen et al., 1988).

1.2. Design-based Stereology

Stereological methods are generally used in biological studies to obtain four numerical data, which are number, length, surface, and volume data (Canan et al., 2002; Odacı et al., 2004; Ünal et al., 2002). The number is the amount of elements that make up a certain population and is a fundamental quantity of interest to researchers. Determining the number of objects of interest in biological structures is not as simple as it may seem. Length is an easy-to-understand concept, but it can be difficult to define. When considering objects that bend and twist in space, some difficulties may arise in defining this concept. Surface, although in theoretical geometry this expression means surface area, in the adaptation of stereological methods to biological studies, it refers to an irregular surface area. Volume or profile area is a measure of how an object fills space. The area of objects observed on a plane is called the profile or cross-sectional area (Gundersen, 1986).

For example, the calculation of the number of neurons in the brain, the length of capillaries, the surface area of membranes, and tumor volume can be considered as data that can be obtained through stereological methods (Pakkenberg and Gundersen, 1988). Recently, new concepts have emerged in stereological methods, the most important of which is "design-based" stereology, also known as unbiased stereology. Design-based stereology increases the reliability and efficiency of quantification in biological research (Micro Bright Field Bioscience [MBF Bioscience], 2017). The term "design-based" is used to describe new methods in stereology where probes and sampling schemes are "designed" or pre-determined, and are independent of size, shape, spatial orientation, and spatial distribution. To achieve this independence for the geometric properties to be examined, the basic rules of stereology must be followed and the biases must be eliminated by following these rules correctly (Gundersen et al., 1999). Since traditional stereological methods were "model-based," methods based on the geometric properties of

the objects being studied were used in these models. However, design-based methods eliminate the need for knowledge about the geometry of the objects being examined. More robust data is obtained since possible sources of systematic errors are eliminated in the calculations (MBF Bioscience, 2017).

Different methods have been developed in stereology to obtain different types of data, and these methods are widely used. These include volume calculation from 2D sections using the Cavalieri principle, counting the number of objects in a defined area using dissector (optical and physical) methods, and calculating the total number of objects in the structure of interest using the fractionator (optical and physical) method. Additionally, using stereological methods, measurements of length, width, surface area, and volume of the structure of interest can be obtained (Canan et al., 2002; Gundersen et al., 1988; Odacı et al., 2004).

1.2.1. Systematic Random Sampling

Systematic random sampling (SRS) is a sampling method used in stereological techniques. In sources, it is also referred to as systematic uniform random sampling or systematic sampling. All these terms refer to the same concept. SRS is an excellent method that reduces variance compared to random sampling (also known as simple random sampling in the field of statistics) and is easy to apply (Canan et al., 2002; Gundersen and Jensen, 1987; Gundersen et al., 1999; Odacı et al., 2004; Ünal et al., 2002). In SRS, the researcher selects a fraction f. The fraction f = 1/p is chosen as a whole number. The symbol p is used to indicate repetition. The sets in this case are as follows:

$$S1 = \{S1, Sp+1, S2p+1, ...\}$$

$$S2 = \{S2, Sp+2, S2p+2, ...\}$$

$$...$$

$$Sp = \{Sp, S2p, S3p, ...\}$$

In this method, the set is unbiased as long as it is randomly selected. To add randomness to the selection, a random number between 1 and p is selected as the starting point. An arrangement made according to this rule is given in the following figure (Figure 1).

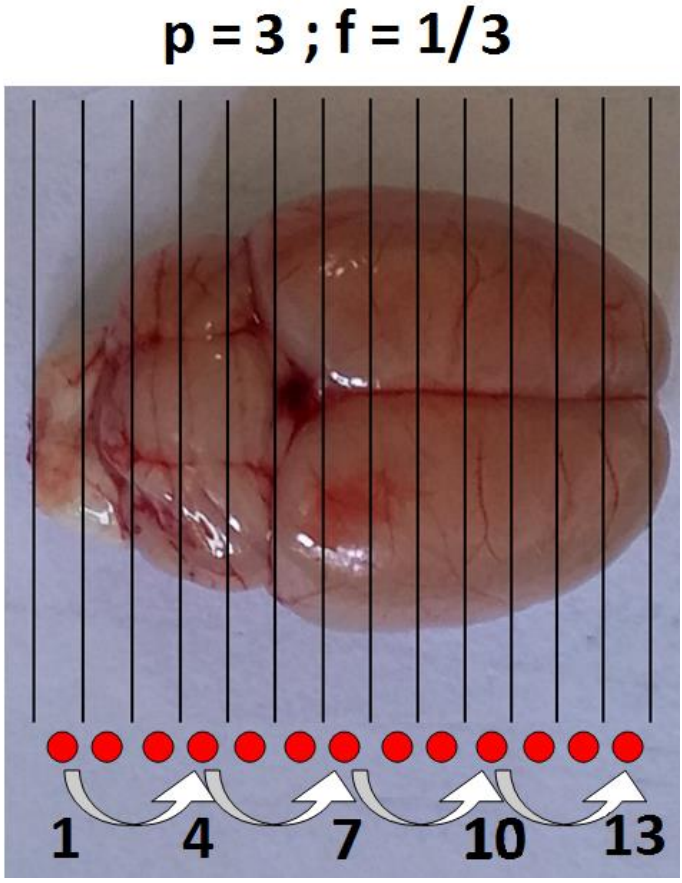

Figure 1. Determining cross-section with systematic random sampling.

