

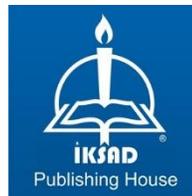
MULTIDISCIPLINARY APPROACH IN MEDICAL SCIENCE I

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PREFACE

Mankind has made observations and research in order to satisfy its curiosity since the day it existed. Today, these research studies also continue. As in every field, scientific studies continue rapidly in the fields of medicine and health sciences. Scientists are working with all their might in order to eliminate the deficiencies or to take the existing knowledge to the next level. In this way, it becomes possible to reach new information and make new discoveries in medicine.

Our book, which includes valuable and important topics, consists of 14 chapters. I fully believe that the topics in the book will contribute to our readers and our scientific community. I sincerely congratulate our writers, which put their valuable work, which they have prepared with great effort, into the service of science and humanity.

I would especially like to express my gratitude to the İKSAD Publishing family, scientific committee, authors and readers who contributed to the preparation, layout and printing of the book.

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CHAPTER 1
CLUSTER HEADACHE

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INTRODUCTION

Trigeminal autonomic cephalalgias (TACs) are a group of 4 different primary headache syndromes. They have a lot of pathophysiological and clinical features in common. Cluster headache (CH), paroxysmal hemicrania, short-lasting unilateral neuralgiform headache attacks and hemicrania continua are 4 different primary headache syndromes. TACs are characterized by frequent, strictly unilateral, very intense headache attacks with ipsilateral cranial autonomic symptoms or intrinsic restlessness or both. A distinction can be made between the 4 TACs on the basis of the duration and frequency of the headache attacks (Burish,2019).

CH is a complex disease characterized by chronic head and neck pain which often accompanied by autonomic features. Headache associated with pain in the maxillary arch and teeth is usually localized to the orbit, supraorbital and /or temporal regions. Autonomic symptoms are lacrimation (tearing), conjunctival injection (redness of the sclera), rhinorrhoea, nasal congestion, hyperhidrosis (excessive sweating) and eyelid oedema and usually occur on the ipsilateral side to the pain.

There are episodic and chronic forms of CH. Episodic CH occurs over periods from 7 days to 1 year separated by pain-free periods lasting at least 1 month. Chronic CH (CCH) occurs over the interval of more than 1 year without remission or with remissions lasting less than 1 month (Wei,2018). CH is a rare headache disorder. The incidence of CH is 0.1% (Rusell,2004). Some studies suggest that CH is an autosomal recessive and an autosomal dominant or multifactorial inheritance pattern inherited disorder (Russel,1995). Verhagen et al. reported that men with migraine and CH more often suffer from symptoms consistent with clinical androgen deficiency than males without a primary headache disorder (Verhagen,2021).

All patients with suspected CH should undergo MRI with or without contrast enhancement and with fine slices through the region of the pituitary gland to determine whether conditions are present that could cause a secondary headache with a cluster phenotype (De Pue,2016).

The symptoms of CH are similar to those of other TACs, but they can be differentiated by the duration and frequency of attacks and therapeutic response to indomethacin.

When migraine is associated with ipsilateral cranial autonomic features and when CH is associated with photophobia, phonophobia, nausea or vomiting, It can be difficult to distinguish between these two diseases. Characteristics that are consistent with side-locked migraine rather than CH are the longer duration of untreated attacks with migraine and the differences in patient activity during the attack (Lai,2009). Because secondary headaches with a cluster phenotype cannot be differentiated from primary CH based on clinical features alone, a diagnostic evaluation is required.

Although the pathogenesis of CH has not been completely elucidated, the SPG has traditionally been considered to be involved in the pathophysiology of CH (Robbins, 2016; Van Kleef,2009).

Neuroimaging studies have suggested the hypothalamus as attack generator in CH. CH involves activation of the parasympathetic outflow from the superior salivary nucleus of the facial nerve, predominantly through the sphenopalatine ganglion (SPG) (Obermann, 2018).

The SPG is located in the pterygopalatine fossa. It is suspended from the maxillary nerve by the pterygopalatine nerves, inferiorly it is connected to the greater and lesser palatine nerves, and posteriorly it is connected to the vidian nerve (Robbins, 2016). Efferent branches of the SPG form the posterior lateral nasal and pharyngeal nerves, as well as the pharyngeal branch of the maxillary nerve. There are also orbital branches reaching the lacrimal gland (Robbins, 2016; Cappello,2020). The medial wall of the pterygopalatine fossa communicates with the nasal cavity via the sphenopalatine foramen, which transmits the sphenopalatine artery, the nasopalatine nerve and the posterior superior nasal nerves (Cappello,2020).

MANAGEMENT

Pharmacological treatment of CH can be divided into acute attack abortion and prophylaxis, but most patients receive both types of treatment (Schoenen,2022).

The acute attack of CH is managed with oxygen, triptans, and verapamil (Schoenen,2022).

Preventive treatments of CH is managed with verapamil, lithium, anticonvulsants (Ekbohm, 1981; Bussone,1990; Schoenen,2022).

Topiramate (Forderreuther,2002;Huang,2010), valproic acid (Hering,1989) and gabapentin

(Schuh-Hofer,2007;Vukovic,2009) have a probable efficacy in the prophylaxis of episodic and chronic cluster headache.

Oral ergotamine has been used for the treatment of CH attacks for >50 years and is probably effective within 15 minutes if administered early in the attack. Intravenous administration was shown to abort severe attacks (Nagy,2011).

The nasal installation of lidocaine is probably effective in at least one third of patients when administered within 15 minutes of attack onset (Mills,1997).

Subcutaneous octreotide is effective in the treatment of acute attacks when given within 15 minutes of attack onset (Matharu,2004).

CCH accounts for about 10% of patients with cluster headache, and it usually lacks the circadian pattern typical of the episodic cluster. Patients with CCH are often resistant to pharmacological management.

In recent years, there have been a series of reports on the treatment of CCH via the pterygopalatine ganglion. One type of pterygopalatine ganglion treatment is SPG RFA, which blocks pain signalling by denaturing pterygopalatine ganglion proteins. The other type is pterygopalatine ganglion pulsed radiofrequency treatment (PRF) (Wei,2018;Shah ,2020; Narouze,2010; Li,2018).

Percutaneous radiofrequency ablation of the sphenopalatine ganglion (SPG RFA) is a quick and simple technique that has proven its efficacy in episodic CH, having been used in a short series of refractory chronic cluster headache with variable results (Shah,2020).

Percutaneous SPG RFA was described by Salar et al in 1987 (Salar,1987). Although the pathogenesis of CH has not been completely elucidated, the SPG has traditionally been considered to be involved in the pathophysiology of CH (Robbins,2016; Van Kleef). SPG RFA is a method used to destroy painful nerves with heat (Salgado-López,2018; Tolba,2019;Shah,2020). The SPG RFA device uses high frequency (ranges 300-500kHz) to create charged molecular oscillation which generates heat by the friction of ions and radio waves. When the needle tip heats to 80 degrees C for 60 to 90 seconds, it produces the local tissue damage and loss of myelinated fibers. This temperature reliably produces an 8-10 mm affected area(Shah,2020).

The anesthesia was administered at a level that gave comfort to both the patient and surgeon during the electrical test stimulation and lesioning procedure. Because of the vagal reflex, atropine was used for bradycardia. Nitroglycerine dermal patches were applied to patients who were predisposed to cardiac ischemia and acute hypertension.

If the position is correct, the paresthesia occurs on the endonasal level. If the maxillary nerve was stimulated, paresthesia occurs on the cheek, upper teeth, or upper lip. The needle should be repositioned caudal and medially. If major or minor palatine nerves were stimulated, the paresthesia occurs on the hard palate. The needle should be moved posterior, medial, and superiorly.

Once stimulation is achieved and prior to lesioning, 0.2-0.4 mL of contrast agent is injected under real-time fluoroscopy to rule out intranasal or intravascular spread. After stimulation and proper positioning are confirmed, 0.4 mL of lidocaine 2% is injected, and two radiofrequency lesions are carried out at 80°C for 90 seconds. After lesioning, 0.4 mL of bupivacaine 0.5% and 5 mg of triamcinolone are injected with the aim to prevent postprocedure neuritis.

The patients were typically discharged on the day of surgery. Overnight hospitalization was recommended in patients with poor medical status to observe pain alleviation and vital functions after the procedure was completed. All medications previously provided for pain control were discontinued after the patient had undergone SPG RFA.

Radiofrequency lesioning of the SPG can result in permanent or, more commonly, temporary hypesthesia or dysesthesia in the palate, maxilla, or posterior pharynx and dryness of the eye. Precise needle placement with the use of real-time fluoroscopy and electrical stimulation prior to attempting radiofrequency lesioning may reduce the incidence of adverse events.

Single-centre reports on small groups of patients have shown that SPG RFA treatment in patients with CCH can quickly relieve pain without significant side effects. However, a randomised controlled trial is still necessary to evaluate whether SPG RFA treatment is a viable treatment option for patients with CCH who are not responding to drug treatment.

Li J et al reported that pterygopalatine ganglion pulsed radiofrequency (PRF) in patients with refractory chronic cluster headache (CCHr) can quickly relieve pain without significant side effects (Li,2019). However, a randomised controlled trial is still necessary to evaluate whether PRF treatment is a viable treatment option for patients with CCHr.

Substantial progress has been achieved in the management of CH using invasive and noninvasive neuromodulation techniques, which have given new hope to patients with refractory CH. However, large sham controlled trials are scarce.

Neurostimulation of the vagal nerve, supraorbital nerve, occipital nerve and sphenopalatine ganglion, transcranial magnetic stimulation and deep brain stimulation have been investigated for the treatment of migraine and/ or CH (Vyas,2019; Nowacki,2020; Ho,2017; Schwedt, 2015). Whereas invasive methods of neurostimulation would be reserved for patients with very severe and treatment refractory migraine or CH, noninvasive methods of stimulation might serve as useful adjuncts to more conventional therapies (Farmer,2021). Jürgens TP et al reported that SPG stimulation is an effective acute therapy in 45% of patients, offering sustained effectiveness over 24 months of observation (Jürgens,2017). However, the potential utility of each type of neurostimulation has yet to be completely defined (Schwedt,2015).

Hypothalamic deep brain stimulation is a neuromodulation technique that has been used to treat drug refractory patients with CH (Leone,2001) and is based on the activation of the posterior hypothalamus during attacks (May,1998).

International treatment guidelines are available for CH (May,2006;Robbins,2016). Lademann et al. reported that the superiority of guide line adherent treatment compared with non guideline treatment in CH (Lademann,2016).

A committee of the International Headache Society published the first edition of the Guidelines for Controlled Trials of Drugs in CH in 1995. These have not been revised. Schoenenet et al. reported that an updated version of the International Headache Society Guidelines for Controlled Clinical Trials in CH is warranted (Schoenen,2022).

REFERENCES

- Burish MJ, Rozen TD(2019).Trigeminal Autonomic Cephalalgias.Neurol Clin. 37 :847-869.
- Bussone, G. et al.(1990). Double blind comparison of lithium and verapamil in cluster headache prophylaxis. Headache 30, 411–417.
- Cappello ZJ, Potts KL. (2021). Anatomy, Pterygopalatine Fossa. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing
- De Pue, A., Lutin, B. & Paemeleire, K(2016) Chronic cluster headache and the pituitary gland. J. Headache Pain 17, 23.
- Ekbom, K. (1981). Lithium for cluster headache: review of the literature and preliminary results of long-term treatment. Headache 21, 132–139.
- Farmer AD, Strzelczyk A, Finisguerra A, Gourine AV, Gharabaghi A, Hasan A, Burger AM et al.(2021). International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). Front Hum Neurosci Mar 23;14
- Forderreuther, S., Mayer, M. & Straube, A.(2002). Treatment of cluster headache with topiramate: effects and side-effects in five patients. Cephalalgia 22, 186–189.
- Hering, R. & Kuritzky(1989) A. Sodium valproate in the treatment of cluster headache: an open clinical trial. Cephalalgia 9, 195–198.
- Ho KWD, Przkora R, Kumar S.(2017). Sphenopalatine Ganglion: Block, Radiofrequency Ablation and Neurostimulation - A Systematic Review. J Headache Pain 18:118.
- Huang, W. Y., Lo, M. C., Wang, S. J., Tsai, J. J. & Wu, H. M.(2010) Topiramate in prevention of cluster headache in the Taiwanese. Neurol. India 58, 284–287.
- (IHS) HCCotIHS.(2018). Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. Cephalalgia.38: 1–211.
- Jürgens TP, Barloese M, May A, Láinez JM, Schoenen J, Gaul C, Goodman AM, Caparso A, Jensen RH. (2017).Long-term effectiveness of sphenopalatine ganglion stimulation for cluster headache. Cephalalgia. Apr;37(5):423-434.

- Lademann, V., Jansen, J.-P., Evers, S. & Frese, A.(2016). Evaluation of guideline-adherent treatment in cluster headache. *Cephalalgia* 36, 760–764.
- Lai, T.-H., Fuh, J.-L. & Wang, S.-J.(2009). Cranial autonomic symptoms in migraine: characteristics and comparison with cluster headache. *J. Neurol. Neurosurg. Psychiatry* 80, 1116–1119.
- Leone, M., Franzini, A. & Bussone, G.(2001).Stereotactic stimulation of posterior hypothalamic gray matter in a patient with intractable cluster headache. *N. Engl. J. Med.* 345, 1428–1429.
- Li J, Ren H, Wang B, Wu D, Luo F. (2019). Multicentre, prospective, randomised, controlled, blinded-endpoint study to evaluate the efficacy and safety of pterygopalatine ganglion pulsed radiofrequency treatment for cluster headache: study protocol. *BMJ Open* 23;9(3):e026608.
- Loomba V, Upadhyay A, Kaveeshvar H.(2016). Radiofrequency Ablation of the Sphenopalatine Ganglion Using Cone Beam Computed Tomography for Intractable Cluster Headache. *Pain Physician* Sep-Oct 19(7):E1093-6. PMID: 27676681
- Matharu, M. S., Levy, M. J., Meeran, K. & Goadsby, P. J.(2004). Subcutaneous octreotide in cluster headache: randomized placebo-controlled doubleblind crossover study. *Ann. Neurol.* 56, 488–494.
- May, A. et al (2006). EFNS guidelines on the treatment of cluster headache and other trigeminal-autonomic cephalalgias. *Eur. J. Neurol.* 13, 1066–1077.
- May, A., Bahra, A., Büchel, C., Frackowiak, R. S. J. & Goadsby, P. J.(1998). Hypothalamic activation in cluster headache attacks. *Lancet* 352, 275–278.
- Mills, T. M. & Scoggin, J. A.(1997). Intranasal lidocaine for migraine and cluster headaches. *Ann. Pharmacother.* 31, 914–915.
- Nagy, A. J., Gandhi, S., Bhola, R. & Goadsby, P. J.(2011). Intravenous dihydroergotamine for inpatient management of refractory primary headaches. *Neurology* 77, 1827–1832.
- Narouze S.(2010). Role of Sphenopalatine Ganglion Neuroablation in the Management of Cluster Headache. *Curr Pain Headache Rep*14: 160-3.
- Narouze S, Kapural L, Casanova J, Mekhail N.(2009). Sphenopalatine Ganglion Radiofrequency Ablation for the Management of Chronic Cluster Headache. *Headache* 49: 571-7.

- Nowacki A, Schober M, Nader L, Saryyeva A, Nguyen TK, Green AL, Pollo C, Krauss JK, Fontaine D, Aziz TZ. (2020). Deep Brain Stimulation for Chronic Cluster Headache: Meta-Analysis of Individual Patient Data. *Ann Neurol.* 88: 956-969.
- Obermann M, Holle D, Nagel S.(2018). Functional Neuroimaging in Trigeminal Autonomic Cephalalgias. *Ann Indian Acad Neurol.*21(Suppl 1):S51-S56.
- Robbins MS, Robertson CE, Kaplan E, Ailani J,Charleston L, Kuruvilla D, et al. (2016). The sphenopalatine ganglion: anatomy, pathophysiology, and therapeutic targeting in headache. *Headache.* 56: 240-58.
- Robbins, M. S., Starling, A. J., Pringsheim, T. M., Becker, W. J. & Schwedt, T. J. (2016) Treatment of cluster headache: the American Headache Society evidencebased guidelines. *Headache* 56, 1093–1106.
- Russell M. B(2004). Epidemiology and genetics of cluster headache. *Lancet Neurol.* 3, 279–283.
- Russell, M. B., Andersson, P. G., Thomsen, L. L. & Iselius, L.(1995). Cluster headache is an autosomal dominantly inherited disorder in some families: a complex segregation analysis. *J. Med. Genet.* 32, 954–956.
- Salar G, Ori C, Iob I, Fiore D. (1987). Percutaneous thermocoagulation for sphenopalatine ganglion neuralgia. *Acta Neurochir.* 84: 24-8.
- Salgado-López L, de Quintana-Schmidt C, Belvis Nieto R, Roig Arnall C, Rodríguez Rodríguez R, Álvarez Holzapfel MJ, Molet-Teixidó J.(2019). Efficacy of Sphenopalatine Ganglion Radiofrequency in Refractory Chronic Cluster Headache *World Neurosurg.* Feb;122:e262-e269.
- Schoenen J, Snoer AH, Brandt RB, Fronczek R, Wei DY, Chung CS, Diener HC, Dodick DW, Fontaine D, Goadsby PJ, Matharu MS, May A, McGinley JS, Tepper SJ, Jensen RH, Ferrari MD; Guidelines of the International Headache Society for Controlled Clinical Trials in Cluster Headache; IHS Standing Committee for Clinical Trials; IHS cluster headache trial guideline subcommittee.*Cephalalgia.*2022 Oct 21:3331024221120266.. Online ahead of print.PMID: 36268950
- Schuh-Hofer, S., Israel, H., Neeb, L., Reuter, U. & Arnold, G.(2007) The use of gabapentin in chronic cluster headache patients refractory to first-line therapy. *Eur. J. Neurol.* 14, 694–696.

- Schwedt TJ, Vargas B. (2015). Neurostimulation for Treatment of Migraine and Cluster Headache. *Pain Med.* Sep;16(9):1827-34.
- ShahRJ, Dixon B, Padalia D.(2020). Sphenopalatine Ganglion Radiofrequency Thermocoagulation In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; Jan–.2020 Feb 13.
- Tolba R, Weiss AL, Denis DJ.(2019) Sphenopalatine Ganglion Block and Radiofrequency Ablation: Technical Notes and Efficacy *Ochsner J Spring* 19:32-37.
- Van Kleef M, Lataster A, Narouze S, Mekhail N,Geurts JW, Van Zundert J. (2009).Evidence-based interventional pain medicine according to clinical diagnoses. *Cluster headache. Pain Pract.* 9: 435-42.
- Verhagen IE, Brandt RB, Kruitbosch CMA, MaassenVanDenBrink A, Fronczek R, Terwindt GM.J(2021).Correction to: Clinical symptoms of androgen deficiency in men with migraine or cluster headache: a cross-sectional cohort study.*Headache Pain.* Nov 9;22(1):135.
- Vukovic, V., Lovrencic-Huzjan, A., Budisic, M. & Demarin, V.(2009). Gabapentin in the prophylaxis of cluster headache: an observational open label study. *Acta Clin. Croat.* 48, 311–314.
- Vyas DB, Ho AL, Dadey DY, Pendharkar AV, Sussman ES, Cowan R, Halpern CH.(2019). Deep Brain Stimulation for Chronic Cluster Headache: A Review. *Neuromodulation.* 4: 388-97.
- Wei DY, Yuan Ong JJ, Goadsby PJ. (2018) .Overview of Trigeminal Autonomic Cephalalgias. Nosologic Evolution, Diagnosis, and Management. *Ann Indian Acad Neurol* 2: 539-44.

CHAPTER 2

Therapeutic usage of Mesenchymal Stem Cells (MSCs) in Ophthalmology

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INTRODUCTION

Mesenchymal stem cells (MSCs) are regarded as multi-potent fibroblastic cells originally isolated from bone marrow. Following, MSCs have been isolated from other tissues, such as adipose tissue, oral tissue, cord blood, dental pulp and they differentiate into multiple cell types. Recently, MSCs have many functions, from tissue repair, regeneration to immune modulation. Thus, MSCs have drawn attention in cell-based therapies and regenerative medicine for many years.

Regenerative and immunomodulatory mechanisms of mesenchymal stem cells Tissue repair and regeneration

Tissue regeneration is one of the most important role of MSCs. During tissue repair, pro-inflammatory and chemotactic factors are released from damaged cells and they increase the immigration and activation of immune cells. Pro-inflammatory mediators release both directly from damaged tissues and activated immune cells and lead to the homing of MSCs to the site of injury. The homing and tissue regenerative capacities of MSCs are still investigated. There are two possible mechanism. One of them may be through paracrine modulation of the tissue microenvironment other way is transdifferentiation. The paracrine factors produced by MSCs are discrete, and the tissue repair mechanism are complex. MSCs have been released a variety of growth factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF)-1, transforming growth factor (TGF- β), fibroblast growth factor (FGF), angiopoietin-1 and stromal cell-derived factor (SDF-1). These growth factors cause to the proliferation of endothelial cells, fibroblasts, macrophages and other tissue-intrinsic progenitor cells. Although, MSC-associated factors remain partly unidentified, the transplantation of stem cells for their paracrine tissue regenerative effects is a feasible treatment option.

Immunomodulatory ability

One of many amazing functions of MSCs is that it can regulate the recipient immune response by modulating the maturation and the function of multiple immune cells. According to Ren et al., MSCs home to inflamed microenvironments where, upon arrival, their immunosuppressive function is induced. Thus, the MSC immunoregulatory function is understood as adjustable; it is amplified in response to high concentrations of specific inflammatory cytokines, and reduced in their absence. Given that the local cytokine neighbourhood varies considerably during disease progression, the immunoregulatory role of MSCs may fluctuate correspondingly.

MSCs have a capacity that can be used to control proliferation and function of T cells, B cells and NK cells, also can be used to decrease production of cytokines. IL-6, IL-10, and TGF- β are the examples of immunosuppressive factors secreted by MSCs. Other factors involved in MSC-induced immune tolerance include indoleamine 2,3-dioxygenase and Fas ligand. MSCs require preliminary activation through exposure to IFN gamma. Recently, it has been suggested that the immune-suppression effects of MSCs are modulated by toll-like receptor signalling. By contrast, TLR4-stimulation resulted in expression of pro-inflammatory mediators, collagen deposition and a reversal of the suppressive mechanisms of T-cell activation. However, differences between animal and human immune systems should be considered, benefits seen in animal studies may not work for humans. As a summary, the immunomodulatory activity of MSCs is known to be contingent on their origin, their microenvironment and their target cells.

Under light of this knowledge, we look at to the therapeutic potential of MSCs in ocular surface and retina.

MSCs-based therapies for ocular surface disease

Corneal wound healing after injury

In corneal stroma, long-standing inflammatory activity cause to the differentiation of transparent corneal fibroblasts into opaque myofibroblasts, which finally synthesize disorganized extracellular matrix and the loss of corneal clarity. Lan et al. have demonstrated the capability of MSCs to home to injured corneal tissue. The investigators showed that endogenous MSCs are activated into blood following corneal injury, and that exogenously applied MSCs home to the damaged cornea using thermal cauterization in a murine model. Intravenously injected *ex vivo* expanded red Q-dot-labeled or GFP+ bone marrow-derived MSCs were demonstrated to home to the injured cornea but not to the normal cornea with epifluorescence microscopy. Crucially, long-term survival of these labeled MSCs, at 50 days following injury, were established by the authors. Contrastly, Roddy et al. indicated that MSC-derived TSG-6 which referred to the anti-inflammatory activity of MSCs, were observed in injured rat corneas at 1 day or 3 days after i.v. or i.p. administration of hMSCs in a rat corneal injury model. These contrary datas may be associated with the potential issues of integration and engraftment when cells are transferred across the xeno-species, with the use of human MSCs in a rat model of corneal injury. The specific locating of murine MSCs has been demonstrated by Omoto et al. in a murine model of corneal transplantation, in which MSCs were found in wideness at the transplanted cornea, but not in contralateral tissues at 3 days post transplantation. An inflammatory neighborhood promotes the secretion of HGF by MSCs, and moreover it was demonstrated that the ability of MSCs to restore corneal transparency is dependent on their HGF expression in a murine model of mechanical corneal injury. Additionally, topical administration of HGF alone (without MSCs) has been shown to restore corneal transparency after injury, thus an important paracrine mechanism by which MSCs contribute to the repair

of injured corneal tissue was characterized. MSC paracrine pathways at the cornea have been highlighted in further studies. In a murine model of mechanical corneal injury, the researchers demonstrated that application of fibrin gel containing stem cells derived from human corneal stroma reduced the infiltration of neutrophils and myeloperoxidase expression. Furthermore, a cell-cell contact in MSC immunoregulation was reported as co-culture of MSCs and neutrophils was discovered to suppress neutrophil expression of the tissue-damaging enzymes myeloperoxidase. Also, it was demonstrated that treatment with MSCs resulted in decreased myeloperoxidase expression, lower neutrophil frequencies at ocular surface and normalization of corneal tissue structure compared to untreated injured controls in a murine model of mechanical corneal injury. These results indicate that MSCs limit tissue damage by suppressing neutrophil effector functions with direct cell-cell contact during corneal wound healing.

Basu et al. enlarged mesenchymal cells from limbal biopsies of human cadaveric cornea-scleral rims (limbal biopsy-derived stromal cells; LBSCs), and engrafted these cells into murine corneas following stromal injury. Fifty thousands of LBSCs were applied to the wounded cornea in a solution of fibrinogen at the time of mechanical debridement of epithelium and stroma. Although the number of LBSCs in the cornea reduced over time, half of the engrafted LBSCs remained in situ at 4 weeks. The researchers showed that LBSCs decreased the constitution of opaque scar tissue and cooperate regeneration of the damaged stroma, so the potential of autologous stem cell-based approaches for the treatment of corneal opacification was demonstrated.

The ability of MSCs to build up stromal transparency investigated in Lumican-null mice. Lumican-null mice have congenitally cloudy corneas due to defective collagen fibril and disorganized stromal structure. Du et al. demonstrated that injection of

50,000 MSCs derived from adult human corneal stroma into the corneas of Lumican-null mice reduced stromal thickness and haze. Also, Lumican was distributed throughout the posterior stroma, and regular collagen structure was seen without fibril aggregates after injection of human corneal stromal stem cells at twelve weeks post-injection. Interestingly, it was shown that, a unique gene expression of lumican and keratocan could be achieved in those MSC injected mice. Another study using a penetrating corneal trauma model in rats, demonstrated that MSCs derived from bone marrow and adipose tissue of rats could survive in corneal tissue following injection into the anterior chamber. Also after 8 weeks, the gene expression pattern of these cells was similar to that of corneal keratocytes, with the cells expressing the cornea-specific proteoglycan keratocan, aldehyde dehydrogenase (ALDH) and CD34. And in that study, it was shown that the keratocyte density had been increased in the MSCs injected groups when compared controls in *in vivo* confocal microscopy.

Chemical injuries

Corneal chemical burns are the most common eye injury and require urgent evaluation and treatment in order to maintain corneal clarity. Acid burns such as hydrofluoric and sulfuric acids usually deteriorate, clot and sediment corneal protein after contact. This will put a barrier to prevent advanced penetration of the acid to deeper of the cornea. But alkali injuries, such as sodium hydroxide, ammonium hydroxide, penetrate the cornea via membrane lipid saponification and collagen matrix denaturation. The injured tissue progress to liquefactive necrosis, which initiates proteolytic enzyme release leading to further damage. Levels of antioxidant enzymes decrease and antioxidant/pro-oxidant imbalance occurs in the impaired corneal epithelium. It has been showed that MSCs protect antioxidant properties that significantly suppress corneal inflammation and neovascularization. In studies using an alkali burned rabbit cornea model, rabbit bone marrow-MSCs were

found to promote corneal re-epithelialisation, suppress inflammation, restore antioxidant balance and reduce neovascularization.

MSCs derived from different sources have been revealed to have therapeutic functions attributing soluble paracrine factors, immunosuppressive and immunomodulatory roles. MSCs' anti-inflammatory role was found to be the key factor in corneal regeneration. In many animal models, subconjunctivally injected MSCs reduced corneal oedema, opacity and neovascularization. Also, this was found in an association with an increased level of the anti-inflammatory cytokine TGF- β and decreased expression of proinflammatory cytokine including TNF- α . Also VEGF and MMP-2 expression decrease and anti-angiogenic cytokine Thrombospondin-1 (TSP-1) expression upregulate which results in reduced corneal neovascularization. A study used BM-MSCs grown on a nanofiber scaffold on the rabbit cornea demonstrated reduction in the inflammatory response and neovascularization by a decrease in the number of CD3+ cells, iNOS expression and caspase-3 levels in the cornea, resulting in reduced corneal opacity. Interestingly, it was found that MSCs' efficiency can seem with and/or without cell engraftment. Based on the knowledge the therapy with MSCs at corneal chemical burns can support and enhance healing. There is no adequate evidence of which mechanism is more superior.