According to the design in the figure, p represents the number of cycles. In this design, p = 3, which means one out of every 3 sections will be selected. f is the fraction rate and represents the selected section rate in each cycle. In the design shown, since one section is selected in every 3 sections in each cycle, the value of f is 1/3. In the example shown in the figure, the first section randomly selected from the first three sections represents the random part of the system, and the advancement by p ratio after the first section is selected represents the systematic part of the system. The section selection should continue in the same way in the application. For example, if one section was selected using systematic random sampling from the first 3 sections in the figure, the next section to be selected would be the 4th section. The application continues with the same sampling and the sections

corresponding to the samples are included in the study. In this example, the selected sections continue as 1, 4, 7, 10, 13... (Gundersen and Jensen, 1987; Gundersen et al., 1999).

1.2.2. Particle Counting

The determination of the total number of particles or numerical density per unit volume (NV) in biological structures is an indispensable parameter for many studies. Especially in the nervous system, the numbers of nerve cells, which are the fundamental functional elements, often come up as important data in research. The concept of number, which is interpreted as an important expression of the relationship between structure and function, is one of the research data that is commented on for many research topics such as organ development, seasonal functional changes, effects of chemicals, and physical treatments, adaptive specialization, learning and memory, disease formation mechanisms, and aging. Despite the importance of the concept of numbers, a method that would keep particle counts within an acceptable margin of error could not be established for many years (Canan, 2003).

To count particles, cross-sections of the structures of interest need to be taken. However, these cross-sections, when evaluated only visually, are two-dimensional images that cannot exemplify the concept of zero-dimensional number, which is distributed in a three-dimensional environment. Attempting to reach the particle count by directly counting the projections of particles in the sections is one of the first methods tried. However, it did not take long for the researchers dealing with counting to realize that the number of projections and the number of particles are not directly related. The particle count is a zero-dimensional quantity that needs to be sampled independently of three-dimensional effects such as particle size or orientation. It is zero-dimensional because regardless of the dimensional properties of any particle (size, length, diameter, volume, etc.), its value is 1 when it comes to number. Thus, the number is independent of dimensional properties. However, the cross-sections obtained from the structures are considered two-dimensional planes and sample particles in a closely related manner with their dimensional properties. A series of equally spaced cross-sections passing through a structure samples large particles more than small ones, and those perpendicular to the sectioning direction more than those parallel to it. Thus, the chance of a particle being hit

by a section depends on its diameter, height (volume), and orientation (Mayhew, 1996)

In fine histological sections, the presence of more nuclei than expected or more cells than nuclei is a good example of the relationship between particle size and the probability of sampling.

Although sections are considered as two-dimensional planes, they are slices with finite thickness, and their thickness should also be taken into account. If the section images are evaluated as two-dimensional planes by neglecting their thickness, the effect of thickness on the number of particles can lead to counting errors. As the section thickness increases, the frequency of particles appearing in the sections will also increase. Because histological sections are transparent, this increase is manifested as an increase in the number of projections. The phenomenon that occurs depending on the section thickness is called over-projection (formerly known as the Holmes Effect). After the detection of the Holmes Effect, researchers have tried to overcome this problem by using various correction factors. The most famous and perhaps most widely used is Abercrombie's correction factor. In his famous study published in 1946, Abercrombie focused on counting errors that arise due to section thickness and particle size. According to Abercrombie, the actual number of particles counted should be equal to the product of the number of particles or particle fragments obtained from the count and a correction factor expressed as section thickness / (section thickness x particle height). This expression is formulated as follows (Canan, 2003).

$$N = \frac{N_S\ xt}{tx\ \overline{D}}$$

N: total particle count;
Ns: counted particles;
t: section thickness;
D: average particle diameter.

1.2.3. Dissector

The dissector was first described by Sterio in 1984. This method can also be described as a virtual 3-dimensional stereological probe used in particle counting. The basic principle of the dissector is to find the "endpoints" of particles, which are the parts where particles first appear or are

last seen along the cutting direction. Regardless of the shape and orientation of each particle, this method assumes that each particle has only one endpoint in a specific direction, allowing for an accurate determination of the true particle count (Canan, 2003).

1.2.3.1. Physical Dissector

The dissector is the first form of stereology that was introduced (Sterio, 1984). In this method, two consecutive or spaced-apart sections are taken, and the particles that are present in one section but not in the other are counted. The distance between the two sections is referred to as the dissector height, and the counting of particles or the tips of particles that can be sampled along the dissector height yields the numerical density (NV) of the studied particles.

The fundamental implementation of the dissector involves comparing two sections that are separated by a distance that is less than the minimum particle height. This requirement ensures that the particles are not skipped or missed between the sections without being sampled. The chance of a particle being hit by one section and missed by the adjacent parallel section depends on the height of the particle in the sectioning direction, whereas the probability of being cut by one section and not by the next adjacent one is equal for all particles of any size (Pakkenberg and Gundersen, 1988). Based on this relationship, the total number of particles (or their numerical density) in a defined volume can be calculated.

$$N = \frac{\sum Q^{-}}{h.\sum a(are)}\, V(ref)$$

Here, N represents the total number of particles, Q- represents the number of particles counted using the dissector method, h represents the distance between the sections (i.e., the height of the dissector), a(are) represents the area of the unbiased counting frame used in the measurement, and V(ref) represents the total (or reference) volume of the studied structure.

A dissector particle is a name given to particles that can be counted using the dissector method. In the physical dissector, one of the section pairs taken for counting is used as the sample section (reference section) and the other as the observation section (look-up section). These sections are

examined, and particles that are found in the sample section but not in the observation section are included in the count as dissector particles and represented by the symbol "Q-". These are the particle ends that can be sampled within the dissector height (Canan, 2003).