Dry eye Syndrome

Ocular surface inflammation is the main cause of DES. In animal studies, the authors found MSCs reduced inflammation and improved tear production. Also, MSCs were shown to home within the conjunctival epithelium and Meibomian glands. But, it was not fixed whether this function was adjusted by topically injected MSCs' differentiation and production of trophic factors. In another study with a dry eye model in rats reported that a one-week topical application of MSCs increased aqueous tear volume and got improvement in ocular

surface evaluation tests. Also it was demonstrated that topical application of MSCs can decrease inflammation by their anti-inflammatory effects.

Limbal deficiency syndrome

LSCD is the loss of the stem cells located at the limbus, which hinder epithelial cells' natural regenerative capacity. In LSCD, corneal epithelium deteriorates and conjunctivalisation comes forward and it results loss in transparency of cornea, scarring and blindness. Autologous limbal grafts can be transplanted from the healthy eye to restoration of corneal transparency successfully in a unilateral LSCD patients. But, patients with bilateral LSCD can only get an allograft limbal transplantation from a donor which may reduce its success. MSCs gained attention in the treatment of LSCD due to their anti-inflammatory and transdifferentiating effects, irrespective of their origin. Dental pulp-derived MSCs grown on amniotic membrane improved corneal transparency in a rabbit LSCD model and showed well distributed epithelial cell layer. In animal models with LSCD showed a reduction in inflammation but the evidence whether MSCs transdifferentiated to epithelial cells was controversial.

Ocular graft-versus-host disease

Ocular GVHD causes inflammation of the ocular surface and lacrimal glands and goes to cicatricial damage of the conjunctiva, meibomian glands and finally cornea. It develops nearly 40–60% of patients undergoing allogeneic hematopoietic stem cell transplantation. In a murine model of GVHD, the therapeutic effect of subconjunctival transplantation of MSCs were investigated. The authors reported that GVHD increased infiltration of CD3⁺ T cells into the cornea and this effect was disappeared after treatment with MSCs. Also it was found that subconjunctival administration of MSCs decreased Pax6 expression which contributes to development of keratinizing squamous metaplasia in GVHD mice. So, it was suggested that MSCs might

suppress ocular surface inflammation, inhibit squamous metaplasia and provide corneal clarity in ocular GVHD.

Allergic Conjunctivitis

The pathogenesis of allergic conjunctivitis is predominantly an IgE-mediated hypersensitivity reaction. Activation of mast cells induces enhanced tear levels of histamine, tryptase, prostaglandins and leukotrienes. Su et al. reported that topical administration of MSCs were effective in decreasing IgE production, histamine release, enrichment and deactivation of mast cells in a murine model of experimental allergic conjunctivitis. Besides, treatment with MSCs was showed reduced clinical inflammation scores (scored by slit lamp examination of conjunctival edema, redness, lid swelling, tearing). Also, infiltration of inflammatory cells and eosinophil accumulation in the MSCs treatment group was decreased on histological examination of conjunctival tissue. Analysis of conjunctival tissue using real-time PCR and ELISA showed increased expression of the inflammatory cytokines TNF- α and IL-4 in mice with experimental allergic conjunctivitis, but this effect was decreased by treatment with MSCs. Further research is required to detail the mechanisms by which TNF- α -stimulated, MSCs apply their antiallergic effects, but researches provide valuable evidence that MSCs have regenerative properties in this setting.

Mesenchymal Stem Cells in Retinal degenerative diseases

In the retina, various cell types, such as photoreceptor cells, bipolar cells, horizontal cells, amacrine cells and retinal ganglion cells (RGCs) play an essential role in the transformation process of light signals to electrical signals. Photoreceptor cells are two types -rods and cones- in the human retina. Cones are concentrated in the macula, which provides high-resolution central vision and rods are located mainly in the peripheral retina. Bipolar cells transport light signals from photoreceptor cells and to RGCs. Horizontal and amacrine cells are

responsible for coordinating this transport. Ultimately, all the signals are collected in the optic nerve and transmitted to the brain. Any cell degeneration in this route and synaptic interruption can cause permanent visual defect or blindness.

Retinal degenerative diseases are characterized by retinal cell loss, such as RPE and/or photoreceptor cell loss in AMD and retinitis pigmentosa (RP), and RGC death in glaucoma. Retinal cells don't have self-repair ability so degenerating of any retinal cells cause irreversible impairment of visual acuity. After transplantation of autologous RPE from the peripheral retina into the submacular space in patients with macular degeneration provided an improvement of visual acuity and after that it was suggested RPE transplantation might be a future therapeutic modality for retinal degenerative diseases. However, this treatment option is limited by a shortage of cell sources and by genetic defects in patients' autologous cells. Instead of this, SCs thought to be a good cell source.

Pluripotent stem cells

Currently, there is no ideal stem cell, however various types of stem cells have been explored as potential therapy for retinal dysfunction. Pluripotent stem cells, such as embryonic stem cells and induced pluripotent stem cells, have unlimited potential to differentiate and expand. So, they have some of the ideal features of stem cells for retinal regeneration and these pluripotent stem cells have received a lot of attention in recent years. However, these cells have not been explored in clinical trials since more long-term preclinical safety studies are needed.

Besides, preclinical studies have shown that pluripotent stem cells can be differentiated into cells expressing features of retinal pigment epithelial (RPE) cells, retinal progenitor cells and retinal ganglion cells. However, these cells do not incorporate into the retina following intravitreal injection. The RPE cells derived from embryonic

stem cells and induced pluripotent stem cells had been transplanted into the subretinal space and shown to slow down retinal degeneration in animal models. Thus, this is the route of delivery being explored in clinical trials.

Mesenchymal stem cells

The transplantation of MSCs derived from both adipose tissue and bone marrow has neuroprotective effects in RGC degenerative animal models. In vitro studies showed that mesenchymal stem cells can gain features of RPE cells. Mesenchymal stem cells were reported to differentiate into photoreceptors, RPE, and express neuronal markers following local or systemic administration in animal studies of retinal degeneration and retinal ischemia but whether the study findings represent true differentiation or fusion with pre-existing photoreceptors is unclear. Many reports declared a protective effect of subretinal injection of mesenchymal stem cells in animal models of retinal degeneration even though the incorporation of these mesenchymal stem cells into the retina itself was limited. Rescue of retinal degeneration was reported following intravitreal injection of MSCs, but the effect was less prominent and shortlived considered that noted after subretinal cell injection. In addition, the intravitreally transplanted MSCs formed cellular clusters in the vitreous cavity. Protective effects on retinal degeneration have also been observed following injection of bone marrow derived MSCs intravenously in a rat model. In an animal model of retinal ischemia-reperfusion injury, intravitreal administration of MSCs had a protective effect on ganglion cell loss, and the cells were noted to secrete neurotrophic growth factors for at least 4 weeks following administration. In animal models of diabetic retinopathy, bone marrow derived MSCs were found to integrate into the inner retina, stimulate retinal gliosis and improve ERG amplitude. However, long-term studies of intravitreal and subretinal transplantation of human bone marrow- derived MSCs noted that some of these human

cells integrated into other ocular structures and passed through the blood-retinal barrier to migrate into non-target tissue (choroid), raising some long-term safety concerns.

The route delivery for MSCs also affects the survival time the therapeutic effect of MSCs in host tissue. In subretinal transplantation, retinal function can be significantly better for up to 20 weeks, whereas this improvement can be found for only up to 12 weeks in intravitreally injected eyes when compared controls. In animal models with retinitis pigmentosa, transplantation of bone marrow-derived MSCs can protect ONL cells and prolong photoreceptor survival. Thus, subretinal injection of MSCs may benefit both RGCs and photoreceptor cells, but the strength and duration of the protective effect is unknown. Several possible mechanisms related to protective effect of MSCs transplantation have been described. Those characteristics of MSCs have been linked to the paracrine effects which exerted by the transplanted cells. Local injection of MSCs had neuroprotective effects for different models of retinal degeneration. It is showed that MSCs can express a variety of factors which could protect damaged retina, such as NGF, BDNF, CNTF, IGF1 and bFGF.

Also it was found that MSC transplantation revealed protective effects on optic nerves in a model of glaucoma by directly protecting RGCs and their axons. They speculated that the most likely mechanism for the protection was through the secretion of NTFs by the transplanted cells. Moreover, other paracrine factors produced by MSCs have been linked to anti-inflammatory, immunomodulatory collateral perfusion and neovascularization processes in the ischemic region. However, whether paracrine effect of MSCs is a key mechanism in host retinal cell protection or rescue is still controversial. No doubt that further studies of interactions between MSCs and host tissue may accumulate datas, either from the perspective of understanding the therapeutic mechanism of stem cell or the broad range of application.

Age-related macular degeneration (AMD) and Stargardt's disease (SD)

AMD is a degenerative disease and has a complex pathogenesis includes several genetic and environmental factors. Geographic atrophy and choroidal neovascularization (CNV) occur in advanced form of the disease. Finally, degradation of the retinal pigment epithelium layer and Bruch's membrane, the basement membrane, loss of photoreceptors take place. Damaged RPE layer fails to phagocytose the photoreceptor outer segments and insufficient phagocytosis causes to collecting of a lysosomal protein lipofuscin, which interferes with the proper functioning of the RPE layer. The accumulating cell debris between the RPE layer and Bruch's membrane called as drusen causes RPE detachment which progress towards CNV. CNV is characterized as abnormal leaky capillaries across the choroid to retina that cause to fluid accumulation and hemorrhage at the macula. Stargardt's disease (SD) occurs within the first two decades of human life and is characterized by hereditary macular degeneration. The most common form of this disease involves mutation in the ABCA4 (ATP-binding cassette, subfamily A , member 4) gene, the dysfunction of which causes accumulation of N-retinylidene-N-retinyl-ethanolamine, a major component of lipofuscin, which has a detrimental effect on RPE and photoreceptor cells. Researchers found that subretinal transplantation of human embryonic stem cell-derived RPE cells was well tolerated in AMD and Stargardt's disease. Although iPSCs have drawn attention in preclinical and clinical researches, autologous transplantation of human iPSCs derived RPE cells showed no significant visual improvement in the patients with AMD.

Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is the most common hereditary retinal disease and characterized by progressive photoreceptor loss. At the beginning of the disease destruction of the rod photoreceptors occurs

and cause to loss of night vision and limited peripheral vision. As the disease progress to later stages degeneration of cones cause to loss of central and color vision. The degeneration of photoreceptors in RP is usually associated with gene mutations and ~4500 mutations have been determined in 70 genes up to date. Since, gene replacement through adeno virus vector have resulted in improvement in retinal function in RP models as well as human patients. The first in vivo gene therapy to be approved by Food and Drug Administration (FDA) for RP is, Luxturna. FDA also approved transplantation of an artificial retina which succeeded in recovery of vision in late-stage RP patients. Treatment with MSCs has supported the survival of photoreceptors and showed therapeutic benefits. For example, the transplantation of MSCs to the eyes of rd1 and rd10 mice provided a rescue effect for retinal cells. The application of genetically modified MSCs with an overexpression of BDNF resulted in enhanced antiapoptotic signaling in the retina, and in a reduction of cell damage in the rd6 mouse. Furthermore, the donor cells integrated into the outer retinal layers preferentially. In addition, the combined transplantation of the human retinal progenitor cells and BM-MSCs into the subretinal space provided an effective immunomodulation in the eye of RCS rats and prevented pathological changes more effectively than with a single therapy.

Diabetic Retinopathy

DR is a multifactorial microvascular disease induced by. Abnormal metabolic events triggered by chronic hyperglycemia cause to overproduction of reactive oxygen species (ROS). Loss of pericytes, endothelial cells and neuronal cells in the retina in early or nonproliferative stage of DR (NPDR) and it results in pro-angiogenic and inflammatory responses, forming intra-retinal vasculature abnormalities and hemorrhages in the proliferative stage of DR (PDR). Since PDR characterized by abnormal neovascularization, standard

treatment methods intend to inhibit uncontrolled angiogenesis by anti-VEGF administration. Ezquer et al. showed that the local administration of Mouse MSCs prevented the loss of retinal ganglion cells in diabetic mice. Also levels of neurotrophic factors, such as NGF, bFGF and GDNF were increased in the eyes treated with MSCs. Although donor MSCs were found integrated into the host retina, the differentiation of MSCs into retinal cells wasn't observed. In other studies, the intravitreal administration of human umbilical cord derived-MSCs attenuated capillary damage in streptozotocin-induced DR and increased levels of BDNF and NGF in the treated eyes. Donor MSCs also restored the visual function measured by ERG. Yang et al. showed that the treatment with human MSCs improved the integrity of the blood-retinal barrier and improved DR in diabetic rats. Slightly enhanced levels of BDNF in the retina were also achieved after the administration of neural stem cells differentiated from umbilical cord MSCs, thus suggesting that this type of cells originated from MSCs may represent a suitable option for neuroprotection in DR. It has been shown that the treatment of BM-MSCs from mice with streptozotocin-induced diabetes with Wharton's jelly extract (containing a number of growth factors and other cytokines) significantly improved their proliferative abilities and therapeutic potential. It suggests that the preconditioning of diabetic MSCs could improve their therapeutic properties.

Clinical Trials with MSCs for retinal diseases

Attempts at treatments for cone-rod dystrophy were probably part of the first scientific programs seeking a treatment method for RP. In 2005, researchers from Brazil (clinical trial number: NCT01068561) have investigated the use of autologous stem cells to treat RP. In this study, 107 autologous mononuclear cells isolated from the bone marrow were injected into the vitreous cavity of five patients. No side effects associated with cell application were detected, but unfortunately

the therapeutic effect of the cell therapy was not confirmed. Most of the cells used in the treatment, however, were hematopoietic cells, and according to various sources, MSCs constitute only 0.02–0.0017% of all mononuclear cells in bone marrow aspirate. Therefore, the lack of a therapeutic effect in this study may be due to an insufficient number of MSCs. And secondly, autologous MSCs used in this study could have the same genetic defect as the patients' retinal cells, and thus they would not have positive and long-lasting effects.

In SCOTS (Stem cell ophthalmology treatment study) and SCOTS 2 (clinical trial NCT01920867), seventeen patients with bilateral visual loss due to Retinitis Pigmentosa (RP) underwent autologous bone marrow derived MSCs treatment. According to the results, meaningful visual acuity improvements (all eyes capable of LogMAR vision showed an average of 31% improvement in vision over baseline) or stability in RP that were of statistical significance, duration of disease did not appear to affect the ability of eyes to respond and safety was confirmed at 6 months follow-up. However, Satarian et al reported that intravitreal injection of autologous BMSCs in three patients suffering from advanced RP, resulted in improvement in visual acuity in only two of the patients. The third patient developed vitreal and pre-retinal fibrosis two weeks after transplantation which progress to total tractional retinal detachment at the end of the three months follow-up period.

A prospective, nonrandomized clinical study (ChiCTRONC-16008055) analyzed the safety and effectiveness of intravenous injection of autologous BMSCs in patients with diabetic retinopathy. The study included 10 patients with severe NPDR and 7 patients with PDR. In results, only eyes in the NPDR group had the macular thickness reductions and a significant improvement in BCVA from baseline, while those in the PDR group did not at 6 months follow-up time.

In two separate clinical trials involving 12 AMD patients, Limoli et al transplanted autologous adipose-derived stem cells (ADSCs) from the stromal vascular fraction of the adipose tissue along with platelets obtained from platelet-rich plasma and adipose stromal cells of the orbital fat in the subcleral space. The study reported a significant improvement in retinal functionality as observed by increased electroretinogram values with no adverse effects. Six months follow up revealed that 19 out of 36 (52.78%) eyes exhibited better vision, 14 eyes (38.89%) showed no change in functionality, and three eyes (8.33%) got worsened. The eyes which have thicker macula prior to the treatment were seen to show greater improvement in vision and thus, high number of residual cells can lead to more interaction with paracrine factors secreted by ADSCs and chorio-retinal cell membrane receptors, allowing improving in vision quality.

In Phase I clinical trial on 4 Asian patients with traumatic optic neuropathy underwent sub-tenon transplantation of human placenta-derived MSCs (PD-MSCs). It was found safe without any adverse inflammatory or proliferative side effects. PD-MSCs had a protective effect on RGCs, rescued the expression of Tuj1 and GFAP, which was concurrent with improved visual acuity.

Oner et al tested the safety and efficacy of subretinal implantation of ADSCs in 11 patients suffering from end-stage RP and found neither improvement nor adverse effects in most of the patients. However, ocular complication was observed in 5 patients and one patient suffered from CNV. In another phase II study conducted with patients with dry AMD (4 patients) and Stargardt's macular dystrophy (4 patients), Oner et al found an improvement in visual acuity, visual field and multifocal electroretinography (mf-ERG) readings after suprachoroidal ADSCs transplantation. And no ocular or systemic complications were occurred in these patients during the 6 month follow up.

An open label, phase III clinical trial (NCT04224207) recently reported by Özmert and Arslan. In this study, Wharton gel derived-

MSCs. were injected in the sub-tenon space in 32 patients diagnosed with RP. A significant enhancement in mean BCVA, outer retinal thickness values and mf-ERG results and decrease in the visual field mean deviation value was reported in the 6 month follow up period. Any severe ophthalmic or systemic complication wasn't reported.

The use of AD- and BM-derived MSCs to treat certain ocular diseases is scientifically proven and evidence-based. Applying techniques are simple and reproducible, so this will provide a quick and effective introduction into routine clinical practice. Thus, ongoing scientific research and activities to introduce MSCs into a clinical practice offer great opportunities for cell-based therapy and highlight an essential role of MSCs in the evolution of medicine.

Bibliography

- Basu, S., Hertszenberg, A.J., Funderburgh, M.L., Burrow, M.K., Mann, M.M., Du, Y., Lathrop, K.L., Syed-Picard, F.N., Adams, S.M., Birk, D.E., Funderburgh, J.L. (2014). Human limbal biopsy-derived stromal stem cells prevent corneal scarring. *Science Translational Medicine*, Dec 10;6(266):266ra172.
- Camargo, F.D., Green, R., Capetenaki, Y., Jackson, K.A., Goodell, M.A. (2003). Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nature Medicine*, (9):1520-1527.
- Demirayak, B., Yüksel, N., Çelik, O.S., Subaşı, C., Duruksu, G., Unal, Z.S., Yıldız, D.K., Karaöz, E. (2016). Effect of bone marrow and adipose tissue-derived mesenchymal stem cells on the natural course of corneal scarring after penetrating injury. *Experimental Eye Research*, (151):227-35.
- Du, Y., Carlson, E.C., Funderburgh, M.L., Birk, D.E., Pearlman, E., Guo, N., Kao, W.W., Funderburgh, J.L. (2009). Stem cell therapy restores transparency to defective murine corneas. *Stem Cells*, 27(7):1635-42.
- Ezquer, F., Ezquer, M., Arango-Rodriguez, M., Conget, P. (2014). Could donor multipotent mesenchymal stromal cells prevent or delay the onset of diabetic retinopathy? *Acta Ophthalmologica*, 92(2):e86-95.
- Hertszenberg, A.J., Shojaati, G., Funderburgh, M.L., Mann, M.M., Du, Y., Funderburgh, J.L. (2017). Corneal stromal stem cells reduce corneal scarring by mediating neutrophil infiltration after wounding. *PLoS One*. 12(3):e0171712.
- Holan, V., Palacka, K., Hermankova, B. (2021). Mesenchymal Stem Cell-Based Therapy for Retinal Degenerative Diseases: Experimental Models and Clinical Trials. *Cells*. 10(3):588.
- Jin, Z.B, Gao, M.L., Deng, W.L., Wu, K.C., Sugita, S., Mandai, M., Takahashi, M. (2019). Stemming retinal regeneration with pluripotent stem cells. *Progress in Retinal and Eye Research*. (69):38-56.
- Limoli, P.G., Limoli, C., Vingolo, E.M., Franzone, F., Nebbioso, M. (2021). Mesenchymal stem and non-stem cell surgery, rescue, and regeneration in glaucomatous optic neuropathy. *Stem Cell Research and Therapy*. 12(1):275.
- Martínez-Carrasco, R., Sánchez-Abarca, L.I., Nieto-Gómez, C., Martín García, E., Sánchez-Guijo, F., Argüeso, P., Aijón, J., Hernández-Galilea, E., Velasco, A.

- (2019). Subconjunctival injection of mesenchymal stromal cells protects the cornea in an experimental model of GVHD. *Ocular Surface*. 17(2):285-294.
- Mittal, S.K., Mashaghi, A., Amouzegar, A., Li, M., Foulsham, W., Sahu, S.K., Chauhan, S.K. (2018). Mesenchymal Stromal Cells Inhibit Neutrophil Effector Functions in a Murine Model of Ocular Inflammation. *Investigative Ophthalmology & Visual Science*. 59(3):1191-1198.
- Oner, A., Gonen, Z.B., Sinim, N., Cetin, M., Ozkul, Y. (2016). Subretinal adipose tissue-derived mesenchymal stem cell implantation in advanced stage retinitis pigmentosa: a phase I clinical safety study. *Stem Cell Research and Therapy*. 7(1):178.
- Özmert, E., Arslan, U. (2020). Management of retinitis pigmentosa by Wharton's jelly-derived mesenchymal stem cells: prospective analysis of 1-year results. *Stem Cell Research and Therapy*. 11(1):353.
- Ren, G., Zhang, L., Zhao, X., Xu, G., Zhang, Y., Roberts, A.I., et al. (2008). Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2:141–50.10.1016
- Roddy, G.W., Oh, J.Y., Lee, R.H., Bartosh, T.J., Ylostalo, J., Coble, K., et al. (2011). Action at a distance: Systemically administered adult stem/progenitor cells (MSCs) reduce inflammatory damage to the cornea without engraftment and primarily by secretion of TNF- α stimulated gene/protein 6. *Stem Cell* (29):1572–9
- Omoto, M., Katikireddy, K.R., Rezazadeh, A., Dohlman, T.H., Chauhan, S.K. (2014). Mesenchymal stem cells home to inflamed ocular surface and suppress allosensitization in corneal transplantation. *Investigative Ophthalmology & Visual Science*. 55(10):6631-8.
- Sahu, A., Foulsham, W., Amouzegar, A., Mittal, S.K., Chauhan, S.K. (2019). The therapeutic application of mesenchymal stem cells at the ocular surface. *Ocular Surface*. 17(2):198-207.
- Su, W., Wan, Q., Huang, J., Han, L., Chen, X., Chen, G., Olsen, N., Zheng, S.G., Liang, D. (2015). Culture medium from TNF- α -stimulated mesenchymal stem cells attenuates allergic conjunctivitis through multiple antiallergic mechanisms. *Journal of Allergy and Clinical Immunology*. 136(2):423-32.

Weiss, JN., Levy, S. (2018). Stem Cell Ophthalmology Treatment Study: Bone marrow derived stem cells in the treatment of Retinitis Pigmentosa. *Stem Cell Investigation*. 5:18.

Zuk, P.A., Zhu, M., Mizuno, H., Huang, J., Futrell, J.W., Katz, A.J. et al. (2001). Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Engineering*. 7:211–228.

CHAPTER 3

AN OVERVIEW OF CAD/CAM SYSTEMS IN PROSTHODONTICS AND RESTORATIVE DENTISTRY

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INTRODUCTION

The increase in aesthetic, short treatment time expectations of patients, physicians in terms of comfort and time have increased the use of technology in dentistry. Dr. Mörmann first developed the ceramic reconstruction system (CEREC) in 1985, which was a new approach for same day restorations at dental clinics. Historically, this system was the first CAD/CAM system in the field of dentistry (Baroudi & Ibraheem, 2015). CAD/CAM is a term for “computer-aided design” and “computer-aided manufacturing”.

In dentistry, CAD/CAM systems can be used in inlay and onlay restorations, laminate restorations, partial and full crowns, removable prostheses, fixed prostheses, implant-supported prostheses, abutment, hybrid prostheses and crown-bridge infrastructure design and manufacture, maxillofacial prostheses (Ronay et al., 2011; Kanazawa et al., 2011). The purpose of the development of CAD/CAM systems is to eliminate the disadvantages of conventional impression methods, to design a three-dimensional restoration suitable for natural tooth anatomy and functions, to increase the restoration quality (mechanical properties, aesthetics, physical durability) and to restore restorations in a shorter time (Christensen, 2001). In CAD/CAM systems, instead of the conventional impression, the optical impression of the prepared abutment tooth is taken in the mouth, instead of the wax modeling stage, the restoration is designed on the computer with the appropriate computer program and the production phase is carried out in a computer-aided milling device (Miyazaki et al., 2009; Aeran et al., 2014).

1. CAD/CAM SYSTEMS

- Computer-Aided Impression (CAI)
- Computer-Aided Design (CAD)
- Computer-Aided Manufacturing (CAM)

1.1. CAI

In the field of dentistry, “scanning” means taking impression of the prepared tooth, opposing arch and adjacent tissues with three-dimensional and converting into digital data (Irfan et al., 2015). Obtaining and collecting data from the prepared tooth has two methods (optical and mechanical scanners) in different CAD/CAM systems (Beuer et al., 2008; Kalpana et al., 2015).

1.2. CAD

There are different CAD software programs in the market to design the data by transferring the data to the virtual environment after scanning the teeth or taking impressions. In most systems, the restoration, which is designed automatically, has the opportunity to be modified by the dentist. The software programs are specific for CAD/CAM systems and cannot be exchanged. After the design of the restoration is completed, the CAD software transfers the obtained virtual model to the CAM unit that will produce the restoration using special commands (Kalpana et al., 2015; Goswami et al., 2014).

1.3. CAM

CAM is the shaping and production phase, which is under computer control. In the first systems the prefabricated blocks were cut by burs, diamonds or diamond discs. In the following years, the technology got additional methods started to be used as a new CAM approach (Kalpana et al., 2015; Goswami et al., 2014; Marques et al., 2021). These methods are;

1.3.1. Subtractive Method

It is a method in which the desired form of the restoration is formed by abrading the material from the block placed in the CAM unit. After the completion of the restoration design, the data obtained for production is calculated automatically. Almost any restorations can be produced with the subtractive method, but a large part of the material is abraded.

1.3.1.1. Copy Milling Technique

The main working principle of the copy milling technique is to produce a copy of the model placed in the reading unit by scraping it in the other unit of the system. Special burs and discs in the engraving unit shape the ceramic block while the special non-abrasive scanner tips circulate on the modeling in the copying section. Celay and Zirkozahn Systems are the systems that work with the copy-milling technique.

1.3.2. Additive Method

In the additive (rapid prototyping) CAM approach, instead of cutting the prefabricated block to create the restoration, the system combines the restoration by sintering it from a cuvette with ceramic or metal powders and continues this until the restoration is complete. So no residual material is left. In this system, the restoration design is similar to the subtractive CAM devices (Davidowitz & Kotick, 2011). It was reported to be advantageous over traditional production techniques in terms of production speed, reliability and economy.

1.3.2.1. Stereolithography (SLA)

In the technique, which was first introduced by Charles W. Hull in 1986, the three-dimensional model of the desired shape is created in the CAD program and the model is divided into fine pieces by the software. Concentrated ultraviolet light scans the liquid photopolymer in the vessel, resulting in a solid model of the first layer. As a result of the laser scanning process that continues layer by layer, the resin layer is polymerized or cross-linked each time to form other layers and the model is completed.

1.3.2.2. Selective laser sintering (SLS)

In the selective laser sintering (SLS) method, the material in powder form, which is stacked on top of each other in the form of layers, is connected by the heat energy generated as a result of the laser beam. After the first powder layer is sintered with a laser, the piston in the production section goes down and covered with a new powder layer. Laser sintering of powder layers continues until the desired

pattern is obtained (Kalpana et al., 2015; Goswami et al., 2014; Marques et al., 2021; Davidowitz & Kotick, 2011).

1.3.2.3. Selective electron beam melting (SEBM)

This method is similar to the SLS method. However, the high-density energy used in the system causes the powder particles to completely melt and products with higher density are obtained. It has been stated that the terminology used is sometimes confused due to the lack of a consensus about the limitations of the methods. For this reason, the production of materials from polymer and ceramic, generally refers to the term SLS, while the term selective laser melting is used for metals.

In addition to subtractive and additive CAM systems, there are also CAD/CAM systems using the combination of these two systems (Torabi et al., 2015; Abduo et al., 2014). Examples of these systems are Procera. In Procera, the larger metal dies (to compensate for the shrinkage that will occur in final sintering) is milled according to the data obtained after scanning. Porcelain powder is sent to the milled metal die with high pressure with the additive system. The restoration obtained in larger sizes than it should be is taken from the metal stump and sintered so that it can be returned to its real dimensions and densified (Miyazaki et al., 2009; Marques et al., 2021).

2. ADVANTAGES and DISADVANTAGES of CAD/CAM SYSTEMS

CAD/CAM applications have brought many advantages. It has eliminated traditional impression methods. Restorative materials with better properties were obtained in a shorter time. It has greatly reduced the probability of error and prevented possible cross-contaminations that may result from indirect restorations (Christensen, 2001). With the use of CAD/CAM systems in dentistry, the condensation, melting processes of ceramic materials have decreased relatively. One of the advantages of CAD/CAM systems is that there is no loss of time for both patients and physicians as applications can be made in a single session. In this way, the necessity of preparing temporary crowns is

eliminated. As restorations are designed with CAD software, technicians' work becomes easier. One of the factors limiting the use of CAD/CAM restorations is the production cost. Although many new systems have been developed, it is still not economical to use CAD/CAM systems. The use of single-color blocks can cause problems with aesthetic expectations. However, this problem is about to be overcome with the development of blocks of different colors. Transferring teeth with deep subgingival margins to the computer environment can also be a problem, so it becomes necessary to perform a good gingival retraction. In addition, there is a need for an appropriate teamwork as professional personnel who know this technology are needed (Christensen, 2001; Duret & Preston, 1991).

3. MATERIALS USED in CAD/CAM SYSTEMS

3.1. Feldspathic Ceramic Blocks

These blocks, which were first used with CAD/CAM systems, contain 30% homogeneously dispersed feldspar particles of 3-4 μm in the glass matrix. Their fracture strength is approximately 150 MPa, and their modulus of elasticity is 45-63 GPa (Fasbinder, 2012). It is used in inlays, onlays, veneers, partial or full crowns for the anterior and posterior regions (Denry & Kelly, 2008).

3.2. Lithium Disilicate Glass Ceramics

Lithium disilicate crystals are used at a rate of 70% in the material, and the superstructure ceramic consists of fluorapatite crystals (Michel & Lewis, 2011). These blocks are partially crystallized because the milling of this material is difficult and its brittleness is high. There is a wide range of indications, including thin veneers (0.4mm), veneers, inlays, onlays, crowns, three-unit bridges that do not cover molars, hybrid abutments, the superstructure of these abutments, veneers of zirconium oxide infrastructure (Taskonak et al., 2005).

3.3. Leucite Reinforced Glass Ceramic Blocks

The leucite crystal phase 1-5 μm in size constitutes 30-40% of the silicate glass matrix volume. The semi-permeability and abrasion effect of the material is similar to natural teeth and its bending strength is approximately 160 MPa (Kelly et al., 1996). It is suitable for single tooth restorations including anterior and posterior crowns, partial crowns and laminate veneers (Giardano, 2002).

3.4. Hybrid Ceramic Blocks

These blocks consist of ceramic and polymer. The polymer network consists of surface-modified polymethylmethacrylate (Michel & Lewis, 2011). It is suitable for use in single-tooth restorations (inlay, onlay, veneer, crown), especially in molar areas where high chewing forces occur and in teeth with minimal preparation. The polymer network structure of the material absorbs intraoral stresses and provides the elasticity required for minimally invasive restorations. Its elasticity of 30 Gpa is very close to natural dentin. It has two options: HT (high translucent) and T (translucent) (Taskonak et al., 2005). Hybrid ceramic blocks are containing 61% filler by mass (Kelly, 1996).

3.5. Zirconium Reinforced Lithium Disilicate Ceramic Blocks

These blocks contain 8-12% ZrO_2 . The fracture resistance of these ceramics is 210 MPa after milling, while it increases to 420 MPa after crystallization (Raigrodski, 2004). Anterior and posterior crowns, crowns on implants, veneers, inlays and onlay restorations can be produced with this material (Perea-Lowery et al., 2020).

3.6. Nanoceramics

Nanoceramics are composed of ceramic particles and a resin matrix containing urethane-dimethacrylate (UDMA). There are silica nanomers with a diameter of 20 nm and zirconia nanomers with a diameter of 4-11 nm in the structure. The silane resin added to the structure during the production phase of the blocks provides the chemical connection between the matrix and the nanomer (Fradeani et al., 2005). They can be used in the production of inlays, onlays, lamina veneers, crowns and implant crowns (Lauvahutanon et al., 2014).

3.7. Polymer CAD/CAM Blocks

It can be used for the production of temporary restorations that can be used on the implant with a two-body bridge restoration, therapeutic restorations to correct temporomandibular joint problems or the occlusal plane.

3.8. Composites

It contains 85% by mass and zirconium-silica fillers approximately 0.6 μm in size. It can be used in inlays, onlays, veneers and full crowns.

3.9. Stabilized Zirconia

Zirconia is a crystalline phase of the structure that is formed by the regular composition of 0.4 μm particles. The crystals in its structure can be found in 3 phases: monoclinic (room temperature - 1170 °C), tetragonal (1170 °C – 2370 °C) and cubic (2370 °C – 2680 °C). Transitions between these phases lead to volumetric changes. For example, when transitioning from the tetragonal phase to the monoclinic phase, a volume increase of 3-5% is observed (Manicone, 2007; Li, 2014).

Since the monoclinic phase is not a stable phase, metal oxides are added to ensure the stability of this phase at room temperature. By incorporating yttrium oxide into the structure of zirconia, polycrystalline zirconia is obtained. In this way, the mechanical properties of zirconia are also improved. While the fracture toughness is 5-10 MPa, the flexural strength is around 900-1400 MPa (Miyazaki, 2013).

Although zirconia has been used as a prosthetic infrastructure material to be layered with ceramic since the day it was first produced. It is now possible to produce monolithic restorations from zirconia. Ready-to-use blocks and discs are available as a monochromatic uniform material colored by infiltration. With this; there is also an increasing trend in the use of polychromatic CAD/CAM blocks and discs produced to mimic dentin-to-enamel color changes. In addition, manufacturers have enabled the production of increased translucency in contrast to the highly opaque feature of zirconia (Gracis, 2015).

4. CURRENT CAD/CAM SYSTEMS

4.1. Lava System

Semi-sintered zirconium oxide blocks are used in the CAD/CAM system, which was launched in 2002 by 3M ESPE company (Piwowarczyk et al. 2005). Lava substructures are produced from semi-sintered zirconium oxide blocks and are abraded to a larger size to compensate for 20% polymerization shrinkage. With the Lava system, anterior and posterior crowns and three or four-unit bridge prostheses, inlays and fixed prostheses can be produced. In the lava system, the model obtained in the laboratory is scanned in three-dimensional with the Lava Scan optical scanner. After the data is transferred to the computer, the infrastructure planned for the restoration is designed according to the system parameters and widely abraded from semi-sintered zirconium oxide blocks in the Lava Form milling unit (Piwowarczyk et al., 2005).

4.2. Everest System

Everest system consists of 3 units: a scanning and design unit, an etching unit and a sintering machine (Piwowarczyk et al., 2005). With the Everest system, inlay, onlay, laminate veneer restorations, anterior and posterior crown prostheses and bridge prostheses up to 14-unit can be produced (Piwowarczyk et al., 2005; Leinfelder et al., 1989). Everest system, unlike many systems, wears 5-axis technology.

4.3. Procera System

Procera system was first developed in 1986 to produce titanium substructures for use in the construction of crown and bridge prostheses. Approximately ten years later a new version was introduced that can sintered pure and high-strength aluminum oxide (99.9%) substructures (Polansky et al., 1999). In order to use the Procera system; a special scanner, a special software that evaluates the scanned information and an advanced computer with an internet connection are required (Andersson, & Oden, 1993).

4.4. Cercon System

Cercon system, unlike other CAD/CAM systems, has only a CAM unit and no computer-aided three-dimensional design is made in the system. Infrastructure production is made with the CAM system by using the wax modeling design prepared by the dental technician. The first developed Cercon system consists of the main machine and a sintering machine (Andersson, & Oden, 1993). Main machine includes a laser scanner and etching unit. The data obtained after scanning the wax modeling are transferred to the etching unit. The infrastructure is obtained from semi-sintered zirconium oxide blocks by grinding with special tungsten carbide burs. At this stage, the abrasion should be done in 25-30% larger size to meet the shrinkage after sintering (von Schroeter et al., 2004). With the Cercon system; single crown, four or five-unit bridge prostheses, over-implant crown and bridge prostheses can be prepared. Recently, with the production of larger blocks, it has been possible to make 5-6-unit bridge prostheses. In 2005, the CAD unit was included in the system and a new Cercon system was started to be used (von Schroeter et al., 2004).

4.5. CICERO

CICERO, an abbreviation of the words “Computer Integrated Ceramic Reconstruction”, is based on optical scanning, ceramic sintering and production of restoration based on CAM principles. As in Procera, in this system, the production phase is done in the laboratory (Yöndem & Aykent, 2008; van der Zel et al., 2001).

4.6. DC-Zircon System

This system consists of three parts: (1) fully automatic, optical scanner working with laser projection, (2) special software and (3) an etching unit). Fully sintered Y-TZP blocks (DC-Zircon) are used in the system. Since fully sintered zirconia blocks are used, the etching process of a single crown takes 2 hours. There is no sintering process after the etching stage (Giordano, 2002).

4.7. CEREC System

CEREC, an abbreviation of the words “Ceramic” and “Reconstruction”, was designed by Siemens. The system was first used by Mörmann to make ceramic inlays in 1985. It was designed with 3 axes. It was not preferred due to the disadvantages of not being able to be adequately shaped and not providing sufficient marginal edges (Deany, 1996; Mörmann & Bindl, 2002). By using the new CEREC system, it can be used for inlay, onlay, laminate veneer restorations, anterior and posterior crown prostheses (Giordano, 2006). Compared to other systems; its lower cost, ability to process blocks other than zirconia, and the ability to color the zirconia infrastructure with six different color options are the advantages that make the use of the system widespread (Martins et al., 2012).

4.8. Celay System

Celay system was developed in 1987 based on copy milling technique as an alternative to computer-aided methods. Celay system works with the logic of a precision copy milling device, which is also used in the key-making system. Restoration is obtained by etching technique from prefabricated ceramic blocks without any computer support (Mörmann, 2006; De Munck, 2005).

This system consists of two units. The model prepared from blue photopolymerized composite material is placed on the left side of the device, and the Celay Zirconia block to be etched is placed in the right side compartment (Mörmann, 2006). With the Celay system, it is possible to manufacture inlay, onlay, laminate veneer restorations, crown prostheses and three-unit bridge prostheses.

4.9. Zirkozahn System

Zirkozahn system consists of a special software program, a scanning unit and a milling unit. The data obtained after scanning the plaster model in 5-axes by the scanner unit with a laser reader tip is transferred to the etching unit via the special software program of the system. An unsintered zirconia block suitable for the planned prosthetic restoration is placed in the etching unit. In the system, the zirconia block placed in the etching unit is shaped according to the restoration

designed on the special software. To meet the shrinkage that will occur after the sintering process, the substructure, which is abraded 20% larger, reaches its real dimensions by being subjected to the sintering process at approximately 1500 °C for 8 hours (Neves, 2014).

5. DIGITAL WORKFLOWS in DENTISTRY

Developing technology and increased patient expectations have brought digital dentistry to an important position. The stages in digital dentistry processes are changing with various workflows.

5.1. Traditional Digital Workflow

This workflow starts with conventional impressions but is completed with digital production.

- The dentist takes impressions with the conventional method using an impression tray and impression material,
- The impression tray send to the laboratory by the dentist,
- The laboratory technician scans the plaster model with an extraoral scanner to create a three-dimensional virtual digital model of the entire dental arch,
- The technician designs the prosthesis using the CAD/CAM system and sends this file to the milling machine,
- The milling machine creates the prosthesis,
- The prosthesis is applied to the patient's mouth by the dentist and adjusted to the occlusion with the necessary corrections.

This workflow is the oldest of the digital impression techniques and sometimes the plaster casting step can be skipped by directly scanning the impression tray.

5.2. Digital Workflow

The second type of workflow is called the “Digital Workflow”. This workflow starts with a digital intraoral impression but can be completed conventionally if desired. The digital workflow can be maintained by a dentist with a stand-alone intraoral scanner not equipped with a milling unit. In the digital workflow concept, the steps followed for prosthesis construction are as follows;

- The dentist takes the digital impression through an intraoral scanner,
- The digital data send to the laboratory by the dentist,
- The laboratory uploads the digital file and uses a special software program to mark the margins,
- A stereolithographic model is created using the CAD/CAM system,
- The technician can proceed with his preferred finishing procedure,
- All ceramic restorations are fully digitally designed and milled via the CAD/CAM system,
- The final restoration is sent to the dentist to be applied to the patient.

5.3. Fast digital workflow

The third workflow concept is the “Fast Digital Workflow”. This concept can be implemented where the dentist has an intraoral scanner working in conjunction with the milling machine in the clinic.

In the fast digital workflow concept, the steps followed for prosthesis construction are as follows;

- The dentist takes the digital impression through an intraoral scanner,
- The dentist designs the restorations in special CAD software and sends the obtained data to the milling unit for production,
- The final restoration is prepared in a short time by the milling unit,
- The dentist applies the restoration to the patient in the same session.

REFERENCES

- Abduo, J., Lyons, K., & Bennamoun, M. (2014). Trends in computer-aided manufacturing in prosthodontics: a review of the available streams. *International journal of dentistry*, 2014, 783948.
- Aeran, H., Kumar, V., Seth, J., & Sharma, A. (2014). Computer Aided Designing-Computer Aided Milling in Prosthodontics: A Promising Technology for Future. *IJSS Case Report & Reviews*,1(1), 23-27.
- Andersson, M., & Odén, A. (1993). A new all-ceramic crown. A dense-sintered, high-purity alumina coping with porcelain. *Acta odontologica Scandinavica*, 51(1), 59–64.
- Baroudi, K., & Ibraheem, S. N. (2015). Assessment of Chair-side Computer-Aided Design and Computer-Aided Manufacturing Restorations: A Review of the Literature. *Journal of international oral health : JIOH*, 7(4), 96–104.
- Beuer, F., Schweiger, J., & Edelhoff, D. (2008). Digital dentistry: an overview of recent developments for CAD/CAM generated restorations. *British dental journal*, 204(9), 505–511.
- Christensen G. J. (2001). Computerized restorative dentistry. State of the art. *Journal of the American Dental Association* (1939), 132(9), 1301–1303.
- Davidowitz, G., & Kotick, P. G. (2011). The use of CAD/CAM in dentistry. *Dental Clinics of North America*, 55(3), 559–69.
- De Munck, J., Van Landuyt, K., Peumans, M., Poitevin, A., Lambrechts, P., Braem, M., & Van Meerbeek, B. (2005). A critical review of the durability of adhesion to tooth tissue: methods and results. *Journal of dental research*, 84(2), 118–132.
- Deany I. L. (1996). Recent advances in ceramics for dentistry. *Critical reviews in oral biology and medicine : an official publication of the American Association of Oral Biologists*, 7(2), 134–143.
- Denry, I., & Kelly, J.R. (2008). State of the art of zirconia for dental applications. *Dent Mater*,24,299-307.
- Duret, D., & Preston, J.D. (1991).CAD/CAM imaging in dentistry. *Curr Opinion Dent*, 1(2),150–4.
- Fasbinder, DJ. (2012) Chairside CAD/CAM: an overview of restorative material options. *Compend Contin Educ Dent*, 33(1),50, 52-58.

- Fradeani, M., D'Amelio, M., Redemagni, M., & Corrado, M. (2005). Five-year follow-up with Procera all-ceramic crowns. *Quintessence international* (Berlin, Germany : 1985), 36(2), 105–113.
- Giardano, R.A. (2002) Dental ceramic restorative systems. Fasbinder DJ. Restorative material options for CAD/CAM restorations. *Compend Contin Educ Dent*, 23,911- 916.
- Giordano R. (2002). A comparison of all-ceramic restorative systems. *Journal of the Massachusetts Dental Society*, 50(4), 16–20.
- Giordano R. (2006). Materials for chairside CAD/CAM-produced restorations. *Journal of the American Dental Association* (1939), 137 Suppl, 14S–21S.
- Goswami, R., Arora, G., & Priya, A. (2014) CAD/CAM in Restorative Dentistry: A Review. *BBB*, 2(4),591-597.
- Gracis, S., Thompson, V. P., Ferencz, J. L., Silva, N. R., & Bonfante, E. A. (2015). A new classification system for all-ceramic and ceramic-like restorative materials. *The International journal of prosthodontics*, 28(3), 227–235.
- Irfan, U., Aslam, K., & Nadim R.(2015). A review on cad cam in dentistry *J Pak Dent Assoc*,24(3),112- 116.
- Kalpana, D., Harish, G., Mahesh, P.C., Suhasaria, S., Madhuri, V., & Brunda, K. (2015). CAD / CAM in dentistry- a Review. *Int J Research Dent*, 5(2),14-21.
- Kanazawa, M., Inokoshi, M., Minakuchi, S., & Ohbayashi, N. (2011). Trial of a CAD/CAM system for fabricating complete dentures. *Dental materials journal*, 30(1), 93–96.
- Kelly, J. R., Nishimura, I., & Campbell, S. D. (1996). Ceramics in dentistry: historical roots and current perspectives. *The Journal of prosthetic dentistry*, 75(1), 18–32. [https://doi.org/10.1016/s0022-3913\(96\)90413-8](https://doi.org/10.1016/s0022-3913(96)90413-8) .
- Lauvahutanon, S., Takahashi, H., Shiozawa, M., Iwasaki, N., Asakawa, Y., Oki, M., Finger, W. J., & Arksornnukit, M. (2014). Mechanical properties of composite resin blocks for CAD/CAM. *Dental materials journal*, 33(5), 705–710.
- Leinfelder, K. F., Isenberg, B. P., & Essig, M. E. (1989). A new method for generating ceramic restorations: a CAD-CAM system. *Journal of the American Dental Association*, 118(6), 703–707.
- Li, R. W., Chow, T. W., & Matinlinna, J. P. (2014). Ceramic dental biomaterials and CAD/CAM technology: state of the art. *Journal of prosthodontic research*, 58(4), 208–216.

- Manicone, P. F., Rossi Iommetti, P., & Raffaelli, L. (2007). An overview of zirconia ceramics: basic properties and clinical applications. *Journal of dentistry*, 35(11), 819–826.
- Marques, S., Ribeiro, P., Falcão, C., Lemos, B. F., Ríos-Carrasco, B., Ríos-Santos, J. V., & Herrero-Climent, M. (2021). Digital Impressions in Implant Dentistry: A Literature Review. *International journal of environmental research and public health*, 18(3), 1020.
- Martins, L. M., Lorenzoni, F. C., Melo, A. O., Silva, L. M., Oliveira, J. L., Oliveira, P. C., & Bonfante, G. (2012). Internal fit of two all-ceramic systems and metal-ceramic crowns. *Journal of applied oral science : revista FOB*, 20(2), 235–240.
- Michel, A., & Lewis, HA. (2011). Repairing Worn Dentition with Lithium-Disilicate Glass-Ceramic. *Inside Dentistry*, 7 (3).
- Miyazaki, T., Hotta, Y., Kunii, J., Kuriyama, S., & Tamaki, Y. (2009). A review of dental CAD/CAM: current status and future perspectives from 20 years of experience. *Dental materials journal*, 28(1), 44–56.
- Miyazaki, T., Nakamura, T., Matsumura, H., Ban, S., & Kobayashi, T. (2013). Current status of zirconia restoration. *Journal of prosthodontic research*, 57(4), 236–261.
- Mörmann W. H. (2006). The evolution of the CEREC system. *Journal of the American Dental Association* (1939), 137 Suppl, 7S–13S.
- Mörmann, W. H., & Bindl, A. (2002). All-ceramic, chair-side computer-aided design/computer-aided machining restorations. *Dental clinics of North America*, 46(2), 405–8.
- Neves, F. D., Prado, C. J., Prudente, M. S., Carneiro, T. A., Zancopé, K., Davi, L. R., Mendonça, G., Cooper, L. F., & Soares, C. J. (2014). Micro-computed tomography evaluation of marginal fit of lithium disilicate crowns fabricated by using chairside CAD/CAM systems or the heat-pressing technique. *The Journal of prosthetic dentistry*, 112(5), 1134–1140.
- Perea-Lowery, L., Gibreel, M., Vallittu, P. K., & Lassila, L. (2020). Characterization of the mechanical properties of CAD/CAM polymers for interim fixed restorations. *Dental materials journal*, 39(2), 319–325.
- Piowarczyk, A., Lauer, H. C., & Sorensen, J. A. (2005). The shear bond strength between luting cements and zirconia ceramics after two pre-treatments. *Operative dentistry*, 30(3), 382–388.

- Polansky, R., Arnetzl, G., Smetan, M., Haas, M., & Lorenzoni, M. (1999). The production of Cerec restorations from a plaster cast. *International journal of computerized dentistry*, 2(1), 37–44.
- Raigrodski, A.J. (2004) Contemporary all ceramic fixed partial dentures: a review. *Dent Clin North Am*, 48,531-544.
- Ronay, V., Sahrman, P., Bindl, A., Attin, T., & Schmidlin, P. R. (2011). Current status and perspectives of mucogingival soft tissue measurement methods. *Journal of esthetic and restorative dentistry : official publication of the American Academy of Esthetic Dentistry ... [et al.]*, 23(3), 146–156.
- Taskonak, B., Mecholsky, J. J., Jr, & Anusavice, K. J. (2005). Residual stresses in bilayer dental ceramics. *Biomaterials*, 26(16), 3235–3241.
- Torabi, K., Farjood, E., & Hamedani, S. (2015). Rapid Prototyping Technologies and their Applications in Prosthodontics, a Review of Literature. *Journal of dentistry (Shiraz, Iran)*, 16(1), 1–9.
- van der Zel, J. M., Vlaar, S., de Ruitter, W. J., & Davidson, C. (2001). The CICERO system for CAD/CAM fabrication of full-ceramic crowns. *The Journal of prosthetic dentistry*, 85(3), 261–267.
- von Schroeter, P., Jürgensen, B., & Zöllner, M. (2004). Cercon move--a navigation aid for dental CAD applications. *International journal of computerized dentistry*, 7(4), 371–377.
- Yöndem, I., & Aykent, F. (2008).Dental ceramics made by using computer technology (CAD/CAM), *J Dent Fac Hacettepe Uni*, 32(3),79-86.

CHAPTER 4

NORMAL PRESSURE HYDROCEPHALUS

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INTRODUCTION

Normal pressure hydrocephalus (NPH) is a rare, but progressive neurological disorder. It is mainly characterized by gait disturbance, cognitive impairment and urinary incontinence. It is caused by the accumulation of cerebrospinal fluid (CSF) in the ventricular space without an increase in intracranial pressure. Its prevalence is reported between 0.2% and 5.6%. NPH can be congenital or acquired with ventriculomegaly or impaired CSF absorption. It is classically divided into two forms as idiopathic NPH, in which there is no identifiable cause, and secondary NPH caused by several underlying mechanisms include trauma, subarachnoid hemorrhage, malignancy, stroke, meningitis and intracerebral hemorrhage. This chapter begins with the definition and history of NPH. Etiology, epidemiology, pathophysiology, clinical features, diagnosis, treatment and prognosis are discussed in line with the current literature and the chapter ends with future directions.

1. DEFINITION AND HISTORY

For several centuries, hydrocephalus has been considered as a cause of brain dysfunction and movement disorders. The term “normal pressure hydrocephalus (NPH)” was used for the first time by Columbian neurosurgeon Salomon Hakim in 1964 (Hakim & Adams, 1965). It meant “Hydrocephalus Syndrome in Adults with ‘Normal’ CSF Pressure”. Today, NPH is defined as ventriculomegaly without increased intracranial pressure in patients presenting with urinary incontinence, cognitive disturbance and gait difficulty.

Idiopathic normal pressure hydrocephalus (iNPH) is defined as a disease without identifiable cause that causes cognitive loss, gait ataxia, and/or urinary incontinence and ventriculomegaly. Ventricular system is a common location for accumulation of CSF and hydrocephalus also involves an increase in CSF in the extraventricular subarachnoid cavity. Ventriculomegaly is defined as an increase in CSF content in the ventricular system. NPH may be multifactorial including congenital causes, impaired CSF absorption and vascular disease.

2. ETIOLOGY

The main etiological factors for NPH include congenital factors, vascular factors and impaired cerebrospinal fluid absorption (Figure 1).

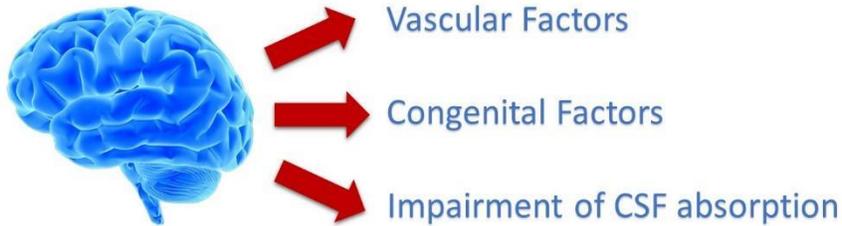


Figure 1. Etiological factors of NPH

2.1. Congenital Factors

Hydrocephalus is divided into two categories: communicating and obstructive. Communicating hydrocephalus is characterized by increased intracranial CSF in the absence of an obstructive cause. NPH is a part of communicating hydrocephalus in which CSF pressure does not increase. NPH syndrome can be primary without any identifiable cause (idiopathic NPH [iNPH]) or secondary due to several conditions that are known to disrupt CSF absorption. Clinical symptoms of primary or secondary NPH respond to surgical treatment. Some surgical CSF diversion procedures include ventriculopleural shunt, ventriculoperitoneal shunt, lumboperitoneal shunt etc.

Obstructive hydrocephalus is also known as noncommunicating NPH and is defined as NPH secondary to blockage of CSF flow through the ventricular and subarachnoid spaces related to acquired or congenital causes. Studies have shown that in more than 10% of the cases, these patients have a larger head size (Kreff et al. 2004; Wilson & Willimas, 2007; Graff-Radford & Jnes, 2019). This indicates that congenital factors may have a crucial role in the occurrence of NPH.

2.2. Vascular Factors

Animal studies have shown that vascular mechanisms are associated with the development of hydrocephalus. It has been shown in a study that pulse pressure increased in the ventricles of sheep using a balloon resulted in hydrocephalus within hours (Pettorossi et al., 1978). Human studies also have demonstrated that vascular disease alters CSF dynamics, contributing to the occurrence of NPH. Epidemiological studies have associated NPH with vascular diseases (Graff-Radford et al., 2017; Jaraj et al., 2016). Atherosclerosis Risk in Communities (ARIC) study reported that baseline systolic pressure and pulse values were associated with enlargement of ventricles due to accumulation of CSF (Graff-Radford et al., 2013). In a study by Tisell et al. 14 hydrocephalus patients had vascular risk factors and were treated with shunting (Tisell, 2005).

2.3. Impaired CSF Absorption

The mechanisms leading to increased CSF volume are poorly understood in iNPH and this condition can possibly occur due to numerous etiologies that alter CSF dynamics such as production of flow or absorption. It has been proposed that decreased CSF absorption leads to increased CSF volume over time, resulting in an increased ventricular volume to preserve normal intracranial pressure. It has been reported in the studies that there is a strong relationship between poor CSF absorption and good outcome after shunting surgery (Boon et al., 1997). In addition, white matter lesions, and a history of diabetes mellitus and hypertension have been associated with iNPH (Jaraj et al., 2016). Recently, a fluid exchange has been suggested between CSF and interstitial space fluid in the brain parenchyma. This could be possible due to water channels attached to the end of glial cells (Ringstad, Vatnehol and Eide, 2017; Iliff et al., 2012; Brinker et al., 2014). Clinicians should be aware of modifiable risk factors in the impairment of CSF absorption (Figure 2).