1.2.3.2. Cavalieri's Principle

Cavalieri's Principle, developed by Italian mathematician Bonaventura Cavalieri, is a principle stating that if two objects have the same base area and height, then their volumes are equal. For example, if two pyramids have the same base area and height, then according to Cavalieri's Principle, their volumes are equal. Similarly, if two cylinders have the same base area and height, then their volumes are also equal. Cavalieri's Principle has various applications in geometry and mathematical analysis, particularly in volume calculations used in integration.

Cavalieri's Principle also plays an important role in biological studies, particularly in measuring and comparing the volumes of biological tissues. For example, Cavalieri's Principle can be used to measure the volume of a tumor. The volume of the tumor can be calculated by adding up the areas of tissue sections using Cavalieri's Principle. Similar-shaped and sized sections are chosen using Cavalieri's Principle, and their areas are calculated and added together. This calculation provides an estimate of the tumor volume. Similarly, Cavalieri's Principle can be used to compare the volumes of biological tissues. For instance, the volume of an organ can be calculated by adding up the areas of similar-shaped and sized sections, and this calculation can help compare the volumes of the same organ in different organisms or under different conditions (Puri, 2014).

In addition, Cavalieri's Principle is used in morphological analyses. For example, to measure the volume of an object using Cavalieri's Principle, similar-shaped and sized sections are chosen, and information about the object's volume and shape can be obtained by examining the differences in the areas of these sections.

According to this principle, the structure whose volume is to be calculated is divided into serial sections from beginning to end. In each section, the area of the projections belonging to the structure is calculated.

Area calculations can be performed using expensive image analysis systems, or they can be obtained with the same reliability by using a gridded area measurement ruler. Gridded area measurement rulers are patterns consisting of points representing specific areas. The area calculation can easily be performed by randomly placing these patterns on the sectional images of the structure or on the surface whose area is to be calculated, and counting the number of points falling within the relevant area. When the total number of points falling within the area is multiplied by the area represented by each point, the area of the relevant region can be obtained.

$$A = \sum P.a(p)$$

Here, P represents the number of points, and a(p) represents the area represented by a single point on the section.

After calculating the area of the section image, multiplying the total area with the average section thickness gives the total volume of the structure of interest. Depending on the situation, this calculation can be performed by either calculating separate volume values (area x thickness) for each section and summing them, or by summing the area values and multiplying them by the average section thickness. Generally, the second method is more practical when making microscopic area calculations (Canan, 2003).

1.2.3.3. Unbiased Counting Framework

In situations where particle counts are done using particle projections, the projections that appear in the sections must be delimited by a certain area in order to be counted. The issue of how to delimit the projections arises. In the old methods traditionally applied, a square or rectangular frame was placed on the projections, and the particles that fell directly into this frame were counted, which has been a widely used method. Later, it was found that this type of counting overestimated the particle count, so a counting frame that was simply divided in half along one of its diagonals was used instead. The counting rule for this frame was that particles falling on one half of the frame were counted while those falling on the other half were not. However, it was later understood that this frame was also far from giving the true particle count, and a neutral counting frame was introduced to eliminate counting

errors caused by counting frames. The reason for these counting errors caused by counting frames is attributed to the "edge effect." The edge effect refers to the problem of how particles that intersect with the frame edges should be evaluated, even though there is no problem counting particles that fall within the frame. The neutral counting frame, which was finally introduced by Gundersen in the 1970s, has been shown to be the most reliable in eliminating the edge effect through calculations (Gundersen, 1977).

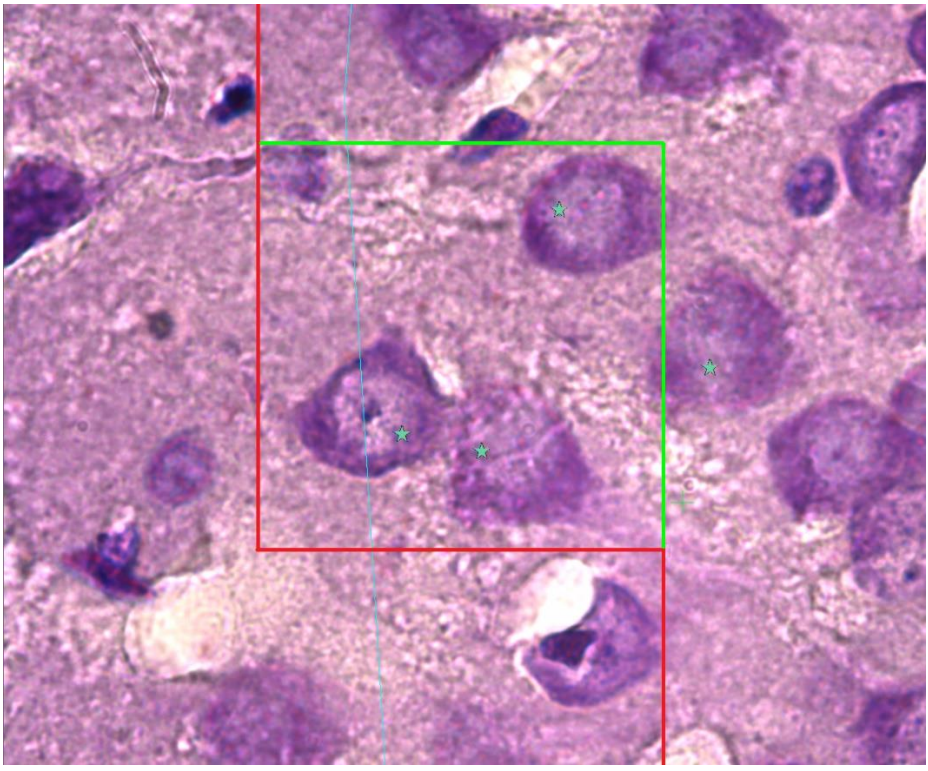

Figure 2. Unbiased counting frame during neuron counting. In the image, the red lines represent the forbidden edges, and the green lines represent the free edges. The star indicates the pyramidal neurons included in the counting (Cresyl fast violet, × 100), and the blue line represents the boundary of the hippocampus.