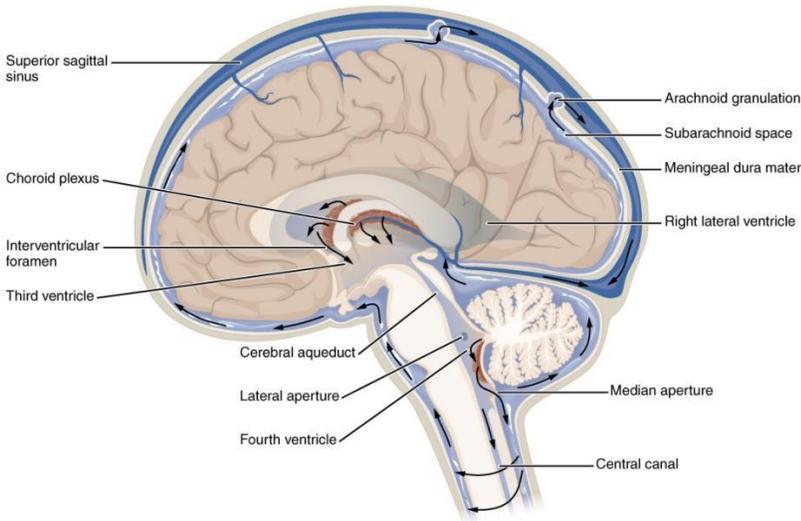


Figure 2. Traditional view of the circulation of CSF. By OpenStax - <https://cnx.org/contents/FPtK1zmh@8.25:fEI3C8Ot@10/Preface>, CC BY 4.0, <https://commons.wikimedia.org/w/index.php?curid=30147960>

3. EPIDEMIOLOGY

NPH is a rare condition compared with other causes of dementia in elderly such as Alzheimer disease. The incidence of NPH has differed among studies from 2 to 20 per million per year (Klassen & Ahlskog, 2011; 15. Kuriyama et al., 2017). Inherent difficulties in diagnosing NPH make estimation of its incidence difficult. The need for lumbar puncture for diagnosis compromises the conduction of population based studies. In a population-based study reported from Norway with 220,000 individuals, the prevalence of NPH was found as 21.9/100000 and the incidence as 5.5/100000 (Brean & Eide, 2008). In their study, Jaraj et al. used clinical examination and neuroimaging and found the prevalence of NPH as 0.2% in individuals between 70-79 years old and 5.9% in those aged over 80 years (Jaraj et al, 2016). In the summer of three studies from Japan conducted by Mori et al., the prevalence of iNPH was reported as 1.1% in persons aged over 60 years (Mori et al, 2012). However these studies are mainly based on radiological criteria, namely ventriculomegaly and disproportionate widening of CSF spaces (Passos-Neto et al., 2022). In another study

from Brazil, Vale and Miranda found NPH in 5.38% of patients with dementia (Bech-Azeddine et al., 2001). In the population based Mayo Clinic Study of Aging, ventriculomegaly was detected on MRI of persons aged over 70 years (Graff-Radford et al., 2017). NPH is equally common in both sexes (Marmarou et al., 2005).

These differences possibly reflect different NPH definitions or diagnostic criteria among studies. On the other hand, there is a need for standard criteria that will enable study of incidence and prevalence of iNPH. The results vary among populations and communities. Details of risk factors, age, gender and comorbidities should be recorded to establish diagnosis and treatment services especially for the growing elderly population (Rosseau, 2011).

4. PATHOPHYSIOLOGY

NPH is divided into two different forms as idiopathic NPH (iNPH), in which there is no identifiable cause, and secondary NPH that can be resulted from various pathologies including trauma, subarachnoid hemorrhage, malignancy, stroke, meningitis and intracerebral hemorrhage (Daou et al., 2016). It is suggested that these conditions lead to an inflammatory process of the arachnoid granulations with altered CSF flow dynamics and reduced CSF reabsorption, causing ventricular enlargement (Espay et al., 2017).

Symptoms and pathogenetic mechanisms caused by various genes may be collected under the general term of iNPH. In a study, sleep disordered breathing has also been proposed as a pathologic mechanism for iNPH (Román et al., 2019). The gait disturbance in iNPH is an impairment of the phased activation of muscle group, rather than disturbance of primary pyramidal tract. Subcortical dopaminergic pathways also contribute to gait disturbance when impaired.

The locus of dysfunctioning iNPH is unknown, although periventricular projection and the frontostriatal system are held responsible regarding dementia (Rosseau, 2011). Findings described for the pathophysiology of iNPH include fibrosis and thinning of the arachnoid membranes and meninges, rupture of the ventricular ependymal gliosis, and inflammation of arachnoid granulations (Espay

et al., 2017). Of patients with iNPH, 10-20% present with increased head circumference, suggesting that iNPH may be based on a congenital hydrocephalus that becomes symptomatic over years (Takada et al., 2003).

The most commonly detected underlying mechanisms in secondary NPH include subarachnoid and intraventricular hemorrhage from trauma or aneurysm and prior chronic or acute meningitis. Other rarely reported causes include Paget's disease at skull base and achondroplasia (Hebb & Cusimano, 2001).

5. CLINICAL FEATURES

NPH is classically manifested with three features including gait disturbance, cognitive dysfunction and urinary incontinence (Adams et al. 1965). It is not necessary for a patient to have all these features together, but gait disturbance must be prominent. These manifestations are believed to originate from dysfunction of supplementary motor areas on the frontal lobe (Lenfeldt et al., 2008). A patient with NPH can present only with the complaint of gait disturbance without the other two features.

5.1. Gait Disturbance

Gait disturbance is the first clinical characteristic of NPH. The NPH gait is characterized by short steps, step height, reduced speed, and difficulty in turning 180 or 360 degrees and impaired dynamic equilibrium. Patients are most vulnerable to falls when trying to turn. Patients may also have difficulties in walking on stairs or standing up from a chair. The posture is commonly forward leaning with spontaneous falling as demonstrated by the pull test (Stolze et al., 2001).

5.2. Cognitive Dysfunction

The cognitive dysfunction in NPH develops over time and usually occurs after gait disturbance. Patients typically exhibit both frontal and subcortical features including psychomotor slowing, decreased attention and concentration, impaired executive function and apathy. Executive function is impaired early in the course of the disease

and may be unresponsive to treatment. Cortical features such as aphasia are rare.

The Mini-Mental State Examination and other simple cognitive tests can be used to assess cognitive dysfunction in NPH. “True” in the absence of other neurodegenerative disorders, the cognitive profile of NPH can only be obtained from studies on autopsy proven NPH.

5.3. Urinary Incontinence

At early stage of NPH urinary urgency rather than incontinence may be prominent. On the other hand, slow walking may delay patients from reaching the bathroom timely. In later stages of NPH, urinary incontinence is accompanied by a lack of concern that is likely resulted from the impairment in the frontal lobe. In iNHP urinary urgency and incontinence are difficult to distinguish from other causes of such urological dysfunction.

6. Differential Diagnosis

Variations in clinical manifestations makes accurate diagnosis of iNPH complicated and the frequency of comorbidities complicates both diagnosis and treatment. Differential diagnosis of NPH includes numerous disorders that are less or more common in elderly. The clinical symptoms of NPH may not be specific and may resemble other neurodegenerative diseases. Some of the disorders to be considered in the differential diagnosis of NPH are shown in Figure 3.



Figure 3. Differential diagnosis of NPH

Sometimes a different condition may coexist with NPH and the presence of comorbidity is a significant predictor of response to shunting therapy in iNPH (Bech-Azeddine et al., 2001). The presence of comorbidity can worsen prognosis of iNPH. Among iNPH patients approximately 75% also have Alzheimer's disease or cerebrovascular dementia (Kiefer & Unterberg, 2012). Studies have shown that 40-75% of iNPH patients have beta-amyloid or other typical findings of Alzheimer's disease (Bech-Azeddine et al., 2001).

It is of paramount importance to detect signs of possible NPH in the evaluation of neurodegenerative diseases and to determine the patient who may benefit from shunting therapy. The elderly people mostly have these diseases and it is almost impossible to observe "pure" NPH in these patients (Williams & Malm, 2016). Peripheral neuropathy, lumbar stenosis, vestibular dysfunction and hypertension may accompany iNPH or complicate the diagnosis. Patients in whom ventriculomegaly is accompanied by cerebrovascular disease, cerebral atrophy and neurodegenerative disorders are not likely to well-respond to CSF shunting procedure (Rosseau, 2011).

7. DIAGNOSTIC PROCEDURES

7.1. Gait Disturbance Assessment

Gait disturbance is the first clinical characteristic of NPH and the most responsive feature to treatment with shunting. The NPH gait presents with short steps, reduction in speed, impaired equilibrium upon turning and reduced step height (Marmarou et al., 2005). Patients may also have difficulties in walking on stairs or standing up from a chair. Blomsterwall et al. found improvement in balance mainly due to improved gait in 75% of patients (Blomsterwall et al. 2000). In all patients suspected to have iNPH, alternative causes of gait disturbance such as vestibular dysfunction, osteoarthritis, visual impairment, peripheral neuropathy and side effects of medications should be considered and evaluated. As a subjective evaluation, clinicians should observe step width, height, tandem gait, turning, resting sway and posture (Tsakanikas & Relkin, 2007). Other techniques include observation of gait using videos, 10-m walking test, Berg balance scale,

GAITRite system and Tinetti scale (Gallagher, Marquez and Osmotherly, 2018).

7.2. Urinary Symptoms Assessment

The proximity of some centers, which controls micturition, may cause urinary symptoms in NPH patients (Tsakanikas & Relkin, 2007). Lesions located above the pontine micturition center or its pathways may lead to an absence in control of the bladder, resulting in overactivity without or with incontinence in NPH. Urodynamic testing is the most effective method to assess urinary symptoms. In a urodynamic test, the patient sits on a chair with a saline infusion via a urinary catheter. A second catheter is then placed in the rectum or vagina to simultaneously measure intraabdominal and intravesical pressure, post-void residual, bladder capacity, maximum flow rate and first sensation (Krzastek et al., 2017). Detrusor overactivity is the most common typical finding in patients with iNPH and is possibly the cause of urinary urgency, which is thought to precede urinary incontinence.

7.3. Lumbar Infusion Test

Measurement of intracranial pressure (ICP) is limited with the diagnosis of NPH and thus, the use of CSF dynamic is preferred (Marmarou et al., 2005). In the lumbar infusion test (LIT), ICP is assessed through a continuous infusion of artificial CSF and saline with a lumbar needle into the subarachnoid region. The flow is directed against the ICP and may provide information about the dynamics of CSF. The sensitivity of LIT has been reported between 56% and 100% and specificity between 50 and 90%. The positive predictive value of LIT is 80% and negative predictive value is 16% (Kahlon, Sundbärg and Rehncrona, 2002; Keong et al, 2016).

7.4. Tap Test

A lumbar puncture via a sğinal test with the removal of a certain amount of CSF is used to identify potential patients who possibly respond to shunting. For this purpose, in a tao test 30-50 mL CSF is removed to assess symptom improvements. Improvement from a tap test is assessed clinically through gait, balance, and cognition assessment methods (Gallagher, Marquez and Osmotherly, 2018).

Various studies have reported sensitivity of a tap test between 26% and 62% and specificity between 33% and 100% (Wikkelsø et al., 2013).

7.5. External Lumbar Drainage

External lumbar drainage (ELD) is one of the methods used for the evaluation of NPH (Walchenbach et al. 2002). In ELD, effusion is applied at a rate between 5-30 mL/h with the patient given a horizontal position. It continues over 3 to 5 days. Positive effects of ELD can be demonstrated on MRI. Several studies have reported sensitivity of ELD between 50% and 100% and specificity between 60% and 100% (20).

7.6. Neuroimaging

Imaging techniques used to evaluate NPH include MRI and CT for visualization of ventricular enlargement and radiographies for the assessment of shunt functioning (Lehnert et al, 2011; Børgesen, Gjerris and Sørensen, 1979). MRI is superior over CT in allowing visualization of other markers of NPH and providing information that can exclude other reasons in the differential diagnosis. Nevertheless, CT can be used to exclude NPH and is suitable for screening patients who cannot tolerate MRI.

The main finding of NPH on CT or MRI is ventriculomegaly with no evidence of obstruction at the level of the 3rd or 4th ventricles. Ventriculomegaly is not specific to NPH, but the next step is to examine the degree of cortical atrophy to distinguish NPH from the other age related ventricular enlargement (Graff-Radford & Jones, 2019).

Ventriculomegaly is evaluated with Evans index which is the ratio of the largest width of the frontal horns and the widest measure of the inner table of the skull at that level. The ventricles are considered to be enlarged in an Evan index >0.3 (Relkin et al., 2005).

In patients with NPH, MRI may show a characteristic high-signal around the vertices. However, it is difficult to distinguish this finding from white matter changes in elderly or from that resembling subcortical vascular dementia. The extent of white matter disease may indicate the degree of cognitive impairment (Iddon et al., 1999).

Some parameters of magnetic resonance spectroscopy have been proposed as potentially useful in the diagnosis and follow-up of NPH

patients. However, the value of spectrometry for diagnosis and follow-up response in iNPH remains controversial (Espay et al. 2017).

8. TREATMENT

The patient is considered eligible for ventricular bypass (ventricular shunting) in the case of improved gait and cognition after tap test or continuous CSF drainage when no medical therapy is proven effective for NPH (Mori et al, 2012; Daou et al., 2016; Oliveira, Nitrini and Román, 2019). Ventriculoperitoneal bypass (VPB) is the preferred route for this purpose. After ventricular bypass, ventricular pressure is reduced and leads to improvement in cerebral perfusion (Kockum et al., 2018). Gait disturbance is most responsive to ventricular bypass followed by urinary incontinence and cognitive disorders.

As medical treatment, the use of acetazolamide and osmotic diuretics has been recommended to control hydrocephalus and for treatment of neuropsychiatric symptoms (Ghosh & Lippa, 2014). However, currently there is no guidelines recommending these drugs.

CONCLUSION

NPH is associated with a classic triad of cognitive impairment, gait disturbance, and urinary incontinence. NPH is associated with impaired CSF resorption. Its pathogenesis is unclear. Ventricular shunting is recommended for patients with clinical and MRI evidence of NPH and a positive tap test. In selected patients two-thirds can expect some benefits following ventricular bypass or shunting. However, shunting complications are common, making close follow-up of these patients critical.

REFERENCES

- Adams, R. D., Fisher, C. M., Hakim, S., Ojemann, R. G., & Sweet, W. H. (1965). Symptomatic occult hydrocephalus with "normal" cerebrospinal-fluid pressure. A treatable syndrome. *The New England Journal of Medicine*, 273, 117–126.
- Bech-Azeddine, R., Waldemar, G., Knudsen, G. M., Høgh, P., Bruhn, P., Wildschjødzt, G., Gjerris, F., Paulson, O. B., & Juhler, M. (2001). Idiopathic normal-pressure hydrocephalus: evaluation and findings in a multidisciplinary memory clinic. *European journal of neurology*, 8(6), 601–611.
- Blomsterwall, E., Svantesson, U., Carlsson, U., Tullberg, M., & Wikkelso, C. (2000). Postural disturbance in patients with normal pressure hydrocephalus. *Acta neurologica Scandinavica*, 102(5), 284–291.
- Boon, A. J., Tans, J. T., Delwel, E. J., Egeler-Peerdeman, S. M., Hanlo, P. W., Wurzer, H. A., Avezaat, C. J., de Jong, D. A., Gooskens, R. H., & Hermans, J. (1997). Dutch normal-pressure hydrocephalus study: prediction of outcome after shunting by resistance to outflow of cerebrospinal fluid. *Journal of neurosurgery*, 87(5), 687–693.
- Børgesen, S. E., Gjerris, F., & Sørensen, S. C. (1979). Cerebrospinal fluid conductance and compliance of the craniospinal space in normal-pressure hydrocephalus. A comparison between two methods for measuring conductance to outflow. *Journal of neurosurgery*, 51(4), 521–525.
- Brean, A., & Eide, P. K. (2008). Prevalence of probable idiopathic normal pressure hydrocephalus in a Norwegian population. *Acta Neurologica Scandinavica*, 118(1), 48–53.
- Brinker, T., Stopa, E., Morrison, J., & Klinge, P. (2014). A new look at cerebrospinal fluid circulation. *Fluids and barriers of the CNS*, 11, 10.
- Daou, B., Klinge, P., Tjoumakaris, S., Rosenwasser, R. H., & Jabbour, P. (2016). Revisiting secondary normal pressure hydrocephalus: does it exist? A review. *Neurosurgical focus*, 41(3), E6.
- Espay, A. J., Da Prat, G. A., Dwivedi, A. K., Rodriguez-Porcel, F., Vaughan, J. E., Rosso, M., Devoto, J. L., Duker, A. P., Masellis, M., Smith, C. D., Mandybur, G. T., Merola, A., & Lang, A. E. (2017). Deconstructing normal pressure hydrocephalus: Ventriculomegaly as early sign of neurodegeneration. *Annals of neurology*, 82(4), 503–513.
- Gallagher, R., Marquez, J., & Osmotherly, P. (2018). Gait and Balance Measures Can Identify Change From a Cerebrospinal Fluid Tap Test in Idiopathic Normal Pressure Hydrocephalus. *Archives of physical medicine and rehabilitation*, 99(11), 2244–2250.

- Ghosh, S., & Lipka, C. (2014). Diagnosis and prognosis in idiopathic normal pressure hydrocephalus. *American journal of Alzheimer's disease and other dementias*, 29(7), 583–589.
- Graff-Radford, N., Gunter, J.L., Ball, C.T., Crook, J.E. (2017) Ventriculomegaly is a biomarker of gait and cognitive decline. *Alzheimers Dement*; 13(7 suppl):1092.
- Graff-Radford, N. R., & Jones, D. T. (2019). Normal Pressure Hydrocephalus. *Continuum* (Minneapolis, Minn.), 25(1), 165–186.
- Graff-Radford, N. R., Knopman, D. S., Penman, A. D., Coker, L. H., & Mosley, T. H. (2013). Do systolic BP and pulse pressure relate to ventricular enlargement?. *European journal of neurology*, 20(4), 720–724.
- Hakim, S., & Adams, R. D. (1965). The special clinical problem of symptomatic hydrocephalus with normal cerebrospinal fluid pressure. Observations on cerebrospinal fluid hydrodynamics. *Journal of the neurological sciences*, 2(4), 307–327.
- Hebb, A. O., & Cusimano, M. D. (2001). Idiopathic normal pressure hydrocephalus: a systematic review of diagnosis and outcome. *Neurosurgery*, 49(5), 1166–1186.
- Iddon, J. L., Pickard, J. D., Cross, J. J., Griffiths, P. D., Czosnyka, M., & Sahakian, B. J. (1999). Specific patterns of cognitive impairment in patients with idiopathic normal pressure hydrocephalus and Alzheimer's disease: a pilot study. *Journal of neurology, neurosurgery, and psychiatry*, 67(6), 723–732.
- Illiff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., Benveniste, H., Vates, G. E., Deane, R., Goldman, S. A., Nagelhus, E. A., & Nedergaard, M. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β . *Science translational medicine*, 4(147), 147ra111.
- Jaraj, D., Agerskov, S., Rabiei, K., Marlow, T., Jensen, C., Guo, X., Kern, S., Wikkelsø, C., & Skoog, I. (2016). Vascular factors in suspected normal pressure hydrocephalus: A population-based study. *Neurology*, 86(7), 592–599.
- Kahlon, B., Sundbärg, G., & Rehncrona, S. (2002). Comparison between the lumbar infusion and CSF tap tests to predict outcome after shunt surgery in suspected normal pressure hydrocephalus. *Journal of neurology, neurosurgery, and psychiatry*, 73(6), 721–726.
- Keong, N. C., Pena, A., Price, S. J., Czosnyka, M., Czosnyka, Z., & Pickard, J. D. (2016). Imaging normal pressure hydrocephalus: theories, techniques, and challenges. *Neurosurgical focus*, 41(3), E11.

- Kiefer, M., & Unterberg, A. (2012). The differential diagnosis and treatment of normal-pressure hydrocephalus. *Deutsches Arzteblatt international*, 109(1-2), 15–26.
- Klassen, B. T., & Ahlskog, J. E. (2011). Normal pressure hydrocephalus: how often does the diagnosis hold water?. *Neurology*, 77(12), 1119–1125.
- Kockum, K., Lilja-Lund, O., Larsson, E. M., Rosell, M., Söderström, L., Virhammar, J., & Laurell, K. (2018). The idiopathic normal-pressure hydrocephalus Radscale: a radiological scale for structured evaluation. *European journal of neurology*, 25(3), 569–576.
- Kreffft, T. A., Graff-Radford, N. R., Lucas, J. A., & Mortimer, J. A. (2004). Normal pressure hydrocephalus and large head size. *Alzheimer disease and associated disorders*, 18(1), 35–37.
- Krzastek, S. C., Bruch, W. M., Robinson, S. P., Young, H. F., & Klausner, A. P. (2017). Characterization of lower urinary tract symptoms in patients with idiopathic normal pressure hydrocephalus. *Neurourology and urodynamics*, 36(4), 1167–1173.
- Kuriyama, N., Miyajima, M., Nakajima, M., Kurosawa, M., Fukushima, W., Watanabe, Y., Ozaki, E., Hirota, Y., Tamakoshi, A., Mori, E., Kato, T., Tokuda, T., Urae, A., & Arai, H. (2017). Nationwide hospital-based survey of idiopathic normal pressure hydrocephalus in Japan: Epidemiological and clinical characteristics. *Brain and behavior*, 7(3), e00635.
- Lehnert, B. E., Rahbar, H., Relyea-Chew, A., Lewis, D. H., Richardson, M. L., & Fink, J. R. (2011). Detection of ventricular shunt malfunction in the ED: relative utility of radiography, CT, and nuclear imaging. *Emergency radiology*, 18(4), 299–305.
- Lenfeldt, N., Larsson, A., Nyberg, L., Andersson, M., Birgander, R., Eklund, A., & Malm, J. (2008). Idiopathic normal pressure hydrocephalus: increased supplementary motor activity accounts for improvement after CSF drainage. *Brain : a journal of neurology*, 131(Pt 11), 2904–2912.
- Marmarou, A., Young, H. F., Aygok, G. A., Sawauchi, S., Tsuji, O., Yamamoto, T., & Dunbar, J. (2005). Diagnosis and management of idiopathic normal-pressure hydrocephalus: a prospective study in 151 patients. *Journal of neurosurgery*, 102(6), 987–997.
- Mori, E., Ishikawa, M., Kato, T., Kazui, H., Miyake, H., Miyajima, M., Nakajima, M., Hashimoto, M., Kuriyama, N., Tokuda, T., Ishii, K., Kaijima, M., Hirata, Y., Saito, M., Arai, H., & Japanese Society of Normal Pressure Hydrocephalus (2012). Guidelines for management of idiopathic normal pressure

- hydrocephalus: second edition. *Neurologia medico-chirurgica*, 52(11), 775–809.
- Oliveira, L. M., Nitrini, R., & Román, G. C. (2019). Normal-pressure hydrocephalus: A critical review. *Dementia & neuropsychologia*, 13(2), 133–143.
- Passos-Neto, C., Lopes, C., Teixeira, M. S., Studart Neto, A., & Spera, R. R. (2022). Normal pressure hydrocephalus: an update. *Arquivos de neuro-psiquiatria*, 80(5 Suppl 1), 42–52.
- Pettorossi, V. E., Di Rocco, C., Mancinelli, R., Caldarelli, M., & Velardi, F. (1978). Communicating hydrocephalus induced by mechanically increased amplitude of the intraventricular cerebrospinal fluid pulse pressure: rationale and method. *Experimental neurology*, 59(1), 30–39.
- Relkin, N., Marmarou, A., Klinge, P., Bergsneider, M., & Black, P. M. (2005). Diagnosing idiopathic normal-pressure hydrocephalus. *Neurosurgery*, 57(3 Suppl), S4–v.
- Ringstad, G., Vatnehol, S., & Eide, P. K. (2017). Glymphatic MRI in idiopathic normal pressure hydrocephalus. *Brain : a journal of neurology*, 140(10), 2691–2705.
- Román, G. C., Jackson, R. E., Fung, S. H., Zhang, Y. J., & Verma, A. K. (2019). Sleep-Disordered Breathing and Idiopathic Normal-Pressure Hydrocephalus: Recent Pathophysiological Advances. *Current neurology and neuroscience reports*, 19(7), 39.
- Rosseau, G. (2011). Normal pressure hydrocephalus. *Disease-a-month : DM*, 57(10), 615–624.
- Stolze, H., Kuhtz-Buschbeck, J. P., Drücke, H., Jöhnk, K., Illert, M., & Deuschl, G. (2001). Comparative analysis of the gait disorder of normal pressure hydrocephalus and Parkinson's disease. *Journal of neurology, neurosurgery, and psychiatry*, 70(3), 289–297.
- Takada, L. T., Caramelli, P., Radanovic, M., Anghinah, R., Hartmann, A. P., Guariglia, C. C., Bahia, V. S., & Nitrini, R. (2003). Prevalence of potentially reversible dementias in a dementia outpatient clinic of a tertiary university-affiliated hospital in Brazil. *Arquivos de neuro-psiquiatria*, 61(4), 925–929.
- Tsakanikas, D., Relkin, N. (2007) Normal pressure hydrocephalus. *Semin Neurol*, 27:58–65.
- Tisell M. (2005). How should primary aqueductal stenosis in adults be treated? A review. *Acta neurologica Scandinavica*, 111(3), 145–153.

- Walchenbach, R., Geiger, E., Thomeer, R. T., & Vanneste, J. A. (2002). The value of temporary external lumbar CSF drainage in predicting the outcome of shunting on normal pressure hydrocephalus. *Journal of neurology, neurosurgery, and psychiatry*, 72(4), 503–506.
- Wikkelsø, C., Hellström, P., Klinge, P. M., Tans, J. T., & European iNPH Multicentre Study Group. (2013). The European iNPH Multicentre Study on the predictive values of resistance to CSF outflow and the CSF Tap Test in patients with idiopathic normal pressure hydrocephalus. *Journal of neurology, neurosurgery, and psychiatry*, 84(5), 562–568.
- Williams, M. A., & Malm, J. (2016). Diagnosis and Treatment of Idiopathic Normal Pressure Hydrocephalus. *Continuum* (Minneapolis, Minn.), 22(2 Dementia), 579–599.
- Wilson, R. K., & Williams, M. A. (2007). Evidence that congenital hydrocephalus is a precursor to idiopathic normal pressure hydrocephalus in only a subset of patients. *Journal of neurology, neurosurgery, and psychiatry*, 78(5), 508–511.

CHAPTER 5

MANAGEMENT OF LEFT RENAL WILMS' TUMOR DURING SURGICAL EXPLORATION

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1.HISTORY

The first case of Wilms' tumor (WT) in the literature was published by Thomas F. Rance in 1814 with a different nomenclature (Rance TF, 1814). The first nephrectomy for WT was performed by Jessop in 1877 in a 2-year-old patient in England (Anonymous, 1877). In 1879, William Osler presented a series of 2 cases and his own 4 cases (Osler W, 1879). In 1899, Karl Maximillian Wilhelm 'Max' Wilms published a series of 7 cases and the nomenclature "Wilms' Tumor" was used for the first time in the literature (Wilms M, 1899).

In 1938, William Ladd "the great pioneer pediatric surgeon of North America" standardized the surgical technique but the survival rate in those years was only 20% (Ladd WE, 1938). Due to the low survival rate, different treatment methods were developed over the years. Friedlander defined the radiotherapy in treatment of WT in 1916 and the first steps of neoadjuvant treatment were taken in Europe by administration of radiotherapy before surgery. The addition of actinomycin-D to the protocol, led to a decrease in mortality in the 1950s, and the use of vincristine, another chemotherapeutic agent, in 1963 laid the foundations of today's modern treatment protocols (Macmahon HE et al, 1963).

The National Wilms' Tumor Study Group (NWTSG) in 1969 and the Societe Internationale D'oncologie Pediatrique (SIOP) in 1971 were established to standardize WT treatment.

2.INTRODUCTION

WT is the most common solid renal tumor in children with an incidence of 6-7% and ranks 5th among childhood cancers. According to North American data, the incidence in the pediatric population under 15 years of age is 8.1/1.000.000 and is growing by 650 new cases every year.

According to 2010 Turkish Association of Pediatric Oncology Group's data, 80 new cases are added every year. Although the most common age group is between 2-3 years, 95% of patients are under 10 years of age (Akyuz C et al, 2010).

3. CLINICAL FEATURES

WT usually presents as an asymptomatic mass in the abdomen. Abdominal pain, high fever and weight loss are the most common presenting complaints. Hematuria is also seen in 30% of the patients. Hypertension may also be observed as a result of renin secretion from the tumor. Acute abdomen may develop as a result of rupture or bleeding.

On physical examination, a painless, large, round, smoothly circumscribed mass that does not move with respiration is palpated in the abdomen. Varicocele due to vena cava inferior or renal vein thrombus may be detected (Figure 1). There may be a tendency of bleeding in 8-10% patients due to acquired vWF deficiency. Aniridia, hemihypertrophy and genitourinary anomalies may be found in 20-25% patients.

4. SIGNIFICANCE OF LEFT RENAL WILMS TUMOR IN SURGICAL EXPLORATION

The main goal of Wilms' tumor surgery is accurate staging and safe and complete removal of the tumor before any invasion. There are two different approaches in WT treatment protocols. The main reason for the difference in approaches lies in whether nephrectomy should be performed before or after chemotherapy.

According to the SIOP approach, preoperative chemotherapy reduces the risk of tumor capsule rupture. Tumor response and sensitivity to chemotherapy can be evaluated. Micrometastases can be prevented at an early stage and the need for postoperative radiotherapy decreases. However, it has been reported that non-WT pathologies were detected in 5% of patients who received chemotherapy considering WT.

In the NWTS approach, the priority is diagnosis with tissue sampling. Preoperative chemotherapy changes the extent and histopathology of the tumor and affects the type and the staging. In addition, preoperative chemotherapy has no effect on the overall survival rate of the patient.

The survival rates of both SIOP and NWTS groups were almost identical. Both groups agree that the patient should receive preoperative chemotherapy in the presence of thrombus in the inferior vena cava extending into the right atrium, bilateral disease, in patients with a very large mass at the time of diagnosis, and in special patients in whom the pediatric surgeon thinks that nephrectomy cannot be performed safely.

Surgical treatment is nephroureterectomy through a transperitoneal approach (Figure 2). Surgical margins should be negative. Routine lymph node dissection is not recommended. If WT invasion is suspected, wedge biopsy should be performed from the area. Routine exploration of the opposite kidney is not recommended. Biopsy is recommended in case of doubt. If the patient has received preoperative chemotherapy, the tumor size is small and the surgeon is experienced, nephroureterectomy can be performed laparoscopically.

Surgical removal of a large WT remains a challenging procedure. Certain anatomically based complications have been identified in the surgical removal of large WTs. The problems in surgical procedures stem from the neighbouring anatomical structures rather than the staging and removal of the WT. Intraoperative visceral organ injury (intestines, spleen, liver etc.) and complications related to vascular structures during WT resection occur in approximately 2% of cases. It has been reported that the risk of surgical complications increases during the removal of tumors with a diameter equal to or greater than 10 cm (Davidoff AM et al, 2016).

In this section, we will discuss the complications that may occur during surgical removal of large WTs originating from the left kidney, which may increase morbidity or mortality.

4.1. Complications related to the vascular structures

There are three compartments related to the kidney in the retroperitoneal space. These are perirenal space, anterior pararenal space and posterior pararenal space. The kidney is surrounded by anterior and posterior laminae of the renal fascia and fatty tissue overlying the fascia. There is a medial fixation between the adventitial covering of the renal vessels and the aorta or inferior vena cava

(Skandalakis JE et al, 2006). According to classical knowledge, radical ureteronephrectomy for WT requires removal of the intact Gerota's fascia and Zuckerkandl's fascia covering the kidney (Duffy PG et al, 2006).

During the mobilization and surgical resection of left renal WT, serious injuries may occur to the aorta and its branches and the inferior vena cava in the medial aspect of the Gerota's and Zuckerkandl's fascia. Iatrogenic rupture may occur due to traction of these fascias during the resection. In addition, the aorta may move to the left from its normal position during mobilization of the left renal WTs. Thus, the right renal artery may be displaced behind the left renal vein (Katmawi-Sabbagh S et al, 2007). This malposition increases the possibility of iatrogenic injury. This may lead to bleeding that may be difficult to control. Morbidity and mortality rates are significantly increased in case of hemorrhage over 50 cc/kg during the operation.

Although the left renal vein is located anterior to the aorta in most patients, there may be significant variations. Retroaortic left renal vein can be seen in up to 3% (Aljabri B et al, 2001). It has been reported that if the left renal vascular structures are not correctly identified during the mobilization of the left renal WT, the right renal artery, superior mesenteric artery and even the aorta can be injured (Tröbs RB, 2009). Ritchey et al. reported four patients who underwent superior mesenteric artery ligation during nephrectomy for WT (Ritchey ML et al, 1992). It is noteworthy that WT was of left renal origin in each of these patients.

Adjacent vascular structures such as the aorta, superior mesenteric artery and inferior vena cava are in close proximity of the tumor mass, especially in large tumors. Localization of the tumor may cause the opening of the plane between the aorta and the inferior vena cava. Again, due to lymphatic infiltration, these vascular structures may move away from each other and the classical anatomical vascular appearance may be obscured. This makes these structures vulnerable to injury during removal of left renal WTs.

During a difficult renal hilus dissection, especially in younger patients and patients with large WTs, the mesenteric branches of the aorta (such as the superior mesenteric artery) may be displaced and

stretched over the tumoral mass, making it difficult to distinguish it from a renal artery of similar diameter (Blunt LW et al, 2004). In addition, the presence of hilar lymphadenopathy may also increase the risk of such injury by making dissection difficult (Nevoux P et al, 2008). The incidence of superior mesenteric artery injury during left renal WT surgery may be underestimated. However, the consequences can be mortal. If superior mesenteric artery injury is not recognized intraoperatively, the postoperative clinical presentation of the patient may not lead to the correct diagnosis. Patients may have abdominal distension and early hematochezia. Suspicion is the key for early diagnosis and should be confirmed by doppler ultrasonography or computed tomography angiography. Children may tolerate the situation for a while, possibly with blood circulation from the inferior mesenteric artery (Deb M et al, 2021). Recognition of such a superior mesenteric artery injury requires immediate surgical intervention and restoration of perfusion. In this situation, it has been reported that autologous vascular grafts or synthetic grafts can be implanted to the aorta or anastomosed to the stump of the renal artery of the nephrectomised side under appropriate conditions (Deb M et al, 2021).

In order to avoid vascular injuries during surgical exploration, the left renal vein should be identified first. Following dissection of this vessel, another underlying vessel is exposed, which may be the superior mesenteric artery, aorta, or left or right renal artery. The surgeon should not be in a hurry at this time. If these vascular structures cannot be clearly identified during the surgical procedure or in case of doubt, these vessels should be clamped with vascular bulldog clamps to clarify the anatomy. If the vessels cannot be clearly identified in this way, blind surgical intervention should be avoided. In this case, intraoperative doppler ultrasonography can be used to identify the vessels.

In order to avoid such vascular accidents during the surgery, it is necessary to be aware of these vessels in advance. Doppler ultrasonography is the first choice for cross-sectional imaging in the pediatric age group. However, if ultrasonography is not sufficient due to radiologist or patient-related factors, magnetic resonance imaging is

also sensitive in evaluating the main vascular structures in the abdomen (Smillie RP et al, 2015). Although it is an advantage not to use ionizing radiation in magnetic resonance imaging, pediatric patients may require anesthesia due to the length of the procedure. In this case, computed tomographic imaging can be used as an alternative in difficult cases.

4.2. Complications related to the neighbouring organs

During the mobilization of the large left renal WT, complications from adjacent organs can occur. In an article published by Ritchey et al, surgical complications in primary nephrectomy for WT were reported according to the NTWS report (Ritchey ML et al, 2001). 76 complications occurred in 68 of 534 children. When complications related to neighbouring visceral organs were analysed, it was found that splenic injury occurred in six patients, diaphragmatic tear in two patients, and liver injury in one patient. After the completion of left renal WT resection, the integrity of the left lobe of the liver, spleen and tumor extending to the diaphragm should be checked in cases where the tumor extends to the diaphragm. Since postoperative diagnosis of such solid organ injuries may be difficult in a patient who has undergone surgery, it is important to recognize them intraoperatively.

The pancreas may also be needed to be mobilized in order to create a dissection plane. This may cause postoperative pancreatitis (Roth H et al, 1996). These patients should be followed closely in this respect in the postoperative period. Missed diagnosis of pancreatitis may increase postoperative morbidity and mortality.

5. CONCLUSION

Identifying the main vascular structures separately and avoiding uncontrolled manipulation of adjacent organs during left renal WT surgery, especially in cases with small patient age and large tumor size, will reduce morbidity and mortality by preventing complications in the postoperative period.

6.IMAGES



Figure 1: Left grade 3 varicocele due to left renal Wilms' tumor (Image obtained from the archive of Ali Sayan, M.D.).

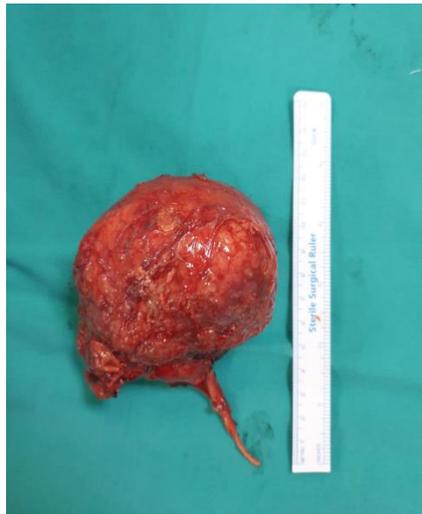


Figure 2: Nephroureterectomy specimen from a left renal Wilms' tumor in a 21-day-old infant (Picture taken from Mehmet Mert, Assist. Prof. Dr.'s archive).

REFERENCES

- Akyuz, C, Yalcin, B, Yildiz, I et al. Treatment of Wilms tumor: a report from the Turkish Pediatric Oncology Group (TPOG). *Pediatr Hematol Oncol*, 2010;27(3),161-178.
- Aljabri B, MacDonald PS, Satin R et al. Incidence of major venous and renal anomalies relevant to aortoiliac surgery as demonstrated by computed tomography. *Ann Vasc Surg*. 2001;15:615–618.
- Anonymous. Extirpation of the kidney. *Lancet*, i (1877), p. 889.
- Blunt LW, Jr , Matsumura J, Carter MF et al. Repair of superior mesenteric artery ligation during left nephrectomy with a native renal vein patch. *Urology*. 2004;64:377–378.
- Davidoff AM, Fernandez-Pineda I. Complications in the surgical management of children with malignant solid tumors. *Semin Pediatr Surg*. 2016 Dec;25(6):395-403. doi: 10.1053/j.sempedsurg.2016.10.003. Epub 2016 Oct 31. PMID: 27989364.
- Deb M, Jayaram H, Arlikar J. Superior Mesenteric Artery Injury during Radical Nephrectomy in an Infant: Delayed Diagnosis and Successful Management. *J Indian Assoc Pediatr Surg*. 2021 May-Jun;26(3):188-191. doi: 10.4103/jiaps.JIAPS_87_20. Epub 2021 May 17. PMID: 34321792; PMCID: PMC8286032.
- Duffy PG, Cuckow P. In: *Operative Pediatric Surgery*. 6th ed. Spitz L, Coran AG, editors. London: Hodder Arnold; 2006. pp. 703–708.
- Katmawi-Sabbagh S, Cuckow P. Mistaken ligation of the right renal artery: a risk in the surgical management of massive left-sided Wilms tumor. *J Indian Assoc Pediatr Surg*. 2007;12:156–157.
- Ladd WE. Embryoma of the kidney (Wilms' tumor). *Ann Surg*. 1938 Nov;108(5):885-902. doi: 10.1097/00000658-193811000-00010. PMID: 17857280; PMCID: PMC1386999.
- Macmahon HE, Bedizel M, Ellis CA. Vincristine (leurocristine) sulfate in the treatment of children with metastatic Wilms' tumor. *Pediatric Division, Southwest Cancer Chemotherapy Group. Pediatrics*. 1963 Nov;32:880-887. PMID: 14075630.
- M. Wilms. *Die Mischgeschwulste. I. Die Mischgeschwulste der Niere*, A. Georgi, Leipzig (1899).
- Nevoux P, Zini L, Villers A et al. Celiac axis and superior mesenteric artery: Danger zone for left nephrectomy. *J Endourol*. 2008;22:2571–2574.

- Osler W. Two cases of striated myo-sarcoma of the kidney. *J Anat Physiol*, 14 (1879), pp. 229-233.
- Rance TF. Case of Fungus Hæmatodes of the Kidnies. *Med Phys J*. 1814 Jul;32(185):19-25. PMID: 30493414; PMCID: PMC5713550.
- Ritchey ML, Lally KP, Haase GM et al. Superior mesenteric artery injury during nephrectomy for Wilms' tumor. *J Pediatr Surg*. 1992;27:612-615.
- Ritchey ML, Shamberger RC, Haase G et al. Surgical complications after primary nephrectomy for Wilms' tumor: report from the National Wilms' Tumor Study Group. *J AmCollSurg*. 2001 Jan;192(1):63-68; quiz 146. doi: 10.1016/s1072-7515(00)00749-3. PMID: 11192924.
- Roth H, Weirich A, Ludwig R et al. Die Resektion des Nephroblastoms: Probleme und Komplikationen –Auswertungen zur Nephroblastom studie SIOP 9/GPOH. *Langenbecks Arch Chir*suppl. 1996;113:1078–1083.
- Skandalakis JE, Skandalakis LJ, Zoras O. Posterior aspect of the abdominal viscera and retroperitoneum. In: Merlini MP, Martin RF, editors. *Multiorgan resection for cancer*. Stuttgart, New York: Thieme; 2006. pp. 26–46.
- Smillie RP, Shetty M, Boyer AC et al. Imaging evaluation of the inferior vena cava. *Radiographics*. 2015 Mar-Apr;35(2):578-592. doi: 10.1148/rg.352140136. PMID: 25763740.
- Tröbs RB. Anatomical basis for Wilms tumor surgery. *J Indian Assoc Pediatr Surg*. 2009 Apr;14(2):50-4. doi: 10.4103/0971-9261.55151. PMID: 20671845; PMCID: PMC2905530.

CHAPTER 6

**PROTEIN MALABSORPTION DISORDERS: THE DIETARY
THERAPY AND THE STUDIES OF NEW PRODUCT
DEVELOPMENT**

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1. INTRODUCTION

In our country, cereal and its products keeps an importance place due to its consumption and its place in economy. Cereals, thanks to its features such as cheapness and easily reachability, being the resource of intensive energy and of protein resource in complete biological value and having neutral taste and aroma, are commonly used in backward regions for preventing from unbalance nourishing human beings (Elgün and Ertugay, 1997).

Although nutrition habits differ for the various reasons, the products obtained from cereals and cereal products have a deniable importance in our nutrition (Battais et al., 2005; Özkaya, 1999).

There are also important effects of cereal and cereal products, which has so great importance on our health. For example, diet which is rich in terms of dietary fiber, taken into body together with cereals, and which has low fat, helps in protecting body against various diseases, especially cardiac diseases, stroke and cancer. However, in return to its positive effects, it is known that cereal and cereal products also lead to the various diseases (Köksel and Demiralp, 1994).

Protein-sensitive diseases are grouped as protein metabolism diseases and protein malabsorption diseases. Protein malabsorption diseases include especially celiac, the diseases such as temporal gluten intolerance, enteropathy sensitive to cow milk protein and enteropathy losing protein.

In celiac disease, as a result of gluten sensitivity, intestinal malabsorption occurs. The main reason for disease is sub-fraction of gluten protein, called gliadin and, depending on taking of foods containing gluten into body, especially vitamins and minerals, a decrease occurs in absorption of the basic nutritional elements that are necessary for body (Battais et al., 2005).

In celiac patients, it is reported that the products rye, barley and triticale which contain prolamine that is a gliadin homologue, also leads to sensitivity (Türksoy and Özkaya, 2006).

Celiac disease is accepted as the only food allergy that can continue lifelong and genetic disease the most seen. The therapy of the disease is in the form of gluten-free diet and, in case of meeting

insufficiency of nutritional element, of putting back nutritional elements of interest, and gluten-free diet has to be sustained lifelong. Although gluten-free diet most of celiac patient apply stops the symptoms forming due to disease and enables intestine to heal up, it is not an exact method of the therapy. Therefore, celiac patients have to keep out from every sorts of gluten-added product (food and cosmetics) (Anonymus, 2011).

In this study, the role of wheat protein in various malabsorption disease was discussed and new studies of product development carried out for celiac patients were compiled.

2. WHEAT PROTEINS

The structure of protoplasm of all livings, basic substance of animal tissues and components of many hormones and enzymes consist of proteins. In making enzyme tissues in body and repairing old cells proteins, proteins play important role (Özkara, 1998).

6-12% of endosperm layer, which firms 80-85% of wheat grains, is protein, and it contains 72% of the protein in wheat. Although aleurone layer forms a part of 7% of wheat grain, it contains 15% in its own structure (Wadhawan, 1988).

There are 10-14% proteins in all grains and 40-50% in embryo. The wheats containing proteins more than 15% are referred to gluten-rich wheats and the ones containing protein less than 15%, gluten-poor wheats. Small grains contain more protein than big ones; summer wheats, than winter wheats and hard wheats, than soft wheats (Özkara, 1998).

2.1. Classification of Wheat Proteins

Although cumulative indicators of consumption of foodstuffs are the amount of per capita calorie, the first indicator of quality of food is amount of per capita protein a day in gram (Arroyave, 1975).

Proteins are generally classified as;

- a. Simple Proteins
- b. Compound Proteins

c. Derivative Proteins

Wheat proteins are assigned to the class of simple proteins and are divided into two as soluble (functional) and insoluble (depot) proteins. While soluble proteins are water –soluble albumin and salty water-soluble globulin, insoluble proteins are glutenin that forms essence and is soluble in weak acid and gliadin that is soluble in alcohol of 80 ° (Seçkin, 1988).

The various nutritional elements in wheat grain are not homogenously distributed. While there are more amount of cellulose, pentosane and mineral substance in the crust part of the grain, in embryo part, there are more amount of lipids, proteins, vitamins, and mineral substances and, in endosperm, starch and protein. Protein content in the grain gradually increase from center to wall (Özkaya and Evren, 1986).

In terms of nutrition, interest in cereal proteins especially arises from the compositions of amino acids they contain and, indirectly, from the existent essential amino acids. This feature comes to our face as determinative elements in evaluating cereal a fodder or human foodstuff (Elgün and Ertugay, 1995).

2.2. Gluten Protein and Its Properties

Depot proteins that are existent in cereals are principally examined in two groups ethanol-soluble prolamins and polymeric glutenins. Polyamines consist of wheat gliadins, rye secalins, barley hordeins, oat avenins and corn zeins that can be freely used in diet of celiac patient (Ciclitira et al., 2005).

It is reported that gluten proteins forming a large part of wheat proteins (80-85% of total proteins) is included in sub-class “polyamines” of depot proteins in cereal grains. Gluten proteins taking place in endosperm form a continuous matrix around starch granulates, shows a feature that is insoluble in water and salty water and consist of two functions as monomeric and polymeric glutenins. It is reported that these functions are almost in equal rates in the grains (Goesaert et al., 2005). However, it is also reported that gliadins are divided into sub-fractions as α , β , γ and ω (Ciclitira et al., 2005). Gliadin, among these

fractions, was found to be toxic for celiac patients and, glutenin, relatively less toxic. Among gliadin fractions, it is reported that while β is the most toxic and γ -gliadin has lower toxicity, that ω -gliadin has the least toxicity (Özkaya, 1999). Toxicity of prolamins in oat is still discussible and, in gluten-free diet, although there is not any a full consensus about the role of oat, while 10% of total proteins in oat consist of prolamins, that this rate rises to 70% in wheat seems to be the reason for that some celiac patients can better tolerate to oat compared to wheat. Besides that prolamins are present in bakery products such as bread, cookie, cake, pastry, prepared by using the flours of the wheat, barley, oat, rye, due to its features such as being thinner, developing texture, absorbing water and oil, they are also contented in ready foods such as meat, sausage and soup (Denery-Papini et al., 1999).

Besides that, gluten is responsible for viscoelastic specifications of dough and its ability to be able to absorb gas during fermentation, it also contributes to texture features of bakery products such as appearance and structure of crumb. Withdrawal of gluten from the formula leads to the various troubles such as less bread volume, easily friable crumb texture, color and other defect of quality (Gallagher et al., 2004).

3. PROTEIN MALABSORPTION DISEASES

3.1. Temporal Gluten Intolerance

In case of seeing gastrointestinal infections, it was reported very less number of cases showing temporal gluten intolerance with the symptoms similar to celiac disease. These phenomena respond very well to gluten-free diet and, after a while, with adding gluten to diet, it does not relapse. Distinguishing this case from celiac disease is important in terms of applying solid and unnecessary dietary therapy to children (Köksal and Gökmen, 2013).

3.2. Celiac Disease (Gluten Enteropathy)

Celiac disease is a food intolerance, seen as a result of taking gluten and similar proteins that are included in cereals in body and called “gluten sensitive intestinal system” (Özkaya, 1999). The main factor in this disease is sub-fraction of gluten that is a wheat protein,

called gliadin. In ill individuals, with consuming foods containing gluten, villi realizing absorption in the internal surface of small bowel shorten and moreover, in some case, it disappears and internal surface of intestine becomes even. It is reported that thickening occurs in uniserial "crypt" cells in the surface of villi and, depending on this, due to decrease of surface area, where absorption actualizes, that nutrient intake becomes difficult (Özkaya, 1999). Disease of interest emerges as a result of interactions of the factors such as genetic and dietary habits, taking breast milk, age beginning age for glutinous foods in supplementary diet and daily amount of consumption. In the period of early childhood (the first 2 ages), symptoms of disease are diarrhea, vomiting, loss of appetite, abdominal distention, weight loss, constipation growth retardation. In old ages, symptoms such as untreatable and non-detectable anemia and decrease in bone mineralization are seen. Just as celiac disease can emerge in any period of the life with typical symptoms, it is also reported that its being to be able to very lightly steer in some patients without giving any symptom makes difficult diagnosis. For diagnosing the disease, it is suggested that it is necessary to identify the levels of anti-gliadin antibodies, endomysium antibodies and transglutaminase antibodies, in case that at least of these antibodies are positive, that it is obligatory to make small bowel biopsy with the suspicion of celiac disease (Urgancı, 2005).

The first data about celiac disease was obtained by Kapodokyah Aretaeus in BC 1st century. Although celiac disease was more prevalent in age 1-5 children in 1888, it was reported that it was defined as chronic dyspepsia that can be in all age groups (Köksal and Gökmen, 2013). As a result of wheat scarcity seen in 2nd World War, it was seen that there was a decrease in complaints of celiac patients (Krause, 1968).

Celiac disease impacts 0.6-1.0% of world population (Biagi et al., 2010). It is reported that this disease is one of the most frequently met of genetic oriented disease in Europe (Kavak, 2002). In a study carried out, it was found that the incidence of disease in our country was 1:115 (Ertekin et al., 2005). It is suggested that incidence of disease is more in the girls compared to the boys and, in first degree relations of the

individuals with celiac, that incidence of celiac is 10% (Köksal and Gökmen, 2013). Sprue is called the form of this disease seen in adults. That the first symptoms of the disease in children is seen emerges with beginning the extra nutrients containing wheat, rye and oat. When extra nutrients of interest are given in low amounts, due to some ignorable cases, it is reported that the patient comes to the clinic with a heavier table and that symptoms begins to disappear with excluding the products such as wheat, barley, rye and oat from diet (Köksal and Gökmen, 2013).

It is suggested that celiac disease affect people very differently and that symptoms may emerge in childhood and adulthood. One of the factors considered that is also effective on disease is duration of breast milk. In the people, who took with relatively longer duration, it is reported that the symptoms of disease emerge later. Another factor that is effective is beginning age for glutinous foods and consumption amount of gluten (Anonymous, 2004).

3 factors that are necessary to be considered in diagnosing celiac disease are as follows:

1. Identification of disorder in absorption process in intestine,
2. As a result of biopsy of small bowel, identification of remarkable changes in mucosae,
3. When gluten-free diet is applied, improvement in clinic response of patient. (Demirdağ, 1985).

The types of celiac disease is stated as classical (typical) form, atypical form, asymptomatic (silence) form, latent-potential cereal disease and temporal gluten intolerance (Kavak, 2002).

The only therapy of celiac disease is to apply dietary treatment. At the end of the therapy, the villus damaged disease, completely healing, can sustain their functions. Healing process changes, depending on the age of the patient and, while this time changes between 3 and 6 months in children, it is seen it can take two years in adults. Gluten-free diet has to be sustained lifelong (Akçelenk, 2004). In case that good response cannot be taken by applying gluten-free diet,

intravenous nutrient supplement is made. In addition, it is reported that medication is preferred some patients (Anonymous, 2004).

It is suggested that diets proposed for celiac patients has to be prepared in such a way that it will provide the balance of energy, protein, fat, vitamin and mineral, considering malnutrition condition of the patient (Köksal and Gökmen, 2013). Diet should contain 25% more calorie than normal requirement of calorie and 6-8 g/kg protein per day. Fat should be given in normal amount, while lactose that is not tolerated in acute period has to be constrained. Cooking methods applied should be carefully selected, red and green cabbage, onion, fried food, spices and cooking oil-added nutrients can be slowly added to diet. After providing a general healing, all gluten-free stuffs can be consumed. The point to be taken into consideration here is that the nutrients are gluten-free (Köksal and Gökmen, 2013).

3.3. Enteropathy

Cows' milk having protein content at high quality contains protein in average rate of 3.0-3.5%. It is known that cows' milk protein is a heterogeneous mix consisting of especially casein whey protein, enzymes and non-protein compounds containing a little amount of protein (Fox et al., 2003). Approximate 80% of total protein consist of casein (inorganic substance, 8%; 92%, protein) and 20%, whey. Casein is a protein, which contains ester-bonded phosphate, proline in high rate and has low solubility at pH 4-5. It has four functions as α , β , γ and κ . Among these, that having one the highest antigenicity is β lacto globulin, and this protein is responsible for the disease of 82% of the patients. Many baby formulas prepared as milk-based are obtained by denaturation and all of them contain β -globulin (Köksal and Gökmen, 2013). Milk protein containing vital amino acids such as leucine, isoleucine, valine, methionine, phenyl amine, threonine, tryptophan, lysine is biologically accepted as high quality protein and, thanks to this, it is suggested that it is used as standard reference in evaluating quality of protein in the other foods (Arabacıoğlu, 1993; Miller et al., 2000; Baysal, 2004).

In this disease seen in the children of the families, which are begun to be postnatally given cow milk, and which have sensitivity to cows' milk in family story, just as vomits are prenatally seen in the baby become difficult nutrition intake, it also causes protein loss. Diarrhea that cannot be stopped in the patient is prevented by excluding cows' milk and milk-based baby formulas from diet and giving soya-based baby formulas. The aim here is to diagnose before impeding the growth and development in child, namely, emerging malnutrition and to fix clinic table by giving foods, in which the main nutrient is breast milk, and which consist of soya-based formulas, vegetable soup, fruit juice, and egg and meat-added nutrients. Another issue that is to be useful in remarking is to also see intolerance against proteins in the other foods in large majority of these babies. In many babies against cow milk proteins, it is stated that intolerance can also develop against soya, wheat and egg proteins. After taking soya protein, the symptoms such as vomit, diarrhea, fever, tachypnea, cyanoses, eczema, bronchiolitis may occur. In these babies, various formulas are used, which contain whey protein-weighted middle-chain fatty acids (Köksal and Gökmen, 2013).

3.4. Protein-Losing Enteropathy

Protein-losing Enteropathy is a disease, which is characterized with ulcerations and excessive destruction of cell, and which is remarkable with loss of more amount of protein from digestion system and holds other diseases in its root. Just as Protein-Losing Enteropathy can arise from digestion system diseases, it can develop, depending on some non-digestion diseases (Proujansky, 2004; Braamskamp et al., 2010).

Protein-Losing Enteropathy may be the cause or symptom of many diseases. To give some examples, it is a case that can be seen in diseases such as allergic gastroenteritis, celiac disease, cystic fibrosis, cow milk protein allergy, tropical sprue, some parasitary diseases, abetalipoproteinemia (Köksal and Gökmen, 2013).

As a result, any disease, which holds any place of gastrointestinal system, can be the cause of Protein-Losing Enteropathy. If there is

more loss of protein in these diseases damaging mucosae of small bowel, hypoalbuminemia occurs. Hepatic protein synthesis is inadequate and, if there are malnutrition, malabsorption and concomitant infections, immune system is damaged. As a result of hypoalbuminemia, edema forms. Since malnutrition is resulted with loss of appetite, retardation of growth and development, and avitaminosis occur (Köksal and Gökmen, 2013). In Protein-Losing Enteropathy, protein loss as well as iron, lipids and traces elements loses form from intestines (Greenwald, 2006). Especially deficiencies of A, D, E, C and K vitamins are the most seen vitamin deficiencies in the patients. In case that oral intakes becomes difficult, it is suitable to give diet that is rich in terms of energy, protein and vitamins as well as formulas containing middle chain fatty acids and to meet protein requirement of the patient by means of energy and protein-modulated formulas (Köksal and Gökmen, 2013).