The neutral counting frame is defined as having two adjacent edges and their extensions as forbidden edges (shown in red in Figure 2), and two adjacent edges as free edges (shown in green in Figure 2) (Canan et al., 2002; Odacı et al., 2004; Ünal et al., 2002). Particles that touch the forbidden edges and their extensions are not included in the count. However, particles that

intersect with and are contained within the counting frame's free edges are included in the count. During the optical dissector application, the virtual volume is created using this neutral counting frame. While moving through the virtual volume created by this frame, particles that appear in the image are counted according to the rules of this counting frame. However, particles that appear outside of the frame, in contact with the red line or its extensions, or within the safety zone, are not included in the count (Figure 2; Sterio, 1994; West, Solomianka and Gundersen 1991).

The unbiased counting frame is currently the most effective and reliable counting frame for particle counting. It has been shown through geometric calculations that this frame accurately counts particle projections (Gundersen, 1977).

1.2.3.4. Optical Dissector

The optical dissector is a stereological method used to count the structures of interest (termed particles or particles in stereology terminology) in a thick tissue section. In the optical dissector, virtual sections are created along short distances through the thick tissue sections (e.g., 30-40 μm in nervous tissue), and counting is performed within the virtual cubes (virtual volume) that are formed. Safety zones, which are areas not included in the counting and determined according to the researcher's preference, are identified at the top and bottom of this virtual volume. The counting is performed in the area between these two zones. A virtual apparatus called the unbiased counting frame is used for the counting to be performed within this area (Kreft and Sterio, 2011).

1.2.4. Calculation of Section Thickness

In the application of stereological methods, the thickness of the section (in light microscopy) is usually measured by calculating the distance the microscope stage travels in the vertical axis (z-axis) during a focus change. This distance change can be achieved either by using the graduations on the microscope's micro or macro screws or by using a tool sensitive to the vertical movements of the stage (such as a micrometer). However, the graduations on the microscope screws are not always reliable due to the backlash between the opposite rotation directions of the screws. Nevertheless, by expanding the

scales on these screws, a more reliable measurement can be achieved (Korkmaz and Tümkaya, 1997).

When determining the thickness of a section or a thickness within a section using a microtome or a similar tool, the upper and lower surfaces of the section must be determined by the researcher with different levels of focus. As the microscope image approaches the upper surface of the section, the point where the first clear image is obtained is the upper surface of the section; the last clear image encountered is the lower surface of the section. The up or down movements made on the microscope stage as it advances optically between these two surfaces will give the section thickness.

When calculating the section thickness using this method, different results may occur due to the differences in assumptions made by researchers in determining the upper and lower surfaces of the section. However, this difference, especially if the sections used are larger than 30 mm and a plastic embedding medium is used, will cause an error margin that is insignificant throughout the entire study (Canan 2003).

1.2.5. Fractionator

The fractionator method in stereology is currently perhaps the most commonly used particle counting (or area, volume, length, etc. calculation) method. Its main advantage is that it is not affected by any shape changes in the tissue, does not require values such as section thickness or shrinkage-swelling amount, and is a highly effective method.

The essence of the fractionator method is to count the particles in a relatively small tissue sample selected by uniform systematic random sampling from any structure. The only requirement is to know the ratio of the sampled tissue amount to the original structure. The particle count value obtained from this small tissue section is then multiplied by only the ratio of this small section to the main structure to obtain the total count. Since only the sampling (dissector) ratio is involved, there is no need to make any assumptions about section thickness, deformation degree, and tissue. The fractionator method is such an easy, unbiased, and effective method that it is not known how biased dissection (fractionation) should be done (Gundersen et al., 1988a, 1988b).

The fractionator method is actually a sampling plan rather than a particle counting method. This composite method, which involves counting according to the rules of the dissector counting method in the tissue sample with the dissector method, is divided into optical and physical depending on the type of dissector application used in particle counting. Although the basic logic is the same in both methods, there are some differences in practice (Canan, 2003).

1.2.5.1. Physical Fractionator

Physical fractionator is a combination of physical dissection and particle counting methods through fragment sampling. Although it is fundamentally applied in the same way, there are some application differences for optical fractionator. In terms of application, it cannot be used in some cases, but the same applies to optical fractionator.

Physical fractionator can be summarized as sampling the structure to be counted by dividing it into smaller physical fragments in certain steps and obtaining the total number of particles from certain fragments that remain after the final sampling. The fundamental logic of the fractionator method lies in systematic random sampling. The only condition that must be followed in the samples taken at various stages from the structure being studied is systematic and random sample selection; that is, a randomly selected initial sample from the interval containing the first m samples, followed by every mth sample selected. Here, m is the predetermined sampling interval (for example, if it is decided to select one out of every 20 sections, m is 20)

Another feature of the fractionator method is systematic sampling at each step. At each step, systematic and random sampling is performed from the available samples as needed. In the next step, a new sampling is made from the samples selected in the previous sampling step. The value of the dissecting coefficient, denoted by "f", should be known for each selected piece or section as a proportion of the structure or samples from which the samples were taken (West et al., 1996).