4. THE STUDIES ON PRODUCTION OF GLUTEN-FREE PRODUCTS FOR CELIAC PATIENTS

Gluten deficiency, depending on decrease of the properties of elasticity and being able to hold gas of the dough, causes the various defects of quality in bakery products and, in order to eliminate these defects, especially in the studies in the area of bakery, it was suggested that the properties of dough was to be able to hold gas could be kept provided that another gel replaced with gluten. Since gluten impeded to decompose thanks to its strong protein networks, preparation of pasta and similar products becomes more difficult in absence of gluten. In cookie production, texture is related to starch gelatinization and crystal sugar rather than protein/starch structure. For this reason, it is stated that defects are rarely encountered during cookie production (Gallagher et al., 2004).

In the recent years, in order to develop the taste, acceptability and shelf life of the products not containing gluten, the studies, in which the possibilities of starch, dairy products, gums, hydrocolloids and gluten-free other proteins to be used are researched, have been carried out (Gobbetti et al., 2007).

The rice starch commonly present in starch and hydrocolloids, commonly used in developing texture and appearance properties of cereal-based products, is commonly used in formulation of gluten-free bakery products. Rice has desirable properties for special diets, which do not contain gluten, has low amount of sodium, and in which easily digestible carbohydrate content is high. But that rice flour does not contain gluten creates problems, when it is used in production of bread. In a study carried out on the role of starch in bread production, it was demonstrated that breads could be prepared by means of components forming starch and gel (Gallagher et al., 2004). In another study carried out, when rice starch of 3-9 is used in bread formulation instead of wheat starch, it was observed that the color crumb became less yellow and crust took a darker color (Gallagher et al., 2002).

In a study carried out, rheological and cooking features of gluten-free noodles prepared with the dry and wet ground rice flour were characterized. The dry ground rice flour, in the conditions of room temperature, with higher starch injury, showed more water absorption feature than the wet ground rice flour. However, pasting results of rice flour suspensions demonstrated that the wet ground rice flour, thanks to perfect swelling power of on starch gelatinization, had higher viscosity value. Similar thermos-mechanic tendency was observed by Mixolab on rice dough system. In the test with plenary expansion, noodle dough prepared with the dry ground rice flour, noodle samples showed higher elongation viscosity, which is related to more strength to expansion of the dry ground rice noodle strips. When rice noodles are cooked, in the dry ground rice noodles, the increased cooking loss was observed, which high solubility, resulted from higher degree of starch injury causes (Lee et al., 2013).

Cassava, known as manioc or tapioca root, is a rhizoid plant growing in tropical regions and containing starch in high rate. Starch of fermented manioc tapioca root was used by some researchers in the production of glute-free bread and cookie (Gallagher et al., 2004).

In a study carried out, the quality of pre-gelatinized flour, obtained from manioc starch and manioc pulp as well as gluten-free pasta, prepared with amaranth flour, was studied. Using pre-gelatinized

flour, natural manioc starch and amaranth flour in the rates of 10:60:30, respectively, was resulted with commercial wheat products in terms of color, texture and nutritional value. The use of manioc pulp increased the amount of diet fiber and it was concluded that it could be used in pasta production as ingredient (Fiorda et al., 2013).

In a study carried out to evaluate the effect of enzymes, hydrocolloids and emulsifiers on rheology of gluten-free dough, thermal characteristics and quality of bread, the breads were prepared in the composition of rice flour, manioc starch, full-oiled soya flour and water of 65-75%. The additives used such as emulsifiers (diacetyl tartaric acid esters of monoglycerides – DATEM and sodium sterol lactate -SSL), enzymes (glycose oxidase and α -amylase) and hydrocolloids (xantan gum, carboxymethylcellulos, alginate and carrageenan). The results demonstrated that adding additive to dough behavior was proved by the different calorimetric and rheological features. In addition, the electrophoretic features of the dough whose proteins are exclude changed with addition of glycose oxidase. These modifications were resulted with the different features such as specific volume, hardness, staling degree, crumbly structure. However, in the breads in the control group, quite better features of quality were observed. The samples of gluten-free breads taken from doughs in control group exhibited a good performance in terms of acceptable volume, crumbly structure, and low stalling rare during storing. In contrast to common view, this study showed that the presence of additives was not obligatory for production of non-gluten bread (Perez et al., 2012).

Buckwheat is a plant similar to bush belonging to *Polygonaceae* family. The seed of buckwheat is similar to cereals in terms of its chemical and structural composition (Pomeranz, 1983; Li and Zhang, 2001). Although digestibility of buckwheat proteins is rather low, thanks to well-balanced amino acid composition, it is suggested that it has a high biological value (Pomeranz and Robbins, 1972). Protein content of buckwheat flour, known that it has the highest protein content following oat, has higher rate than rice, wheat, sorghum and

maize. A large part of buckwheat protein consists of albumin and globulin fractions in contrast to wheat protein (Li and Zhang, 2001).

The absence of gluten in buckwheat made buckwheat flour an important part of gluten-free diets applied to celiac patients (Wijngaard and Arendt, 2006). Descaled buckwheat seed and buckwheat flour is a perfect ingredient in bread and cereal formulations. Immunological analyses show that buckwheat does not contain any detrimental prolamine (Aubrecht and Biacs, 2001).

It is reported that the seeds of buckwheat is ground and can be used in composition of bakery products by mixing the flours of the other cereal and that buckwheat flour can be used in production of noodle, bread, pasta, cake, crepe, cereal for breakfast, cornets and cookie (Marshall and Pomeranz, 1982).

In a study carried out, tarhana (soup with dried yoghurt), which is a traditional fermented agricultural product and is mostly prepared from wheat flour and yoghurt, was produced with buckwheat flour, rice flour and maize starch as gluten-free tarhana. While tarhanas of control group are produced from wheat flour, in-gluten free formulations was used 40% of buckwheat flour, 30% of rice flour and 30% of maize starch for the first formulation instead of wheat flour; for the second formulation, 60% of buckwheat flour, 20% of rice flour and 20% of maize starch. Addition of buckwheat in the rate of 60% increased the content of fat and ash in tarhana samples but negatively affected the brightness value of the samples. In the samples, the use of buckwheat in the increasing rates largely increased the potassium, magnesium and phosphor contents of gluten-free tarhana ($p < 0.05$). Sensorial analyses showed that addition of buckwheat showed that there were some change in density, taste, sourness and sandy structure. The samples of gluten-free tarhana containing 40% buckwheat had the most scores in tarhana and other all acceptability parameters (Bilgiçli, 2009).

In a study, in which the use of buckwheat in noodle production was researched, the noodle samples containing buckwheat flour of 30% showed the highest increase in weight and volume. When the gluten-free noodle samples containing buckwheat flour are compared with control group, high cooking loss was observed. In noodle samples

containing buckwheat of 30%, especially, the amounts of K and Mg were found high. In the result of sensorial analyses, the samples containing buckwheat flour of 20% had the highest scores (Bilgiçli, 2008).

In a study, carried out to identify the rheological, textural and sensorial properties of gluten-free bread formulations based on rice flour and buckwheat flour formulations mixolab was used. According to mixolab profiles obtained, it was stated that the rheological properties of the mix of rice flour and descaled buckwheat flour and of the mix of rice flour and scaled buckwheat flour were similar to wheat flour. Also in both types of mix, the rate of rice flour to wheat flour was arranged as 90:10, 80:20 and 70:30. According to mixolab profiles of the system studied, it was seen that gluten-free products containing descaled buckwheat flour had higher ability to absorb water, lower stability, weaker structure of protein network and lower viscosity compared to those ones containing scaled buckwheat flour. According to the results obtained from sensorial evaluation of the final products, it was concluded that all of gluten-free breads tested (6 formulations) was acceptable as sensorial (Hadnadev et al., 2010).

In the formulation of gluten-free cookie, it was seen that in the study, in which the effects of using buckwheat and lupine flours as well as different sorts of emulsifier (sodium stearyl 2- lactylate - SSL lecithin and SS lecithin), on some qualitative and sensorial properties of cookie are studied, it was seen that using buckwheat flour in cookie formulations reduced cookie diameter and diffusion rate and increased breaking strength. While buckwheat flour increased the amount of cellulose, phytic acid, Fe, K, Mg and P, lupine flour showed an effect increasing the amounts of especially protein, fat, cellulose, Ca, Mg and P. In sensorial evaluation, in terms of general acceptability, it was seen that additions of buckwheat flour of 10% and lupine flour of 15-20% had higher score (Yıldız, 2012).

In this study, for enriching gluten-free cakes, lupine or buckwheat flours were used. As a result, it was seen that while lupine flour increased the protein, calcium, iron, manganese, phosphor and zinc contents of gluten-free cakes, buckwheat flour considerably increased

especially potassium and magnesium contents. As a result of sensorial analysis, it was concluded that addition of lupine of 30% and buckwheat flours was acceptable (Levent and Bilgiçi, 2011).

In a study carried out, the properties of gluten-free crackers made of two types of buckwheat flour (refined and full cereal) was studied. Their approximate compositions, content of basic antioxidant compound (polyphenols and tocopherols), antioxidant activity (radical cleaning activity on 1,1-difenil-2-picrylhydrazyl radicals- DPPH), and sensorial quality were analyzed and these were compared with wheat crackers made of refined and full cereal wheat flour. While rutin and quercetin flavonoids were only identified in buckwheat and wheat crackers, protocatechic acid and ferulic acid were identified in buckwheat and wheat flours. When total content of phenolic and tocopherols is compared with that of wheat crackers, it was found considerably high in buckwheat crackers. Tocopherols that are existent in crackers were found as $\alpha \geq \gamma \geq \delta$ tocopherols. Buckwheat crackers considerably high ($p < 0.05$) radical cleaning activity on DPPH according to IC_{50} . In full cereal buckwheat crackers, when they are compared with full cereal wheat crackers, an important difference was not observed in sensorial quality (Sedej et al., 2011).

In a study, lupine (*Lupinus albus* L.) and buckwheat (*Fagopyrum esculentum* Moench) flours were used in the production of gluten-free bread. Depending on the increase of the rate of lupine flour, it was seen that while the weight, volume, crumb and the yellowness value of (b^*) crust color as well as the amount of water, protein, cellulose, fat, mineral substance (Ca, Cu, Mn, P, Fe and Zn) and essential amino acid, the values of crumb lightness (L^*) and of crumb of 24 and 72 hours decreased (Yarpuz, 2011).

In a study, in which the effects of buckwheat flour and various gums (guar gum, acacia gum, xanthan gum and gum tragacanth) on the production of gluten-free bread was studied, it was seen that addition of gums to buckwheat was considerably effective on the various parameters of quality such as ability of water absorption, ability of fat absorption, emulsion activity. In the cookies prepared, the properties the noise content, diameter, weight and thickness were found high,

while strength was low. Although addition of gums increased sensorial scores, when compared with the samples made with wheat flour, the samples had lower scores. Among gums, using xanthan gum provided development in the properties of cookies such as color, appearance, taste and acceptability (Kaur et al., 2014).

In a study, carried out to study the commonly used 7 gluten-free commercial flours (oat, rice, sorghum, maize, teff, buckwheat and quinoa) (and their nutritional compositions and to compare them wheat flour, in addition to the amounts of all main components of these gluten-free flours, mineral compositions, fatty acid profiles, phytate, polyphenols, and folate content was also determined. In addition, properties of carbohydrates were studied in detail and total amount of injured starch was determined. The contents of amylopectin as well as total soluble and insoluble dietary fiber were also studied. The results showed that rice and maize flour were poor (lower content of protein, fiber and folate), when considered their nutritional values. In return to this, quinoa and buckwheat that are cereal-like as well as teff showed desirable composition of fatty acid and content of high protein. Especially quinoa and teff are characterized by the higher contents of fiber, calcium magnesium and iron. Therefore, these flours can be shown as raw materials having high nutritional density (Arendt et al., 2012).

In a study, in which the use of quinoa flour in production of spaghetti are studied, quinoa and maize flours were used in spaghetti formulations. As a result of analyses made, for obtaining high viscosity values and preferable spaghetti, it was understood that it was necessary to apply short time thermal process and add low amount of quinoa. It was concluded that the suitable combination for final viscosity was obtained by applying short time thermal process to the different contents of quinoa (Amaya-Farfan et al., 2000).

In a study carried out, the use of chestnut flour in production of gluten-free bread, cake and cookie for celiac patients was studied. For this purpose, the samples, prepared by mixing chestnut flour with the sorts of local and import gluten-free flour, were evaluated by the panelists consisting of 22 celiac patients and 38 healthy individuals. In

the study, it was concluded that chestnut flour was a delicious product that can be used for production of cookie, but in terms of cost and general liking, that it was more preferable to use it by mixing with the other gluten-free flours (Seferoğlu, 2012).

In another study, the use of chestnut and rice flour in the different rates for bread production was studied. In this study, in which the effects of the rheological properties and hydrocolloids and emulsifiers on quality parameters of bread were studied, it was seen that the mixes of xanthan gum and guar gum prepared with 30% of chestnut flour and rice flour of 70% and the samples of the samples containing DATEM as emulsifier had the highest score in terms of hardness, specific volume, color and sensorial properties. However, when addition of gum mixes and emulsifier is not taken into consideration, it was seen that the increase in the amount of chestnut flour caused worsening in quality parameters (Sumnu et al., 2010).

In a study, rheological properties of pasta dough were studied by means of creep-recovery tests for the various formulations containing the mixes of guar gum (0.5%), casein (1%) and egg white (1%). For each formulation, non-gelatinized rice semolina was mixed with gelatinized rice semolina in the different rates (0, 25, 50, 75 and 100%). It was identified that gelatinization, proteins and gums had an effect on the rheology of pasta dough. From- creep- recovery data, it was seen that there was an increase in elasticity of the samples as gelatinized fraction increased. However, in the formulation of rice semolina gelatinized in the rate of 75 and 100%, it was seen that the samples produced had a rough texture. From the analysis results obtained, it was detected that the mixes of protein and guar gum could be used as a stabilizer together with pre-gelatinized of 50% sample of rice semolina (Sözer, 2009).

In a study, in order to enrich gelatin-free breads, rice flour, albumin, casein isolates and transglutaminase were used. Protein isolates made a considerably effect on gelatinization and making dough gel and reduced viscosity. Textural properties were firstly affected from addition of casein. Finally, it was concluded that the casein, albumin

and transglutaminase could be used as protein enriching mixes in fermented gluten-free products (Storck et al., 2013).

In another study, it was aimed to evaluate the development, product quality and allergenicity of gluten-free pasta enriched with protein. Pasta was enriched by using high protein flours such as gums as well as soya flour, channa flour, sorghum flour and concentrated whey protein. The pasta prepared was analyzed in terms of quality specifications, and immunological tests such as Dot-Blot and ELISA were applied. Cooking tests showed that cooking loss of gluten-free pasta was a little higher than that in control group, in which *Triticum durum* wheat is used and that adding gums reduced starch loss. Gluten-free pasta showed similar specification to control group in all other quality parameters. Amylose content of all pasta was found lower than control group. In addition, it was observed that less protein and higher protein could be digested. In immunological tests of Dot-Blot and ELISA, it was concluded that the pasta produced was suitable for celiac patients (Susanna and Prabhasankar, 2013).

In a study, in which the buckwheat and linseed flours are used in production of cookie and cake, it was found that the nutritive value of all products produced increased. The most important increase was seen in the protein content, amount of dietary fiber, those containing linseed and α -linoleic acid content. When amino acid composition is considered, it was concluded that amaranth was more useful in gluten-free products (Gambus et al., 2009).

In another study, in the production of gluten-free bread and cookie, the use of unprocessed or exploded amaranth flour was studied. For bread production, the best formulation was the formulation containing 60-70% of exploded and 30-40 % unprocessed amaranth flour and, for cookie production, formulation containing 20% of exploded and 30-13 % full cereal amaranth flour. As a result, in spite of adding hydrocolloids, doughs were found acceptable, and it was seen that gluten-free final products had high nutritional content (Barca et al., 2010).

In a study, wheat flour, made gluten-free by using sourdough lactic acid bacteria fermentation and fungal proteases, was used for the

production of experimental gluten-free pasta (E-GFp) according to traditional process via drying at low temperature. Chemical, technological, structural, nutritional and sensorial properties were characterized and commercial durum wheat pasta (C-DWp) was compared with commercial gluten-free pasta (C-GFp). By means of immunological analyses, it was seen that the remaining gluten concentration in hydrolyzed wheat flour was below 10 ppm. When E-GFp is compared to the other pasta showed quick water absorption and shorter optimum cooking time. Despite the absence of gluten network, when reinforcing pre-gelatinized rice flour is compared with C-GFp, it brought structural properties in E-GFp. In-vitro digestibility of E-GFp was concluded the highest. According to sensorial analyses, the properties of E-GFp were found acceptable (Rizzello et al., 2013).

In another study carried out, in order to develop quality of gluten-free breads, the effects of protease application on gluten-free rice were studied. It was found that the bread, treated by commercial protease obtained from *Bacillus stearothersophilus*, depending on the amount of enzyme added, had higher quality, a good appearance of crumb, high volume and soft structure. Stalling rate was turned out lower in enzyme-applied bread than that in control group. These results showed that enzyme application could be successfully used in increasing quality of gluten-free rice bread via digestion of rice proteins (Kawamura-Konishi et al., 2013).

In another study, in the production of cookie, noodle and pita, rice flour, maize flour, potatoes flour, chickpea flour, maize and potatoes starch were used in the different rates, it was reported that the samples containing 35% rice flour, 35% maize starch, 10% potatoes flour, 10% chickpea flour and 10% potatoes starch were the most enjoyed and acceptable sample as sensorial (Ergin, 2011).

In a study, in production of gluten-free cake, the effects of some vegetable gums on product quality in single or combined way. For this purpose, xanthan gum, guar gum and hydroxypropylmethylcellulose were used as single or dual –trial combinations. As a result, it was identified that cakes made by using vegetable gums in trial combinations gave better results (Yücel, 2009).

In another study, the utilization of taro flour in two different forms (raw or cooked) with shortening 40% or 50% for gluten-free cookie production was investigated. It was reported that, the taro flours in cookie formulation increased the moisture, total ash, protein, Ca, Fe, K, Mg, P, and Zn contents of the gluten-free cookies. When the technological, sensory and chemical properties of the cookies are considered together, it is concluded that taro flour can be used up to 20% with 40% shortening ratio, and up to 40% with 50% shortening ratio (Dilek and Bilgiçli, 2021).

5. CONCLUSION

In this study, protein malabsorption disorders were discussed, and the studies on new product development were tried to be compiled for especially celiac disease.

The only treatment for celiac disease is to follow a lifelong gluten-free diet. The applicability of the diet depends on the variety and deliciousness of the products. In recent studies, alternative products have been tried to be produced for celiac patients.

Although it is not of great physiological importance, gluten protein is of great importance in providing the characteristic properties of bakery products. With the removal of gluten from the formulation, negative effects may occur in parameters such as flavor and texture in the final product. It is thought that more studies should be done in order to eliminate these negative features of gluten-free products and to increase the variety of gluten-free products.

REFERENCES

- Akçelenk, E., 2004, Glutensiz bisküvi üretimi, Bitirme Tezi, *Selçuk Üniversitesi Gıda Mühendisliği Anabilim Dalı*, Konya, 2-8.
- Amaya-Farfan, J., Caperuto, L., C., Camargo, C., R., O., 2000, Performance of quinoa (*Chenopodium quinoa* Willd) flour in the manufacture of gluten-free spaghetti, *Journal of the Science of Food and Agriculture*, 81, 95-101.
- Anonymous, 2004, <http://www.scs.leeds.ac.uk/pfaf/index.html>
- Anonymous, 2011, <http://colyaklayasamak.com.tr/sikligi-nedir.html>
- Arabacıoğlu, Ö., Z., 1993, İçme Sütü Tüketiminin Arttırılması ve Okul Sütü Programları, Ankara.
- Arendt, E., K., Hager, A., Wolter, A., Jacob, F., Zannini, E., 2012, Nutritional properties and ultra-structure of commercial gluten free flours from different botanical sources compared to wheat flours, *Journal of Cereal Science*, 56, 239-247.
- Arroyave, G., 1975, Aminoacid requirements and protein calorie malnutrition.
- Aubrecht, E., Biacs, P., A., 2001, Characterization of buckwheat grain proteins and its products, *Acta Alimentaria* 30 (1), 71–80.
- Barca, A., M., C., Rojas-Martínez, M., E., Islas-Rubio, A., R., Cabrera-Chávez, F., 2010, Gluten-free breads and cookies of raw and popped amaranth flours with attractive technological and nutritional qualities, *Plant Foods for Human Nutrition* 65, 241–246.
- Battais, F., Courcoux, P., Popineau, Y., Kanny, G., Moneret-Vautrin, D. A. and Denery-Paini, S., 2005, Food allergy to wheat: differences in immunoglobulin E-binding proteins as a function of age or symptoms, *Journal of Cereal Science*, 42, 109-117.
- Baysal, A., 2004, Beslenme, *Hatipoğlu Yayınları*, Ankara.
- Biagi, F., Klersy, C., Balduzzi, D., Corazza, G., R., 2010, Are we not over-estimating the prevalence of coeliac disease in the general population? *Annals of Medicine*, 42, 557-61.
- Bilgiçli, N., 2008, Utilization of buckwheat flour in gluten-free egg noodle production, *Journal of Food, Agriculture & Environment*, Vol.6(2), 113 - 115.
- Bilgiçli, N., 2009, Enrichment of gluten-free tarhana with buckwheat flour, *International Journal of Food Science and Nutrition*, 60, 1-8.

- Braamskamp, M., J., Dolman, K., M., Tabbers, M., M., 2010, Clinical practise, Protein-losing enteropathy in children, *European Journal of Pediatrics*, 169, 1179-85.
- Ciclitira, P., J., Ellis, H., J., Lundin, K., E., A., 2005, Gluten-free diet—what is toxic? *Practice&Researh Clinical Gastroenterology*, 19 (3), 359-371.
- Demirdağ, B., 1985, Çocuk sağlığı ve hastalıkları, Cilt 1, 2. baskı, *Türkiye Klinikleri Yayınları*, Ankara.
- Denery-Papini, S., Nicolas, Y., Popineau, Y., 1999, Efficiency and limitations of immunochemical assays for the testing of gluten-free foods, *Journal of Cereal Science*, 30, 121-131.
- Dilek, N.M., Bilgiçli, N., 2021, Effect of taro [*Colocasia esculenta* (L.) Schott] flour and different shortening ratio on physical and chemical properties of gluten-free cookie, *Journal of Food Processing and Preservation*, <https://doi.org/10.1111/jfpp.15894>.
- Elgün, A., Ertugay, Z., 1995, Tahıl İşleme Teknolojisi, Atatürk Üniversitesi, *Ziraat Fakültesi Yayınları* No: 718, Erzurum.
- Elgün, A., Z., Ertugay, 1997, Tahıl İşleme Teknolojisi, Atatürk Üniversitesi, *Ziraat Fakültesi Yayınları* (Ders Notları, 3, Baskı), Yayın No: 297, Erzurum, 376.
- Ergin, A., 2011, Çölyak hastalarına özel bisküvi, erişte ve pide üretimi, *Yüksek Lisans Tezi*, Denizli.
- Ertekin, V., Selimoğlu, M., A., Kardaş, F., Aktaş, E., 2005, Prevalence of celiac disease in Turkish children, *Journal of Clinical Gastroenterology*, 39, 689-91.
- Fiorda, F., A., Soares Jr., M., S., da Silva, F., A., Grosmann, M., V., E., Souto, L., R., F., 2013, Microstructure, texture and colour of gluten-free pasta made with amaranth flour, cassava starch and cassava bagasse, *LWT - Food Science and Technology*, 54, 132-138.
- Fox, P., F., McWeeney, P., L., H., 2003, *Advanced Dairy Chemistry*, Newyork.
- Gallagher, E., Polenghi O, Gormley T., R., 2002, Improving the Quality of Gluten-Free Breads, *Farm and Food*, 12, 8–13.
- Gallagher, E., Gormley, T., R., Arendt, E., K., 2004, Recent advances in the formulation of gluten-free cerealbased products, *Trends Food Science and Technology*, 15, 143–152.
- Gambus H., Gambus F., Pastuszka, D., Wrona, P., Ziobro, R., Sabat, R., Mickowska, B., Nowotna, A., Sikora, M., 2009, Quality of gluten-free supplemented cakes

- and biscuits, *International Journal of Food Sciences and Nutrition*, 60 (S4), 31-50.
- Gobbetti, M., Rizzello, C., G., Cagno, R., Angelis, M., 2007, Sourdough lactobacilli and celiac disease, *Food Microbiology*, 24, 187–196.
- Goesaert, H., Brijs, K., Veraverbeke, W., S., Courtin, C., M., Gebrevers, K., Del cour, J., A., 2005, Wheat flour constituents: how they impact bread quality and how to impact their functionality, *Trends in Food Science & Technology*, 16, 12-30.
- Greenwald, D., 2006, Protein-losing enteropathy, *Gastrointestinal and liver disease*, 557-63.
- Hadnadev, M., Torbica, A., Dapcevic, T., 2010, Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour, *Food Hydrocolloids*, 24, 626-632.
- Kaur, M., Sandhu, K., S., Arora, A., Sharma, A., 2014, Gluten free biscuits prepared from buckwheat flour by incorporation of various gums: Physicochemical and sensory properties, *LWT - Food Science and Technology*, 1-5.
- Kavak, U., S., 2002, Çölyak Hastalığı (36 Hastanın klinik ve laboratuvar bulguları ve tanıda serolojik testlerin yeri), *Hacettepe Üniversitesi Tıp Fakültesi Çocuk Sağlığı ve Hastalıkları Tezi*, Ankara.
- Kawamura-Konishi, Y., Shoda, K., Koga, H., Honda, Y., 2013, Improvement in gluten-free rice bread quality by protease treatment, *Journal of Cereal Science*, 58, 45-50.
- Köksal, G., Gökmen, H., 2013, Çocuk Hastalıklarında Beslenme Tedavisi, *Hatiboğlu Yayınları*, Ankara.
- Köksel, H., Demiralp, M., 1994, Glutensiz Ekmek. *Unlu Mamülleri Dünyası*, 3 (5), 20-27.
- Krause, M., V., 1968, Food, nutrition and diet therapy, *W. B. Saunders Company*, USA.
- Lee, S., Heo, S., Lee, S. M., Shim, J., Yoo, S., 2013. Effect of dry- and wet-milled rice flours on the quality attributes of gluten-free dough and noodles, *Journal of Food Engineering* 116, 213-217.
- Levent, H., Bilgiçli, N., 2011, Enrichment of gluten-free cakes with lupin (*Lupinus albus* L.) or buckwheat (*Fagopyrum esculentum* M.) flours, *International Journal of Food Sciences and Nutrition*, 62(7), 725–728.
- Li, S., Zhang, Q.,H., 2001, Advances in the development of functional foods from buckwheat, *CRC Critical Reviews in Food Science and Nutrition*, 41(6), 451–464.

- Marshall, H., G., Pomeranz, Y., 1982, Buckwheat: description, breeding, production, and utilization, *Advances in Cereal Science and Technology*, 5, 157-210.
- Miller, G., D., Jarvis, K., J., McBean, L., D., 2000, *Handbook of Dairy Foods and Nutrition*, Newyork.
- Özkara, A., 1998, Buğday proteinlerinin özellikleri, *Türkiye Tahıl Sempozyumu*, Ankara.
- Özkaya, H., Evren, R., 1986, Enzimlerin ekmekçilikteki önemi, Ankara Üniversitesi, Ziraat Fakültesi, Yayın No: 5 Basımevi Ankara.
- Özkaya, B., 1999, Tahılların neden olduğu alerjiler ve önemi-2, *Food Hi-Tech*, 82-88.
- Perez, G., T., Sciarini, L., S., Ribotta, P., D., Leon, A., E., 2012, Incorporation of several additives into gluten-free breads: Effect on dough properties and bread quality, *Journal of Food Engineering*, 111, 590-597.
- Pomeranz, Y., Robbins, G. S. 1972, Amino acid composition of buckwheat, *Journal of Agricultural and Food Chemistry*, 20, 270-274.
- Pomeranz, Y., 1983, Buckwheat: Structure, composition, and utilization, *CRC Critical Reviews in Food Science and Nutrition*, 19 (3): 213-258.
- Proujansky, R., 2004, Protein-losing enteropathy, *Pediatric gastrointestinal disease*, 194-202.
- Rizzello, C., G., Curiel, J., A., Coda, R., Limitone, A., Katina, K., Raulio, M., Giuliani, G., Gobbetti, M., 2013, Manufacture and characterization of pasta made with wheat flour rendered gluten-free using fungal proteases and selected sourdough lactic acid bacteria, *Journal of Cereal Science*, 1-9.
- Seçkin, R., 1988, *Deneyisel ekmekçilik ders notları*, Ankara Üniversitesi, Ziraat Fakültesi, Ankara.
- Sedej, I., Sakac, M., Mandic, A., Misan, A., Pestoric, M., Simurina, O., Canadanovic-Brunet, J., 2011, Quality assessment of gluten-free crackers based on buckwheat flour, *LWT-Food Science and Technology*, 44, 694-699.
- Seferoğlu, B., 2012, Çölyak hastalarına yönelik kestane unu ve glutensiz unlarla hazırlanan ekmek, kek ve bisküvi çeşitlerinin duyu analizi ile değerlendirilmesi, *Yüksek Lisans Tezi*, Ankara.
- Sözer, N., 2009, Rheological properties of rice pasta dough supplemented with proteins and gums, *Food Hydrocolloids*, 23, 849-855.
- Storck, C., R., Zavareze, E., R., Gularte, M., A., Elias, M., C., Rosell, C., M., Dias, A., R., G., 2013, Protein enrichment and its effects on gluten-free bread characteristics, *LWT - Food Science and Technology*, 53, 346-354.

- Sumnu, G., Demirkesen, İ., Mert, B., Şahin, S., 2010, Utilization of chestnut flour in gluten-free bread formulations, *Journal of Food Engineering*, 101, 329–336.
- Susanna, S., Prabhasankar, P., 2013, A study on development of gluten-free pasta and its biochemical and immunological validation, *LWT-Food Science and Technology*, 50, 613-621.
- Türksoy, S., ve Özkaya B., 2006, Gluten ve çölyak hastalığı, *Türkiye 9. Gıda Kongresi*, Bolu.
- Urgancı, N., 2005, Çölyak hastalarına ekmeğe zehir oluyor, http://212.174.46.149/w/dergi/basinpdf/kasim2004/18_19_20.pdf.
- Wadhawan, C., K., 1988, Fundamentals studies on vitilaty of gluten for breadmaking, *Thesis University of Monitoba*, Winnipeg M.B.
- Wijngaard, H., H., Arendt, E., K., 2006, Buckwheat, *Cereal Chemistry*, 83 (4), 391–401.
- Yarpuz, D., 2011, Glutensiz ekmeğe üretimi üzerine araştırmalar, *Yüksek Lisans Tezi*, Konya.
- Yıldız, M., 2012, Karabuğday ve lüpen unlarının glutensiz bisküvi üretiminde kullanımı üzerine bir araştırma, *Yüksek Lisans Tezi*, Konya.
- Yücel, R., 2009, Glutensiz kek üretiminde kullanılan bazı zamların kalite üzerine etkisi, *Yüksek Lisans Tezi*, Adana.

CHAPTER 7
MODEL INSECTS FOR DETERMINING THE
PATHOGENICITY OF INFECTIONS

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1. INTRODUCTION

Vertebrates are used in the study and treatment of pathogens that cause disease in humans. Rabbits, rats and some fish species such as zebrafish are the most commonly used experimental animals. Mass production of vertebrate experimental animals under laboratory conditions is very costly. In addition, ethical approval is required for studies conducted to determine the effects of pathogens on vertebrate experimental animals (Tüfek and Özcan 2018; Aci et al. 2020). Therefore, in recent years, researchers have been conducting experiments on alternative experimental models to vertebrate experimental animals. Some invertebrate animals, insect, 3d printers and programs are used as an alternative experimental model to vertebrates (Love 2009; Sertçelik et al. 2018; Tunçsoy et al. 2021)

Insects are experimental animals that can be easily produced under laboratory conditions. Synthetic or semi-synthetic diets are used in the diet of many model insects (Sugeçti and Büyükgüzel; Del Pino et al. 2020; Pascacio-Villafán et al. 2020). The content of artificial diets should be similar to the natural diets of model insects. In addition, the components of these diets should be inexpensive. The most preferred model insects as infection models belong to the order Lepidoptera. For this reason, suitable environments and nutrient contents should be determined for egg, larva, pupa and adult stages in mass production of Lepidopterans under laboratory conditions (Fig 1).

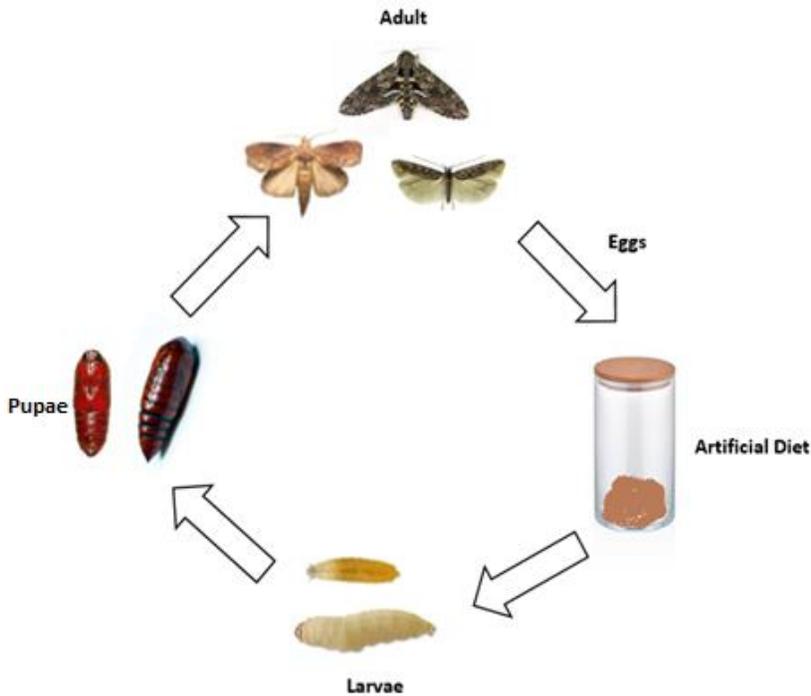


Figure 1. Mass production of model insects of the order Lepidoptera under laboratory conditions

1.1. Model Insects for Determining the Pathogenicity of Infections

Galleria mellonella

G. mellonella is an important pest that causes economic losses in beehives. The life cycle of this insect, which is in the order Lepidoptera, consists of egg, larva, pupa and adult stages (Fig 2). The life cycle is completed in 7-8 weeks (Kwadha et al. 2017; Singkum et al. 2019; Piatek et al. 2021). *G. mellonella* is used as an infection model because of the large surface area of the larvae and the stock culture in a short time. *G. mellonella* is a good infection model to study the immune response and virulence factors of organisms against pathogens such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Candida*

albicans (Fig 2) (Sheehan et al. 2019; Antoine et al. 2021; Sugeçti 2021 a,b; Lam et al. 2022; Meccatti et al. 2022).

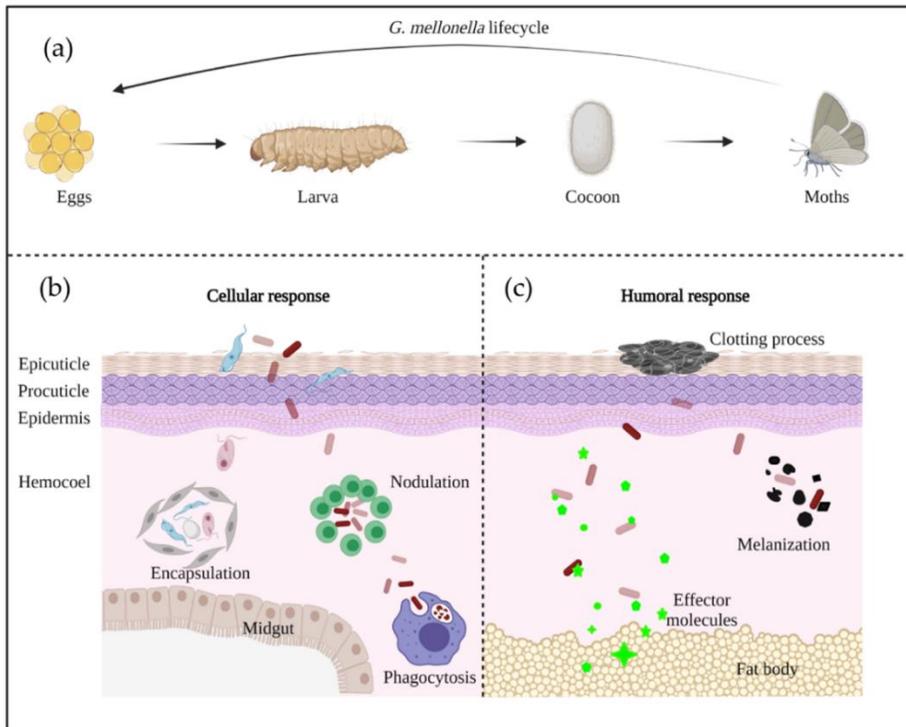


Figure 2. The life cycle (a) and immune system (b,c) of *G. mellonella*. (Tao et al. 2021)

Cellular responses of *G. mellonella* are mediated by clot cells, granular cells, oenocytoids, plasmatocytes, prohemocytes and spherulocytes (Fig 3) (Rajendran et al. 2015; Arteaga et al. 2017). These hemocytes are similar to human phagocytes. These cells are found freely in the hemolymph. It also inhibits the spread of pathogens through processes such as phagocytosis, encapsulation and nodulation in internal organs. Various antimicrobial peptides (AMPs) play a key role against bacterial infections (Moghaddam et al. 2016). In addition, opsonins recognize microbial components such as peptidoglycan, lipoteichoic acid and lipopolysaccharides and stimulate hemocytes to mount an appropriate immune response (Kim et al. 2010).

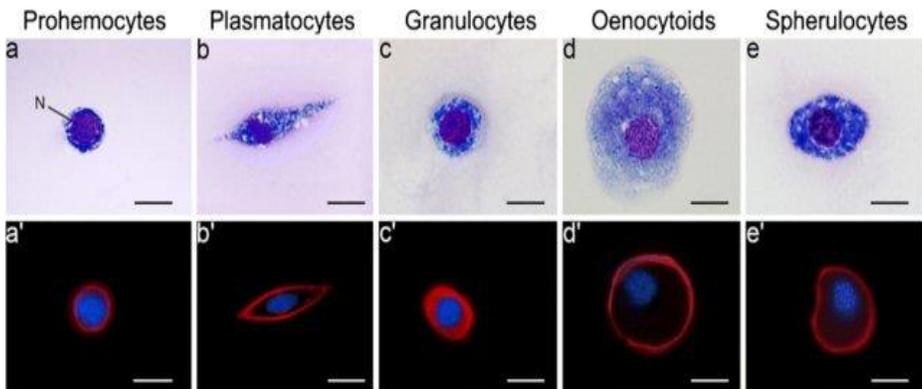


Figure 3. Light (top) and fluorescence (bottom) microscopic images of hemocytes in *G. mellonella* (Arteaga et al. 2017)

Bombyx mori

B. mori is an economically important insect. For this reason, stock culture is widely practiced in many countries (Xiang et al. 2018). In recent years, immune responses of *B. mori* against many bacterial infections have been investigated (Ma et al. 2019; Zhang et al. 2020). One of the most important advantages of using *B. mori* as an infection model is that the genome sequence has been determined. Therefore, this insect is a suitable model for drug evaluation studies (Montali et al. 2022) The silkworm is used as an infection model to evaluate antibacterial drugs against human pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* (Fig 4) (Kaito et al. 2002, Uchida et al. 2014, Montali et al. 2020; Tuba et al. 2021).

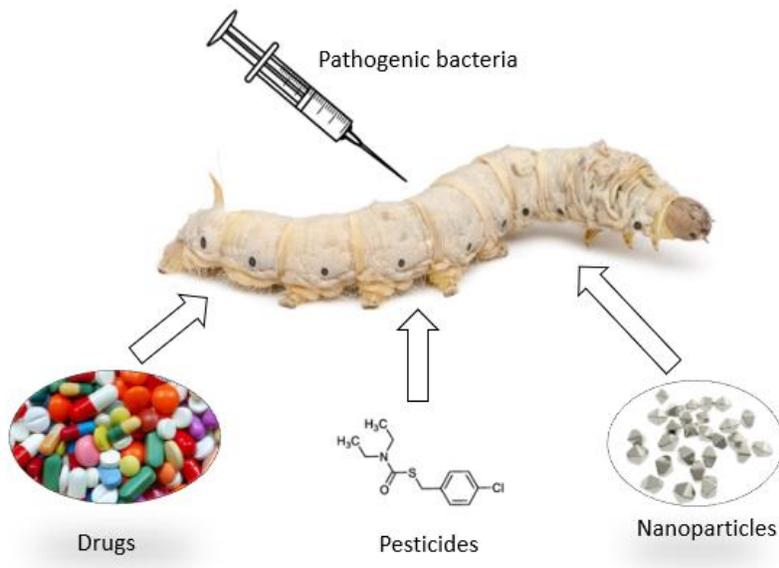


Figure 4. The use of silkworm as a model organism in the evaluation of the effects of biological and chemical agents.

Spodoptera litura

S. litura, called the tobacco caterpillar, is an important pest in agricultural areas. In recent years, interest in methods of controlling this pest has increased (Huang et al. 2014; Cui et al. 2022). In addition, this insect has been reported to be an important infection model (Pei et al. 2021; Wang et al. 2021). In a study, *S. litura* was used as a model to investigate the pathogenicity of *Metarhizium rileyi*. It was determined that *S. litura* infected with *M. rileyi* had both cellular and humoral immunity (Wang et al. 2021).

Manduca sexta

M. sexta, called hornworms, is a pest of the order Lepidoptera that damages tobacco leaves. The life cycle of *M. sexta* consists of 4 stages: egg, larva, pupa and adult (Fig 5). Hornworms larvae are used in many studies on hormones and neurons. In addition, Hornworms are commercially produced as fish food. In recent years, *M. sexta* has been used to study the effects of human pathogens on organisms. In a study, *M. sexta* was used as an experimental model to investigate the effects

of pathogenic bacterium *Bacillus cereus* (Harvie et al. 2005). In addition, *M. sexta* was used as a model organism for the evaluation of virulence factors of important pathogens such as *S. aureus* and *Streptococcus pneumoniae*. (Fleming et al. 2006; Roth et al. 2012). *M. sexta* is an important infection model because microorganisms can be applied to larvae by easy injection.

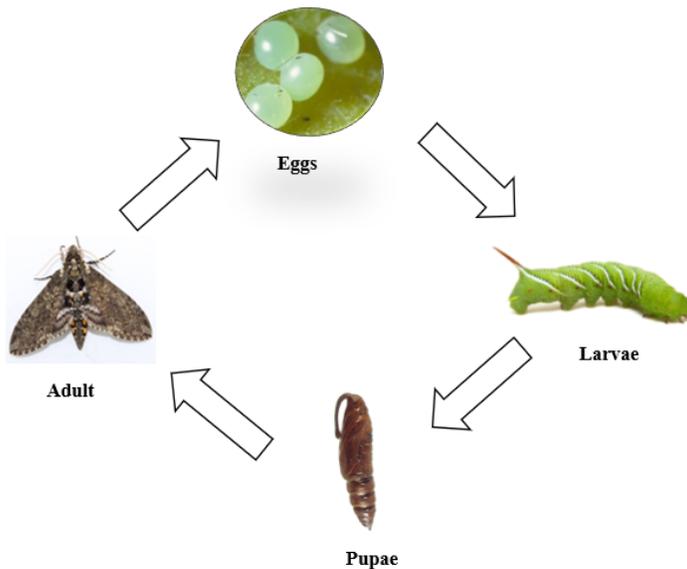


Figure 5. The life cycle of *M. sexta*

2. CONCLUSION

The use of model insects in studies to determine the virulence of pathogens has numerous advantages. Infection models such as *G. mellonella*, *M. sexta*, *B. mori* are particularly important experimental models for the determination of biochemical and physiological processes among the pathogen host. As a result, non-mammalian model organisms can be used as an alternative infection model to vertebrate experimental animals due to ethical concerns and high production costs.

REFERENCES

- Aci, R., Muderrisoglu, S., Duran, U., Shakouri, N., & Çiftci, G. (2020). Content of minerals in muscle tissue of diploid rainbow trout (*Oncorhynchus mykiss*) in freshwater (Derbent Dam) and seawater (The Black Sea). *Journal of Elementology*, 25(4).
- Antoine, C., Laforêt, F., Blasdel, B., Glonti, T., Kutter, E., Pirnay, J. P., ... & Thiry, D. (2021). Efficacy assessment of PEV2 phage on *Galleria mellonella* larvae infected with a *Pseudomonas aeruginosa* dog otitis isolate. *Research in Veterinary Science*, 136, 598-601.
- Arteaga Blanco, L. A., Crispim, J. S., Fernandes, K. M., de Oliveira, L. L., Pereira, M. F., Bazzolli, D. M. S., & Martins, G. F. (2017). Differential cellular immune response of *Galleria mellonella* to *Actinobacillus pleuropneumoniae*. *Cell and tissue research*, 370(1), 153-168.
- Cui, G., Yuan, H., He, W., Deng, Y., Sun, R., & Zhong, G. (2022). Synergistic effects of botanical curcumin-induced programmed cell death on the management of *Spodoptera litura* Fabricius with avermectin. *Ecotoxicology and Environmental Safety*, 229, 113097.
- Del Pino, M., Cabello, T., & Hernández-Suárez, E. (2020). Age-Stage, Two-Sex Life Table of *Chrysodeixis chalcites* (Lepidoptera: Noctuidae) at constant temperatures on semi-synthetic diet. *Environmental entomology*, 49(4), 777-788.
- Fleming, V., Feil, E., Sewell, A. K., Day, N., Buckling, A., & Massey, R. C. (2006). Agr interference between clinical *Staphylococcus aureus* strains in an insect model of virulence. *Journal of bacteriology*, 188(21), 7686-7688.
- Harvie, D. R., Vilchez, S., Steggle, J. R., & Ellar, D. J. (2005). *Bacillus cereus* Fur regulates iron metabolism and is required for full virulence. *Microbiology*, 151(2), 569-577.
- Huang, S. H., Xian, J. D., Kong, S. Z., Li, Y. C., Xie, J. H., Lin, J., ... & Su, Z. R. (2014). Insecticidal activity of pogostone against *Spodoptera litura* and *Spodoptera exigua* (Lepidoptera: Noctuidae). *Pest management science*, 70(3), 510-516.
- Kaito, C., Akimitsu, N., Watanabe, H., & Sekimizu, K. (2002). Silkworm larvae as an animal model of bacterial infection pathogenic to humans. *Microbial pathogenesis*, 32(4), 183-190.
- Kim, C. H., Shin, Y. P., Noh, M. Y., Jo, Y. H., Han, Y. S., Seong, Y. S., & Lee, I. H. (2010). An insect multiligand recognition protein functions as an opsonin for

- the phagocytosis of microorganisms. *Journal of Biological Chemistry*, 285(33), 25243-25250.
- Kwadha, C. A., Ong'amo, G. O., Ndegwa, P. N., Raina, S. K., & Fombong, A. T. (2017). The biology and control of the greater wax moth, *Galleria mellonella*. *Insects*, 8(2), 61.
- Lam, L. N., Brunson, D. N., Kajfasz, J. K., & Lemos, J. A. (2022). Methods for Using the *Galleria mellonella* Invertebrate Model to Probe *Enterococcus faecalis* Pathogenicity. In *Bacterial Virulence* (pp. 177-183). Humana, New York, NY.
- Love, A. C. (2009). Marine invertebrates, model organisms, and the modern synthesis: epistemic values, evo-devo, and exclusion. *Theory in Biosciences*, 128(1), 19-42.
- Ma, L., Zhou, L., Lin, J., Ji, J., Wang, Y., Jiang, H., ... & Lu, Z. (2019). Manipulation of the silkworm immune system by a metalloprotease from the pathogenic bacterium *Pseudomonas aeruginosa*. *Developmental & Comparative Immunology*, 90, 176-185.
- Meccatti, V. M., Figueiredo-Godoi, L. M. A., Pereira, T. C., de Lima, P. M. N., Abu Hasna, A., Senna, L. B., ... & de Oliveira, L. D. (2022). The biocompatibility and antifungal effect of *Rosmarinus officinalis* against *Candida albicans* in *Galleria mellonella* model. *Scientific Reports*, 12(1), 1-8.
- Moghaddam, M. R. B., Tonk, M., Schreiber, C., Salzig, D., Czermak, P., Vilcinskas, A., & Rahnamaeian, M. (2016). The potential of the *Galleria mellonella* innate immune system is maximized by the co-presentation of diverse antimicrobial peptides. *Biological Chemistry*, 397(9), 939-945.
- Montali, A., Berini, F., Brivio, M. F., Mastore, M., Saviane, A., Cappellozza, S., ... & Tettamanti, G. (2020). A silkworm infection model for in vivo study of glycopeptide antibiotics. *Antibiotics*, 9(6), 300.
- Montali, A., Berini, F., Saviane, A., Cappellozza, S., Marinelli, F., & Tettamanti, G. (2022). A *Bombyx mori* Infection Model for Screening Antibiotics against *Staphylococcus epidermidis*. *Insects*, 13(8), 748.
- Pascacio-Villafán, C., Guillén, L., & Aluja, M. (2020). Agar and carrageenan as cost-effective gelling agents in yeast-reduced artificial diets for mass-rearing fruit flies and their parasitoids. *Insects*, 11(2), 131.
- Pei, B., Wang, C., Yu, B., Xia, D., Li, T., & Zhou, Z. (2021). The first report on the transovarial transmission of microsporidian *Nosema bombycis* in

- lepidopteran crop pests *Spodoptera litura* and *Helicoverpa armigera*. *Microorganisms*, 9(7), 1442.
- Piatek, M., Sheehan, G., & Kavanagh, K. (2021). *Galleria mellonella*: The Versatile Host for Drug Discovery, In Vivo Toxicity Testing and Characterising Host-Pathogen Interactions. *Antibiotics*, 10(12), 1545.
- Rajendran, R., Borghi, E., Falleni, M., Perdoni, F., Tosi, D., Lappin, D. F., ... & Nile, C. (2015). Acetylcholine protects against *Candida albicans* infection by inhibiting biofilm formation and promoting hemocyte function in a *Galleria mellonella* infection model. *Eukaryotic cell*, 14(8), 834-844.
- Roth, A., Reichmann, P., & Hakenbeck, R. (2012). The capsule of *Streptococcus pneumoniae* contributes to virulence in the insect model *Manduca sexta*. *Microbial Physiology*, 22(5), 326-334.
- Sertçelik, M., Sugeçti, S., Büyükgüzel, E., Necefoğlu, H., & Büyükgüzel, K. (2018). Diaquabis N, N-dietilnikotinamid-IN1 bis 4-formilbenzoato-IO kobalt II Kompleksinin Model Organizma *Galleria mellonella* L. Lepidoptera: Pyralidae Üzerindeki Toksikolojik ve Fizyolojik Etkileri. *Karaelmas Fen ve Mühendislik Dergisi*, 8(1), 359-364.
- Sheehan, G., Dixon, A., & Kavanagh, K. (2019). Utilization of *Galleria mellonella* larvae to characterize the development of *Staphylococcus aureus* infection. *Microbiology*, 165(8), 863-875.
- Singkum, P., Suwanmanee, S., Pumeesat, P., & Luplertlop, N. (2019). A powerful in vivo alternative model in scientific research: *Galleria mellonella*. *Acta Microbiologica et Immunologica Hungarica*, 66(1), 31-55.
- Sugeçti, S. (2021a). Biochemical and immune responses of model organism *Galleria mellonella* after infection with *Escherichia coli*. *Entomologia Experimentalis et Applicata*, 169(10), 911-917.
- Sugeçti, S. (2021b). Pathophysiological effects of *Klebsiella pneumoniae* infection on *Galleria mellonella* as an invertebrate model organism. *Archives of Microbiology*, 203(6), 3509-3517.
- Sugeçti, S., & Büyükgüzel, K. (2018). Effects of Oxifendazole on Metabolic Enzymes in Hemolymph of *Galleria mellonella* L. (Lepidoptera: Pyralidae) Larvae Reared on Artificial Diet. *Karaelmas Science & Engineering Journal*, 8(2).
- Tao, Y., Duma, L., & Rossez, Y. (2021). *Galleria mellonella* as a Good Model to Study *Acinetobacter baumannii* Pathogenesis. *Pathogens*, 10(11), 1483.

- Tuba, T., Chowdhury, F. R., Hossain, T., Farzana, M., Ahad, I. I., Hossain, M. M., ... & Hossain, M. S. (2021). Klebsiella pneumoniae pathogenicity in silk moth larvae infection model. *FEMS Microbiology Letters*, 368(21-24), fnab159.
- Tüfek, H., & Özkan, Ö. (2018). 4R rule in laboratory animal science. *Commagene J Biol*, 21(1), 55-60.
- Tunçsoy, B., Sugeçti, S., Büyükgüzel, E., Özalp, P., & Büyükgüzel, K. (2021). Effects of copper oxide nanoparticles on immune and metabolic parameters of *Galleria mellonella* L. *Bulletin of Environmental Contamination and Toxicology*, 107(3), 412-420.
- Uchida, R., Hanaki, H., Matsui, H., Hamamoto, H., Sekimizu, K., Iwatsuki, M., ... & Tomoda, H. (2014). In vitro and in vivo anti-MRSA activities of nosokomycins. *Drug Discoveries & Therapeutics*, 8(6), 249-254.
- Wang, L., Wang, J., Zhang, X., Yin, Y., Li, R., Lin, Y., ... & Wang, Z. (2021). Pathogenicity of *Metarhizium rileyi* against *Spodoptera litura* larvae: Appressorium differentiation, proliferation in hemolymph, immune interaction, and reemergence of mycelium. *Fungal Genetics and Biology*, 150, 103508.
- Xiang, H., Liu, X., Li, M., Zhu, Y. N., Wang, L., Cui, Y., ... & Zhan, S. (2018). The evolutionary road from wild moth to domestic silkworm. *Nature Ecology & Evolution*, 2(8), 1268-1279.
- Zhang, Y., Shang, R., Zhang, J., Li, J., Zhu, G., Yao, M., ... & Shen, Z. (2020). Isolation and identification of two *Serratia marcescens* strains from silkworm, *Bombyx mori*. *Antonie van Leeuwenhoek*, 113(9), 1313-1321.

CHAPTER 8

NEUROBLASTOMA

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INTRODUCTION

Central and peripheral nervous system tumors are common among childhood malignancies. They are the second most common tumors worldwide which constitute twenty-five percent of all malignancies and are the most common solid tumors under the age of 15. (Ries, L A G, Smith. M A, Gurney. J Linet M, Tamra T, Young J, et al. *Cancer incidence and survival among children and adolescents: United States SEER Program 1975-1995*. 1999.) This disease group, which has high morbidity and mortality rates during the entire diagnosis and treatment process, has become more prominent, especially with the development of treatment modalities in recent years. (Pizzo PA, Poplack DG. *Principles and practice of pediatric oncology: Lippincott Williams & Wilkins; 2015*.)

Neuroblastoma, a peripheral sympathetic nervous system tumor, is estimated to account for eight to ten percent of all childhood cancers. (Bernstein ML, Leclerc JM, Bunin G, Brisson L, Robison L, Shuster J, et al. *A population-based study of neuroblastoma incidence, survival, and mortality in North America*. 1992;10(2):323-9.) It is the most common extracranial solid tumor of childhood. While it is a spontaneously regressible tumor in the younger age group, it can show a wide spectrum of variations resistant to multimodal therapies as the age increases. It is the most common neoplasm observed in infants. (Izbicki T, Mazur J, Izbicka EJAr. *Epidemiology and etiology of neuroblastoma: an overview*. 2003;23(1B):755-60.)

The prevalence of this disease is 1/7000 and is observed in almost all countries. The boy/girl ratio is 1.1. It accounts for 15% of the mortality due to malignancy in children. The median age is 22 months and 90% of cases are diagnosed within 5 years. (Bernstein ML, Leclerc JM, Bunin G, Brisson L, Robison L, Shuster J, et al. *A population-based study of neuroblastoma incidence, survival, and mortality in North America*. 1992;10(2):323-9. - Izbicki T, Mazur J, Izbicka EJAr. *Epidemiology of neuroblastoma: analysis of a single institution*. 2003;23(2C):1933-8.)