The main aim of the fractionator method, like all stereological methods, is to obtain results within a certain and acceptable margin of error on a small portion of the structure being studied, representing a certain proportion of the

whole structure. Since the method is inherently unbiased, as the number of samples increases, the results will approach the true value more closely. Therefore, the variable that has the greatest effect on the error coefficient should be identified during the preliminary work in order to obtain results within a predetermined (usually less than 10%) margin of error. A preliminary study on at least 5 animals is sufficient to determine the error rate and the factors contributing to it. A preliminary study in a single animal cannot provide an idea about the suitability of the study plan because, as a general rule, the greatest contribution to the variability of the results always comes from the differences between individuals (biological variation). To take this into account, the first thing to do is to start with a preliminary study that can reveal the differences between individuals. If the desired error coefficient is achieved, sampling can be easily performed at each stage (Gundersen and Jensen, 1987).

In the physical fractionator method, the structure being studied is subjected to a series of dissection steps. After the proportions of the systematically and randomly selected pieces are recorded at each step, the remaining samples are embedded into blocks and sectioned until the end. From these sections, samples are taken systematically and randomly as desired. Then, during cell counting under a microscope, sampling can be performed between counting areas (Figure-12). The only requirement is to know the "dissection ratios" of these systematic and random sample selections. After all samples are taken, the particle count obtained from the particle count performed represents how many particles are found in the many parts of the structure being studied. This value is multiplied by the inverse of the sampling rates to calculate the total number of particles. This process can be summarized as follows:

$$N = \frac{1}{f1}.\frac{1}{f2}.\frac{1}{f3}\ldots\frac{1}{fn}.\sum Q^{-}$$

Here, f1, f2, f3... and fn values represent the sampling fractions. For example, if one out of every 5 sections of a sliced structure is taken in the first sampling, then f1=1/5. The product of the inverse of these sampling fractions and the total number of dissector particles (S Q-) will give the total particle count.

As can be understood from its basic logic, the method is completely unbiased. No presupposition is required regarding the structure being studied, and no other data (such as section thickness, deformation constant, etc.) is necessary besides the sampling fractions. When applied correctly, the calculation provides an estimation that approaches the true value as the number of samples increases. The sampling of particles by the dissector method depends solely on their physical existence. They are sampled only if they exist, and all existing particles have an equal chance of being sampled. In addition, it is one of the most effective methods since it allows obtaining results within the desired variability limits in a short time by working with a very small amount of samples (West, Slomianka, and Gundersen, 1991).

1.2.5.2. Optical Fragmentation

It is not possible to isolate cells physically from the surrounding tissues. In studies where cell count data needs to be obtained, it is important to obtain data that is close to the actual number. Therefore, the methods used are also very important. Making the tissue of interest into sections and examining the sections to find the number of cells is a commonly used method. However, in this case, cutting the tissue also leads to the cutting of the cells in the tissue. Therefore, the cell count in the sections is different from the actual number of cells in the tissue and the obtained cell count data will show deviation from the real value if an appropriate method is not chosen.

Two methods are used in design-based stereology to obtain cell count data with a statistically acceptable error close to the actual value. The first method is the dissector method, and this method is called optical dissector (Canan et al., 2002; Pakkenberg and Gundersen, 1988). The method is based on the application of both optical fractionator and sectioning methods at the same time. During the optical fractionator application, cell counting is carried out in the virtual counting areas determined with the SRØ in the thick sections in the X, Y, and Z planes, in compliance with the rules of unbiased counting frames (West, Solomianka and Gundersen, 1991). In the other method, the number of cells counted in all counting areas is divided by the number of examined counting areas, and the average cell density (Nv) in the unbiased virtual counting areas is calculated. The total number of cells is obtained by

multiplying the obtained average cell density by the volume of the examined area (reference volume = Vref). This method is called the Nv x Vref method.

Since Vref is not required for the optical fractionator method for number estimation, it is easier to apply. Optical fractionator is a stereological method widely used in biological sciences to estimate the total particle count in any three-dimensional object, regardless of the shape of the volume of interest. Optical fractionator is suitable for quantifying cells, synapses, glomeruli, and other objects within biological tissues. In the application of the optical fractionator, thick tissue sections are required. A section can be taken in any orientation from the tissue to be analyzed. Cell counts made using the optical fractionator are an unbiased method, as they are not affected by the size, shape, spatial orientation, and spatial distribution of the cells being studied. The optical fractionator is based on a sampling principle of making a series of SRØ to select sections and then using the sectioning principle to sample each section in the X, Y, and Z axes (Canan et al., 2002; Pakkenberg and Gundersen, 1988; West, Solomianka and Gundersen, 1991).

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CHAPTER 18

FUTURE OF THE EMBRYOLOGY & TRANSHUMANISM

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1. INTRODUCTION

Embryology, the study of the development of embryos, has long been a critical area of research with implications on the human species (Gilbert, 2014). In recent years, the advancements in this field have not only revolutionized our understanding of human development but also opened doors to new possibilities for human enhancement. Transhumanism, a movement that seeks to surpass the limitations of the human body and mind through technology, intersects with embryology to form a compelling vision of the future (Bostrom, 2005). This chapter aims to explore the potential of embryology-based transhumanist technologies and their impact on the human species, while examining the ethical, social, and legal implications of such advancements.

From early observations and experiments in embryology to the technological breakthroughs that have transformed human reproduction and health, the history of embryology is rich and varied. Societal attitudes towards embryology and human enhancement have evolved over time, and our understanding of the potential impact of these technologies on the future of humanity has grown (Baylis, 2013). The emergence of powerful gene-editing tools, such as CRISPR-Cas9, has further accelerated the pace of research in this area and generated new opportunities for the application of embryology in the context of transhumanism (Doudna & Charpentier, 2014).

With the advent of these new technologies, it becomes increasingly important to consider not only the scientific aspects of embryology and transhumanism, but also the ethical, social, and legal dimensions of their potential applications (Cavaliere, 2017). As the line between therapeutic intervention and human enhancement becomes blurred, the need for a robust debate surrounding the moral, cultural, and political implications of these advancements is evident(Bostrom, Savulescu, & 2009).

This chapter will provide a comprehensive overview of the future of embryology and transhumanism by examining the latest advancements in embryology, exploring the transhumanist approaches to human enhancement, and discussing the ethical, social, and legal implications of these technologies. Furthermore, the chapter will delve into the challenges and potential solutions in implementing embryology-based transhumanist technologies and envision possible future scenarios.

2. A BRIEF HISTORY OF EMBRYOLOGY AND ITS IMPLICATIONS ON THE HUMAN SPECIES

Embryology has a rich history that dates back to the early civilizations. Ancient Greek philosophers, such as Aristotle, laid the foundation for the field by observing animal development and describing the formation of embryos (Wellner, 1959). As the field of embryology progressed, the invention of the microscope in the 17th century facilitated the study of embryos at the cellular level, leading to the discovery of spermatozoa and eggs (Nicolson, 2016).

Throughout the 18th and 19th centuries, embryologists made significant strides in understanding the principles of embryonic development. The discovery of the mammalian egg by Karl Ernst von Baer in 1827 and the formulation of the cell theory by Theodor Schwann and Matthias Schleiden in 1839 were particularly pivotal in shaping the field (Gilbert, 2014).

The 20th century witnessed groundbreaking advancements in embryology, with the development of in vitro fertilization (IVF) by Robert Edwards and Patrick Steptoe in 1978, marking a turning point in human reproduction (Edwards, 2001). Since then, assisted reproductive technologies (ARTs) have evolved, enabling millions of couples worldwide to conceive (Zegers-Hochschild et al., 2009).

The Human Genome Project, completed in 2003, marked another milestone in our understanding of human biology and embryology, providing an invaluable resource for future research (Collins, Morgan, & Patrinos, 2003). This project, coupled with the rapid advancement of gene-editing technologies, such as CRISPR-Cas9, has transformed our ability to manipulate the human genome, paving the way for novel therapies and interventions at the embryonic stage (Doudna & Charpentier, 2014).

The implications of these advancements on the human species are profound. The widespread use of ARTs has changed the landscape of human reproduction, allowing people to overcome fertility challenges and conceive genetically related children (Zegers-Hochschild et al., 2009). Moreover, the advent of gene-editing technologies has raised the possibility of eliminating heritable genetic diseases, reducing the burden of disease on individuals and society (Ma et al., 2017).

However, these technological advancements also raise ethical concerns about the potential for creating "designer babies" with enhanced traits and the possibility of exacerbating social inequalities through unequal access to these technologies (Ishii, 2017). As we continue to push the boundaries of embryology, it is crucial to consider the societal and ethical implications of these advancements and their potential impact on the human species.

3. CURRENT ADVANCEMENTS IN EMBRYOLOGY AND THEIR POTENTIAL IMPACT ON TRANSHUMANISM

3.1. CRISPR-Cas9 Gene Editing

The development of the CRISPR-Cas9 gene-editing system has revolutionized the field of embryology, enabling researchers to edit the genome with unprecedented precision and efficiency (Doudna & Charpentier, 2014). This powerful tool has the potential to not only cure genetic diseases but also introduce desirable traits in embryos, such as increased intelligence, physical abilities, or disease resistance (Ma et al., 2017). In the context of transhumanism, CRISPR-Cas9 could facilitate the enhancement of human capacities and contribute to the emergence of a new era of human evolution (Bostrom, 2005).

3.2. In Vitro Gametogenesis (IVG)

In vitro gametogenesis (IVG) refers to the generation of functional gametes (sperm and eggs) from pluripotent stem cells in a laboratory setting (Sasaki et al., 2015). This breakthrough technology holds the potential to revolutionize reproductive medicine by overcoming infertility issues, enabling same-sex couples to have genetically related children, and even allowing for the creation of "artificial" gametes from non-reproductive cells (Hendriks et al., 2015). The implications of IVG for transhumanism are significant, as it could enable the production of genetically enhanced embryos, further extending human capacities and contributing to the advancement of the human species (Bostrom, 2005).

3.3. Artificial Wombs and Ectogenesis

Artificial wombs, or bioengineered uterine environments, have been developed to support the growth and development of embryos outside the human body (Romanis, 2020). This technology, known as ectogenesis, could potentially enable the gestation of embryos from conception to birth in an entirely artificial environment. In the context of transhumanism, ectogenesis could facilitate the manipulation of embryonic development in ways that might be impossible in a natural uterine environment, potentially leading to the enhancement of human traits and abilities (Blackshaw & Rodger, 2019).

3.4. Mitochondrial Replacement Therapy (Mrt)

Mitochondrial replacement therapy (MRT) is a novel technique designed to prevent the transmission of mitochondrial diseases from mother to child by replacing the affected mitochondria in the mother's egg with healthy mitochondria from a donor egg (Craven et al., 2010). MRT has sparked intense ethical debate and raised questions about the long-term consequences of altering the human germline. In the context of transhumanism, MRT can be seen as an early example of how genetic interventions at the embryonic stage could pave the way for more extensive enhancements of human traits and abilities in the future (Bostrom, 2005).

3.5. Advances in Stem Cell Research

Stem cell research has made significant strides in recent years, with the discovery of induced pluripotent stem cells (iPSCs) by Shinya Yamanaka and his team in 2006 (Takahashi & Yamanaka, 2006). These cells, which can be derived from adult somatic cells, have the potential to differentiate into any cell type in the body. As a result, iPSCs have opened up new avenues for regenerative medicine and the study of human development. In the context of transhumanism, the potential of stem cells to repair or replace damaged tissues and organs could contribute to life extension and the enhancement of human abilities (Bostrom, 2005).

3.6. Organoids and Tissue Engineering

Organoids are three-dimensional, self-organizing structures derived from stem cells that mimic the organization and function of specific organs (Clevers, 2016). These miniature organ models have been used to study organ development, disease, and regenerative medicine. Tissue engineering, which involves the use of cells, scaffolds, and biochemical factors to create functional tissues and organs, has also advanced significantly in recent years (Langer & Vacanti, 1993). In the context of transhumanism, organoids and tissue engineering could potentially be used to enhance or replace human organs, contributing to the improvement of human abilities and the extension of human life (Bostrom, 2005).

3.7. Chimeras and Interspecies Organogenesis

Chimeras are organisms containing cells from two or more different species. Recent advancements in embryology have led to the successful generation of chimeric animals, such as mice with human cells (Wu et al., 2017). Interspecies organogenesis, the process of generating functional organs from one species within the body of another species, has also been achieved using pluripotent stem cells (Kobayashi et al., 2010). These advancements have the potential to address the shortage of organs for transplantation and contribute to the development of personalized therapies. In the context of transhumanism, chimeras and interspecies organogenesis could lead to the enhancement of human abilities by incorporating beneficial traits from other species (Bostrom, 2005).

4. TRANSHUMANIST APPROACHES TO HUMAN ENHANCEMENT

Recent developments in embryology have shown great potential for furthering our understanding of human development, improving reproductive medicine, and even paving the way for genetic enhancements. Some of these advancements include gene editing techniques, stem cell research, and in vitro gametogenesis. The impact of these advancements on transhumanism cannot be understated, as they may enable us to reshape the human experience by

enhancing our biological capacities and even transforming our social and ethical landscapes (Liao, 2019; Mathews et al., 2015; Pera, 2011).

Gene editing techniques, such as CRISPR-Cas9, have revolutionized the field of genetics, enabling researchers to make precise alterations to DNA sequences (Doudna & Charpentier, 2014). This technology has the potential to correct genetic mutations associated with diseases and may also be employed to introduce desirable genetic traits. As such, CRISPR-Cas9 and other gene editing techniques could play a significant role in the transhumanist pursuit of enhancing human capacities (Mathews et al., 2015).

Stem cell research has also seen remarkable progress in recent years. Pluripotent stem cells, which can differentiate into any cell type in the body, hold immense promise for regenerative medicine, tissue engineering, and the study of early human development (Pera, 2011). Additionally, advances in induced pluripotent stem cell (iPSC) technology allow for the generation of patient-specific stem cells, which can be used to model diseases, develop personalized therapies, and potentially even replace damaged or diseased tissues (Takahashi & Yamanaka, 2006).

In vitro gametogenesis (IVG) is another emerging technology in embryology that involves the creation of functional gametes from pluripotent stem cells (Sasaki et al., 2015). This technique has the potential to revolutionize reproductive medicine by offering new fertility treatments and expanding reproductive options for individuals facing infertility or genetic risks (Liao, 2019). IVG could also have profound implications for transhumanism, as it may enable the generation of designer babies with specific genetic traits, further blurring the line between natural and artificial human enhancement.

The advancements in embryology discussed here demonstrate the potential for these technologies to reshape our understanding of human development and open new doors for human enhancement. However, it is crucial to carefully consider the ethical, social, and legal implications of these advancements as we move forward to ensure that these technologies are developed and utilized responsibly (Liao, 2019; Mathews et al., 2015; Pera, 2011).

5. ETHICAL, SOCIAL, AND LEGAL IMPLICATIONS OF EMBRYOLOGY AND TRANSHUMANISM

5.1. Equity and Access

The potential benefits of embryological advancements and transhumanist technologies raise concerns about equitable access to these enhancements. As these technologies are likely to be expensive, there is a risk that they may only be available to the wealthy, exacerbating existing social and economic inequalities (Bostrom, 2005; Daniels, 2000). To address these concerns, some have argued for the development of policies that ensure fair distribution of enhancements and address potential disparities in access (DELMAS, 2012).

5.2. Safety and Unintended Consequences

The application of new technologies in embryology and human enhancement carries potential risks, such as unintended side effects and long-term consequences that may not be apparent during initial testing (Agar, 2010). For example, genome editing technologies like CRISPR-Cas9 have raised concerns about off-target effects, which could result in unintended genetic alterations (Fu et al., 2013). Ensuring the safety of these technologies will require rigorous testing and regulatory oversight to minimize risks to individuals and society (DELMAS, 2012).

5.3. Identity and Human Dignity

The prospect of modifying human embryos and enhancing human capabilities raises questions about the nature of human identity and dignity (Bostrom, 2005). Critics argue that these interventions may undermine our understanding of what it means to be human and may lead to the devaluation of individuals who do not undergo enhancement (Kass, 2003). Proponents of transhumanism, on the other hand, argue that these technologies can help us overcome biological limitations and achieve our full potential, ultimately enriching human life and experience (Bostrom, 2005).

5.4. Informed Consent and Autonomy

The use of embryological and transhumanist technologies often involves decisions made by parents or guardians on behalf of their children, raising questions about informed consent and autonomy (DELMAS, 2012). Ensuring that individuals can make informed decisions about these technologies requires transparent communication of potential benefits, risks, and uncertainties, as well as the development of policies and guidelines that protect individual autonomy (Schaefer, Kahane, & Savulescu, 2014).

6. CHALLENGES AND POTENTIAL SOLUTIONS IN THE IMPLEMENTATION OF EMBRYOLOGY-BASED TRANSHUMANIST TECHNOLOGIES

6.1. Regulatory Challenges

The development and implementation of embryology-based transhumanist technologies pose significant regulatory challenges. Ensuring the safety and efficacy of these technologies requires the establishment of appropriate regulatory frameworks that take into account the unique ethical, social, and legal implications of these interventions (Buchanan, Brock, Daniels, & Wikler, 2000). Potential solutions may include the development of international guidelines and standards, as well as collaboration between governments, researchers, and industry stakeholders to promote responsible innovation (DELMAS, 2012).

6.2. Public Perception and Acceptance

Public perception and acceptance of embryology-based transhumanist technologies are crucial factors in determining their future development and implementation (Bostrom, 2005). Addressing concerns about the ethical, social, and legal implications of these technologies is essential to foster public trust and support for their responsible use (Caulfield, Rachul, & Zarzeczny, 2012). Potential solutions may involve public engagement initiatives, such as deliberative workshops and educational campaigns, that promote informed dialogue and facilitate public input into the development of policies and guidelines (DELMAS, 2012).

6.3. Balancing Innovation with Precaution

Striking the right balance between promoting innovation and exercising precaution is a critical challenge in the implementation of embryology-based transhumanist technologies (Bostrom, 2005). While it is essential to recognize and address potential risks, an overly cautious approach may impede the development of technologies that could offer significant benefits to individuals and society (DELMAS, 2012). A potential solution may involve the adoption of a proportionate and adaptive regulatory approach that balances the need for safety and efficacy with the desire for innovation (Buchanan et al., 2000).

6.4. Interdisciplinary Collaboration

The development and implementation of embryology-based transhumanist technologies require collaboration among diverse fields, such as biology, medicine, ethics, law, and social sciences (Bostrom, 2005). Encouraging interdisciplinary research and collaboration can facilitate the identification of potential challenges and the development of comprehensive solutions that take into account the complex interplay of scientific, ethical, and social factors (DELMAS, 2012).

7. THE FUTURE OF EMBRYOLOGY AND TRANSHUMANISM: ENVISIONING POTENTIAL SCENARIOS

7.1. Widespread Adoption of Genetic Enhancements

In a future where the ethical, social, and legal challenges of embryology and transhumanism are addressed, genetic enhancements could become widely available and integrated into healthcare systems. This scenario could result in a society where individuals have access to personalized genetic interventions that enhance their physical, cognitive, and emotional capacities, leading to improved quality of life, increased productivity, and reduced disease burden (Bostrom, 2005; DELMAS, 2012).

7.2. Enhanced Humans and the Emergence of New Social Dynamics

As embryology-based transhumanist technologies become more advanced, the distinction between enhanced and non-enhanced individuals may lead to the emergence of new social dynamics. This scenario could involve the development of new social hierarchies based on enhanced abilities, as well as potential conflicts between groups that support or reject the use of such technologies (Hughes, 2004). The challenge in this scenario would be to foster social cohesion and inclusive policies that promote equity and avoid discrimination (Bostrom, 2005).

7.3. Radical life extension and the implications for society

Advancements in embryology and transhumanism could potentially lead to radical life extension, allowing individuals to live significantly longer, healthier lives (de Grey et al., 2002). This scenario would have profound implications for society, including changes in population dynamics, resource allocation, and intergenerational relationships. Policymakers, researchers, and society as a whole would need to adapt to these changes, developing new strategies to manage the challenges and opportunities associated with extended lifespans (Bostrom, 2005).

7.4. The Convergence of Biological and Technological Enhancements

The future of embryology and transhumanism may involve the convergence of biological enhancements with other emerging technologies, such as artificial intelligence, brain-computer interfaces, and nanotechnology (Bostrom, 2005). This scenario could lead to the development of novel human-machine hybrids that challenge our traditional understanding of what it means to be human. In this context, society would need to grapple with the ethical, social, and legal implications of such hybrid entities, and develop new frameworks to address the unique challenges they pose (DELMAS, 2012).

8. CONCLUSION

The interplay between embryology and transhumanism offers promising opportunities for human enhancement, while simultaneously raising critical ethical, social, and legal challenges. Advancements in embryology have the potential to transform human life by improving health, cognition, and emotional well-being. However, these advancements also necessitate addressing the complex ethical, social, and legal implications to ensure responsible development and implementation.

The future of embryology and transhumanism is uncertain, with potential scenarios ranging from widespread adoption of genetic enhancements to the convergence of biological and technological advancements. Addressing these challenges requires interdisciplinary collaboration, thoughtful regulatory frameworks, and public engagement initiatives that promote informed dialogue and responsible innovation.

Ultimately, the future of embryology and transhumanism will be determined by the choices made by individuals, researchers, policymakers, and society as a whole. By grappling with the complex issues raised by these technologies, we can work towards a future that realizes the potential benefits of embryology-based transhumanist technologies in a manner that promotes equity, safety, and human dignity.

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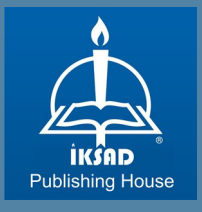

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