Studies to elucidate the etiology are ongoing and no environmental factor (prenatal or postnatal drug exposure, drug use

during pregnancy, virus or radiation) that causes a significant increase in risk has been demonstrated so far. Associations with autosomal recessive anomalies, facial and digestive system anomalies, fetal alcohol syndrome, Beckwith-Wiedemann and Turner syndromes have been reported and the mutated genes found in these conditions have been thought to be associated with neuroblastoma but have not been proven yet. (*Haase GM, Perez C, Atkinson JB. Current aspects of biology, risk assessment, and treatment of neuroblastoma. Seminars in surgical oncology. 1999;16(2):91-104. - Menegaux F, Olshan AF, Reitnauer PJ, Blatt J, Cohn SL. Positive association between congenital anomalies and risk of neuroblastoma. Pediatric blood & cancer. 2005;45(5):649-55. 68 - Maris JM, Matthay KKJJoco. Molecular biology of neuroblastoma. 1999;17(7):2264-.*)

1. HISTOPATHOLOGY

Neuroblastoma originates from primordial neural crest cells. It belongs to the group of small round blue cell tumors. Other tumors in this group are rhabdomyosarcoma, Ewing sarcoma, and Non-Hodgkin Lymphoma. Histologically, Neuroblastoma contains 2 types of cells: neuroblastic ganglionic cells and reactive Schwann stromal cells. It is classified into 3 different morphologic groups. (*Schwab M, Westermann F, Hero B, Berthold F. Neuroblastoma: biology and molecular and chromosomal pathology. The Lancet Oncology. 2003;4(8):472-80.*)

1. Ganglioneuroma (Schwann stroma dominant)
2. Ganglioneuroblastoma (rich in Schwann stroma)
3. Neuroblastoma (Schwann stroma-poor)

These morphological findings are very important in determining the degree of tumor differentiation and treatment modality. Genetic factors have become very important in neuroblastoma as in other malignancies where genetic variations are increasingly valued in determining treatment modalities. (*Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, et al. Revisions of the*

international criteria for neuroblastoma diagnosis, staging and response to treatment. Progress in clinical and biological research. 1994;385:363-9. - Evans AJC. A proposed staging for children with neuroblastoma. 1981;27:324-32. - Castleberry RP, Cantor AB, Green AA, Joshi V, Berkow RL, Buchanan GR, et al. Phase II investigational window using carboplatin, iproplatin, ifosfamide, and epirubicin in children with untreated disseminated neuroblastoma: a Pediatric Oncology Group study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1994;12(8):1616-20. 57. Hiorns M, Owens CJEr. Radiology of neuroblastoma in children. 2001;11(10):2071-81.)

Neuroblastoma can be caused by stalling and derailment of steps involving the maturation of neural crest at different levels. There are 4 main categories that can be addressed about the genetic variables. These can be grouped into the following categories: neural crest induction, neural plate specification, neural crest specification, and sympathoadrenal specification. (Table 1) Although various genes related to the developmental steps have been proven, there are still many ongoing studies in this field. (*Schwab M, Westermann F, Hero B, Berthold F. Neuroblastoma: biology and molecular and chromosomal pathology. The Lancet Oncology. 2003;4(8):472-80.*)

2. CLINICAL FINDINGS

Since neuroblastoma is a tumor of the sympathetic nervous system, its effects can be observed anywhere along this neuronal pathway. The presenting symptom of neuroblastoma is usually asymptomatic abdominal masses, mostly in the abdomen (65%). The probability of tumor detection in the adrenal region is 40% in children and 25% in infants. Cervical and thoracic primary tumor foci are more common in infants. Metastases are present in 75% of the cases at the time of diagnosis, spreading via lymphatic and hematogenous routes. Hematogenous spread is most common to bone marrow, liver and subcutaneous tissues, lung and brain parenchyma (less than 3%), while the lymphomatous spread is most common to regional lymph nodes. (*Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V,*

Castleberry RP, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 1993;11(8):1466-77.)

For these reasons, clinical findings often vary according to the site of metastasis.

Head and neck region - Palpable mass (often unilateral), Horner's syndrome

Thoracic region - Dyspnea, dysphagia, decreased feeding, lung infections, superior vena cava syndrome, and Horner's syndrome

Abdomen - Vomiting, abdominal pain, palpable mass, edema of the lower extremities and scrotum (due to lymphatic compression), Pepper syndrome (massive liver involvement), unresolving diarrhea

Pelvic region - Constipation, urinary retention

Bone involvement - Symptoms such as pain, limping, restlessness may be seen.

3. DIAGNOSIS

According to the International Neuroblastoma Staging System (INSS) criteria, the diagnosis of neuroblastoma is based on light microscopic evaluation of the tumor. Increased urinary catecholamine levels also support the diagnosis. (*Castleberry RP, Pritchard J, Ambros P, Berthold F, Brodeur GM, Castel V, et al. The International Neuroblastoma Risk Groups (INRG): a preliminary report. European journal of cancer (Oxford, England : 1990). 1997;33(12):2113-6.*)

Neuroblastoma is often diagnosed by imaging (plain radiography, computed tomography, MRI) after a suspicious mass. Calcification and hemorrhages observed in the mass often guide further investigations. Prenatal USG is also possible today. PET examinations are currently very useful in confirming the diagnosis with computed tomography and MRI. (*Castleberry RP, Pritchard J, Ambros P, Berthold F, Brodeur GM, Castel V, et al. The International Neuroblastoma Risk Groups (INRG): a preliminary report. European journal of cancer (Oxford, England : 1990). 1997;33(12):2113-6 - Abramson SJ. Adrenal*

neoplasms in children. Radiologic clinics of North America. 1997;35(6):1415-53.)

Tumor markers of neuroblastoma arising from the sympathetic system are homovanillic acid (HVA) and vanillylmandelic acid (VMA). In as many as 95% of cases, the diagnosis is confirmed by an increase in urinary VMA and HVA levels.

INSS criteria for the diagnosis of neuroblastoma:

- 1- Histopathological diagnosis of tumor tissue and/or increased urinary serum catecholamines
- 2- Demonstration of neuroblastoma cells in bone marrow aspiration or biopsy and increased urine/serum catecholamine levels.

Cytogenetic examinations (MYCN, 1p deletion, ploidy) and histopathologic examination should be performed to determine the risk group from the tissue sample taken for diagnosis. According to the recommendations of the Children's Oncology Group (COG), especially these examinations play an important role in determining prognosis and treatment in children under two years of age. Therefore, even in patients diagnosed with bone marrow examinations, tissue samples from the primary tumor should be obtained and further investigations should be performed. (*Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1993;11(8):1466-77. - Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. Progress in clinical and biological research. 1994;385:363-9.*)

In the past, the Evans staging system, the Pediatric Oncology Group staging system, and the TNM system were used for staging; however, these systems have been abandoned and replaced by the International Neuroblastoma Risk Group Staging System (INRGSS).

This system is based on INSS criteria. It is based on the tumor's surgical removal rate and lymph node involvement (Table 2).

In pathological evaluation, neuroblastoma, which is a small blue round cell tumor group, should be differentiated from other group members such as Non-Hodgkin Lymphoma, Ewing sarcoma, and primitive neuroectodermal tumor. It is differentiated from other group members by NSE-specific antibodies and molecules such as synaptophysin revealed by immunohistochemical stains. The stage of differentiation is then determined and pathology classification is made together with the age group.

The place of surgery in the diagnostic algorithm is very important. The gold standard for neuroblastoma is open surgery, as it is based on direct access to the primary tumor, localization, and characterization. If the tumor cannot be removed due to its location or if there is metastatic disease, a biopsy may be sufficient. In cases where a biopsy is taken, a sufficient tissue sample for further examination is necessary to determine the prognostic value. Incisional biopsy (of sufficient size), excisional biopsy, or wedge biopsy of the liver can be taken. It is recommended that the biopsy should be at least two samples of 1x1x1 cm. Laparoscopic surgical methods are preferred for tumors in the thorax or abdomen when appropriate. Thick needle core biopsy is used for diagnosis with increasing frequency. Bone marrow biopsies and aspiration samples should be obtained if there is a suspicion of metastatic disease. (*Kushner BH. Neuroblastoma: a disease requiring a multitude of imaging studies. Journal of nuclear medicine: official publication, Society of Nuclear Medicine. 2004;45(7):1172-88. 76.-Mora J, Gerald WL, Cheung NK. Evolving significance of prognostic markers associated with new treatment strategies in neuroblastoma. Cancer letters. 2003;197(1-2):119-24.*)

4. TREATMENT

Chemotherapy, radiotherapy, and surgery are planned together with a multidisciplinary approach. Current studies suggest that the use of immunotherapeutic agents will play an important role in the future. Clinical presentation changes the approach in determining the treatment

modality because a wide spectrum of clinical presentations can completely change the approach. Prognosis also plays an important role in determining the treatment regimen in diagnosed cases. (Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, et al. *Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. The New England journal of medicine. 1999;341(16):1165-73.*) Clinical factors, tumor markers, and biological factors constitute subgroups in determining the survival rate (Table 3).

The majority of stage 1,2 and 4S diseases are considered low-risk diseases. Tumors in the low-risk group tend to regress and differentiate spontaneously. Treatment-free follow-up is very important in cases diagnosed incidentally or by screening. (Berthold F, Hero B, Kremens B, Handgretinger R, Henze G, Schilling FH, et al. *Long-term results and risk profiles of patients in five consecutive trials (1979-1997) with stage 4 neuroblastoma over 1 year of age. Cancer letters. 2003;197(1-2):11-7.* - Cheung NK, Kushner BH, LaQuaglia MP, Kramer K, Ambros P, Ambros I, et al. *Survival from non-stage 4 neuroblastoma without cytotoxic therapy: an analysis of clinical and biological markers. European journal of cancer (Oxford, England: 1990). 1997;33(12):2117-20.*)

Only surgical treatment is recommended in patients with stage 1 neuroblastoma. Complete tumor resection is aimed, and there are groups that recommend chemotherapy treatment in cases where complete resection cannot be performed due to location.

Although chemotherapy was recommended in stage 2 neuroblastoma patients in the past years, very successful results are obtained with surgery alone today. In a study conducted by the COG group, 4-year event-free survival in patients without poor biological risk factors was 81%, while overall survival after recurrence treatment was 98%. Again, if less than 50% of the mass can be removed in stage 2 patients, success rates with secondary surgery after chemotherapy are encouraging. (Goldsby RE, Matthay KK. *Neuroblastoma: evolving*

therapies for a disease with many faces. Paediatric drugs. 2004;6(2):107-22.)

In patients thought to be stage 4S, follow-up is very important in patients in the low risk group after genetic examination with bone marrow material. Spontaneous regression is over 80% in cases under 6 months. In the presence of life-threatening findings such as pressure symptoms and respiratory distress, evaluation in terms of chemotherapy and radiotherapy is critical.

Primary surgery is for diagnostic purposes in intermediate risk groups. Chemotherapy protocols including cyclophosphamide, etoposide, doxorubicin, and carboplatin are administered for 4-8 cycles depending on the histopathology and biological characteristics of the tumor. Secondary gaze surgery is then performed. Since this group is a very heterogeneous group, very different survival rates are observed according to clinical and biological characteristics. For those with good prognostic markers in the intermediate-risk group, efforts to reduce the duration of chemotherapy are ongoing and the surgical approach plays a particularly valuable role in this area for the future.

The high-risk group consists of patients with advanced age, advanced stage, and poor biological factors. Long-term survival rates are below 45%. Chemotherapies are the mainstay of treatment. Induction, consolidation, and maintenance chemotherapies are administered step by step. The main goal of induction is to reduce tumor burden. The evaluation made after this treatment, which lasts approximately 6 months, is very valuable in terms of prognosis. The aim of consolidation is to eliminate the disease with myeloablation and autologous stem cell transplantation. Since high recurrence rates are observed after induction and consolidation treatments in high-risk patients, treatment is continued for a while to eradicate minimal residual disease. (*Bradfield SM, Douglas JG, Hawkins DS, Sanders JE, Park JR. Fractionated low-dose radiotherapy after myeloablative stem cell transplantation for local control in patients with high-risk neuroblastoma. Cancer. 2004;100(6):1268-75. 82*)

Radiotherapy is used to treat bone or soft tissue lesions and can be used to treat stage 4S neuroblastoma with respiratory distress and inferior vena cava compression. (Castleberry RP, Kun LE, Shuster JJ, Altshuler G, Smith IE, Nitschke R, et al. Radiotherapy improves the outlook for patients older than 1 year with Pediatric Oncology Group stage C neuroblastoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 1991;9(5):789-95. - Haas-Kogan DA, Swift PS, Selch M, Haase GM, Seeger RC, Gerbing RB, et al. Impact of radiotherapy for high-risk neuroblastoma: a Children's Cancer Group study. *International journal of radiation oncology, biology, physics*. 2003;56(1):28-39.)

4.1 The Place of Surgery in Treatment

The main goal in a patient with neuroblastoma is to completely remove the tumor if possible. The removability of the tumor on radiological examination, surgeon experience, and cooperation with the pediatric oncologist are very important in making this decision.

In low-risk groups, removal of the entire mass is essential, and suspicious lymph nodes should be sampled. Positivity in this group has been associated with a decrease in survival rates. Despite a good prognosis, patients in the 4S group may require urgent decompression due to respiratory distress and vascular compression due to diffuse liver infiltration.

The main goal of surgery in the intermediate-risk disease group is to confirm the diagnosis, remove as much of the tumor tissue as possible, identify suspicious metastasis sites and sample these sites if possible. If the tumor cannot be completely removed, surgical resection can be postponed until after several chemotherapies, the main goal here is to prevent the risk of surgical complications.

The aim of the first surgical approach in the high-risk group is to establish the diagnosis and to provide sufficient tissue material for biological indicators. If the tumor location is appropriate, total resection may be attempted; the main goal should be to avoid damage to other organs. There are reports of increased survival rates in stage patients with near-total resection, but there are also reports of no change. After

adequate tumor shrinkage following chemotherapy, a second surgical look is necessary. (Kushner BH, Wolden S, LaQuaglia MP, Kramer K, Verbel D, Heller G, et al. Hyperfractionated low-dose radiotherapy for high-risk neuroblastoma after intensive chemotherapy and surgery. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2001;19(11):2821-8.)

REFERENCES

- Ries, L. A. G., Smith, M. A., Gurney, J., Linet, M., Tamra, T., Young, J, et al. (1999). Cancer incidence and survival among children and adolescents: United States SEER Program 1975-1995.
- Pizzo, P.A., Poplack, D.G. (2015). Principles and practice of pediatric oncology: Lippincott Williams & Wilkins.
- Bernstein, M.L., Leclerc, J.M., Bunin, G., Brisson, L., Robison, L., Shuster, J., et al. (1992). A population-based study of neuroblastoma incidence, survival, and mortality in North America, 10(2):323-9.
- Izbicki, T., Mazur, J., Izbicka, EJAr. (2003). Epidemiology and etiology of neuroblastoma: an overview. 23(1B):755-60.
- Izbicki T, Mazur J, Izbicka EJAr. (2003). Epidemiology of neuroblastoma: analysis of a single institution. 23(2C):1933-8.
- Haase, G. M., Perez, C., Atkinson, J.B. (1999). Current aspects of biology, risk assessment, and treatment of neuroblastoma. Seminars in surgical oncology. 16(2):91-104.
- Menegaux, F., Olshan, A. F., Reitnauer, P. J., Blatt, J., Cohn, S. L. (2005). Positive association between congenital anomalies and risk of neuroblastoma. Pediatric blood & cancer. 45(5):649-55. 68
- Maris, J.M., Matthay, KKJJoco. (1999). Molecular biology of neuroblastoma. 17(7):2264-.
- Schwab, M., Westermann, F., Hero, B., Berthold, F. (2003). Neuroblastoma: biology and molecular and chromosomal pathology. The Lancet Oncology. 4(8):472-80.
- Brodeur, G. M., Pritchard, J., Berthold, F., Carlsen, N. L., Castel, V., Castleberry, R. P., et al. (1994). Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. Progress in clinical and biological research. 385:363-9.
- Evans, AJC. (1981). A proposed staging for children with neuroblastoma. 27:324-32.
- Castleberry, R. P., Cantor, A. B., Green, A. A., Joshi, V., Berkow, R. L., Buchanan, G. R., et al. (1994). Phase II investigational window using carboplatin, iproplatin, ifosfamide, and epirubicin in children with untreated disseminated neuroblastoma: a Pediatric Oncology Group study. Journal of clinical

- oncology: official journal of the American Society of Clinical Oncology. 12(8):1616-20. 57.
- Hiorns, M., Owens, CJEr. (2001). Radiology of neuroblastoma in children. 11(10):2071-81.7.
- Brodeur, G. M., Pritchard, J., Berthold, F., Carlsen, N. L., Castel, V., Castleberry, R. P., et al. (1993). Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 11(8):1466-77.
- Castleberry, R. P., Pritchard, J., Ambros, P., Berthold, F., Brodeur, G. M., Castel, V., et al. (1997). The International Neuroblastoma Risk Groups (INRG): a preliminary report. *European journal of cancer (Oxford, England: 1990)*. 33(12):2113-6.
- Abramson, S.J. (1997) Adrenal neoplasms in children. *Radiologic clinics of North America*. 35(6):1415-53.
- Kushner, B.H. (2004). Neuroblastoma: a disease requiring a multitude of imaging studies. *Journal of nuclear medicine: official publication, Society of Nuclear Medicine*. 45(7):1172-88. 76.
- Mora, J., Gerald, W. L., Cheung, N. K. (2003). Evolving significance of prognostic markers associated with new treatment strategies in neuroblastoma. *Cancer letters*. 197(1-2):119-24.
- Matthay, K. K., Villablanca, J. G., Seeger R. C., Stram, D. O., Harris, R. E., Ramsay, N. K, et al. (1999). Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. *Children's Cancer Group. The New England journal of medicine*. 341(16):1165-73.
- Berthold, F., Hero, B., Kremens, B., Handgretinger, R., Henze, G., Schilling, F. H. et al. (2003). Long-term results and risk profiles of patients in five consecutive trials (1979-1997) with stage 4 neuroblastoma over 1 year of age. *Cancer letters*. 197(1-2):11-7.
- Cheung, N. K., Kushner, B. H, LaQuaglia, M. P., Kramer, K., Ambros, P., Ambros, I. et al. (1997). Survival from non-stage 4 neuroblastoma without cytotoxic therapy: an analysis of clinical and biological markers. *European journal of cancer (Oxford, England: 1990)*. 33(12):2117-20.
- Goldsby, R. E., Matthay, K. K. (2004). Neuroblastoma: evolving therapies for a disease with many faces. *Paediatric drugs*. 6(2):107-22.

- Bradfield, S. M., Douglas, J. G., Hawkins, D. S., Sanders, J. E., Park, J. R. (2004). Fractionated low-dose radiotherapy after myeloablative stem cell transplantation for local control in patients with high-risk neuroblastoma. *Cancer*. 100(6):1268-75. 82
- Castleberry, R. P., Kun, L. E., Shuster, J. J., Altshuler, G., Smith, I. E., Nitschke, R., et al. (1991). Radiotherapy improves the outlook for patients older than 1 year with Pediatric Oncology Group stage C neuroblastoma. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 9(5):789-95.
- Haas-Kogan, D.A., Swift, P.S., Selch, M., Haase, G.M., Seeger, R.C., Gerbing, R.B. et al. (2003). Impact of radiotherapy for high-risk neuroblastoma: a Children's Cancer Group study. *International journal of radiation oncology, biology, physics*. 56(1):28-39.
- Kushner, B.H., Wolden, S., LaQuaglia, M.P., Kramer, K., Verbel, D., Heller, G., et al. (2001). Hyperfractionated low-dose radiotherapy for high-risk neuroblastoma after intensive chemotherapy and surgery. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 19(11):2821-8.
- Kutluk, TJPB. (2004). *Cancer*. First national pediatric cancer registry in Turkey: a Turkish pediatric oncology group study. 43:452
- Cheung, N., Cohn, S. (2005) *Neuroblastoma*. Springer-Verlag.
- Katzenstein, H. M., Kent, P. M., London, W. B., Cohn, S. L. (2001). Treatment and outcome of 83 children with intraspinal neuroblastoma: the Pediatric Oncology Group experience. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 19(4):1047-55.
- Castel, V., Badal, M.D., Bezanilla, J.L., Llombart, A., Ruiz-Jimenez, J. I., Sanchez de Toledo, J., et al. (1995). Treatment of stage III neuroblastoma with emphasis on intensive 74 induction chemotherapy: a report from the Neuroblastoma Group of the Spanish Society of Pediatric Oncology. *Medical and pediatric oncology*. 24(1):29-35.
- Rubie, H., Coze, C., Plantaz, D., Munzer, C., Defachelles, A., Bergeron, C., et al. (2003) Localised and unresectable neuroblastoma in infants: excellent outcome with low-dose primary chemotherapy. 89(9):1605.
- Rubie, H., Hartmann, O., Giron, A., Lemoine, G., Gruner, M., Brugieres, L. et al. (1991). Nonmetastatic thoracic neuroblastomas: a review of 40 cases. *Medical and pediatric oncology*. 19(4):253-7.

Matthay, K. K., Sather, H. N., Seeger, R. C., Haase, G. M., Hammond, G. D. (1989). Excellent outcome of stage II neuroblastoma is independent of residual disease and radiation therapy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 7(2):236-44. 79

Table 1: 4 main categories of the genetic variables

Maturation of neural crest steps	Genes
Neural crest induction	BMP, FGF, STAT3, Wnt, Notch/Delta
Neural plate specification	Gbx2, AP2, Msx1, Pax3, Zic1
Neural crest specification	c-Myc, Id3, Sox9, Sox10, FoxD3, Snail2, Twist1
Sympathoadrenal specification	Phox2a, Phox2b, MASH-1, Hand2, GATA2/3

Table 2: INSS Neuroblastoma Staging System

INSS Stage	Definition
Stage 1	The tumor can be removed completely during surgery. Lymph nodes attached to the tumor removed during surgery may or may not contain cancer, but other lymph nodes near the tumor do not.
Stage 2A	The tumor is located only in the area it started and cannot be completely removed during surgery. Nearby lymph nodes do not contain cancer.
Stage 2B	The tumor is located only in the area where it started and may or may not be completely removed during surgery, but nearby lymph nodes do contain cancer.
Stage 3	The tumor cannot be removed with surgery. It has spread to regional lymph nodes (lymph nodes near the tumor) or other areas near the tumor, but not to other parts of the body.
Stage 4	The original tumor has spread to distant lymph nodes (lymph nodes in other parts of the body), bones, bone marrow, liver, skin, and/or other organs, except for those listed in stage 4S, below.
Stage 4S	The original tumor is located only where it started (as in stage 1, 2A, or 2B), and it has spread only to the skin, liver, and/or bone marrow, in infants younger than one. The spread to the bone marrow is minimal (usually less than 10% of cells examined show cancer)

Table 3: Clinical factors, tumor markers, and biological factors constitute subgroups in neuroblastoma

Prognostic factors	Poor prognosis	Good Prognosis
Clinical factors		
Stage	Stage 3 and 4	Stage 1,2 or 4S
Age	>18 month	<18 month
Tumor markers		
Ferritin	Elevated	Decreased
LDH	Elevated	Decreased
NSE(Neuron spesific enolase)	Elevated	Decreased
Histology	Poor histology	Good histology
Biyologic factors		
MYCN oncogene	Decreased	
Pleuidi	Diploidy	Hyperploidy
1p cromosome deletion	+	-
17q cromosome duplication	+	-
TRK-A veya TRK-C expressions	-	+
CD44 expressions	-	+
Vascularity	Yüksek	Düşük
MRP expression decreasing	-	+

CHAPTER 1
**DEFINITION AND BASIC PRINCIPLES OF FAMILY
MEDICINE**

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INTRODUCTION

This chapter describes the universal definition of Family Medicine and the basic principles of the discipline. WHO currently considers primary health care as the front door of long-term health-care systems. The General Practitioner or Family Physician is the backbone of primary health care. Family medicine focuses ongoing responsibility for complete health care—from the initial contact and assessment to the ongoing management of chronic illnesses. Disease prevention and early detection are critical components of the field. A family physician, who adhering to the principles of the discipline, offers ongoing, comprehensive care to patients of all ages in a individualized way, regardless of the presence of disease or the nature of the clinical condition. In this age of specialization in the field of health as in many fields, worldwide health systems will always need the discipline of Family Medicine that is dynamic, committed to its principles and open to renewal.

1. HISTORY OF FAMILY MEDICINE

Abraham Flexner brought attention to the need for reform (1910) in American medical education more than a century ago. In the same period, it has been stated that new specialized physicians are required in this age of specialization. In 1923, Francis Peabody pointed for the first time at the field of Family Medicine, arguing that the general practitioner should provide broad and individualized healthcare. The notion of specialization in Family Medicine was later stated in Millis and Willard reports published at nearly time each other in 1966. According to Millis' report (The Graduate Education of Physicians), speciality training of Family Medicine should be included in medical education curriculum. Willard's report (Meeting the Challenge of Family Practice) highlighted the need of offering primary health care services. With the influence of these reports, the seeds of academic acceptance of Family Medicine residency training by the American Academy of Family Physicians (AAFP) were sowed in 1971. By 1997, Ian R. McWhinney suggested that primary care physicians be recognized as Family Physicians, and this recommendation became widespread over time and Family Medicine was accepted as a medical specialty. Afterwards, many eminent scientists (including Robert B. Taylor-2002, Allan G. Goroll, and Albert G. Mulley-2006) emphasized on the basic principles that would develop the quality of individual and community health care in future of the Family Medicine.

2. DEFINITIONS OF FAMILY MEDICINE

Until now, definitions of Family Medicine that differ however serve the same aim have been produced. The working group chaired by Leeuwenhorst defined Family Medicine for the first time in 1974 at the European General Practitioner Conference. According to Leeuwenhorst: A General Practitioner is a medical school graduate who offers personal and continuing primary health care to individuals, families, and the public related with a health unit, regardless of age, gender, or illness. In the declaration published by the World Organization of Family Doctors (WONCA) in 1991 defined the general practitioner / family doctor as "a general medical practitioner who accepts everyone who seeks care". By the 2000s, Olesen et al. defined a general practitioner as a specialist physician who works at the forefront of the healthcare system and is educated to take the first steps in providing care for patients' medical problems. In this definition, the physician is capable of making the best use of the health-care system's resources for the benefit of all patients without discrimination. WONCA suggested a new definition of Family Practice, which was first published in 2002, then revised in 2005, and finally in 2011. Unlike prior definitions, this new one offers a definition of specialty for primary health care that includes the evidence foundation and clinical effectiveness of the discipline: "General practice / family medicine is an academic and scientific field with its own educational curriculum, research, evidence basis, and clinical activity, as well as a primary care clinical specialization".

3. BASIC PRINCIPLES OF FAMILY MEDICINE

The basic principles and competencies are indispensable requirements for our discipline of Family Medicine. Despite the discipline's long history, its principles have just recently began to be recognized and learned. In fact, these principles are not and should not be limited to the practice of Family Medicine. These principles can be used by physicians of many different specialties. There are six core independent competences that the family medicine discipline and family medicine specialists should have, as well as twelve basic characteristics on the axis of these. These are crucial components of high GP/FM quality and should serve as a framework for our professionalism.

Famously known as the WONCA Tree, the core competencies basic and characteristics according to the European definition of Family Medicine are presented in Figure 1.

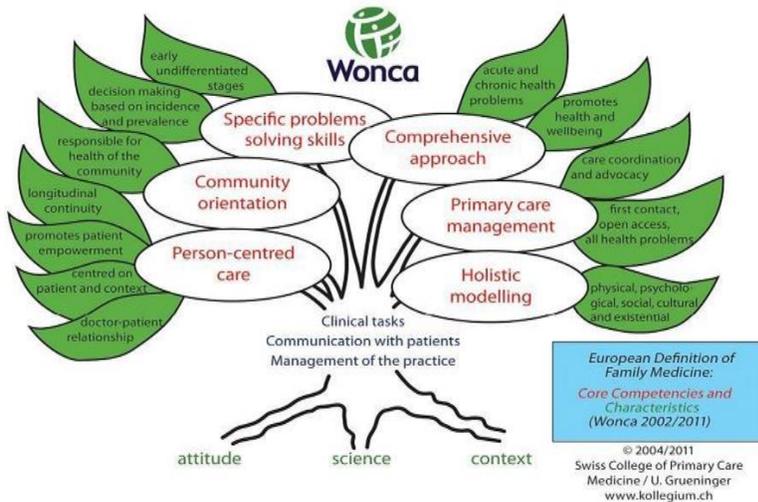


Figure 1: The Wonca Tree (<http://www.woncaeurope.org/>. 2011).

Let's look at the six basic competencies and their twelve major features under the subheadings briefly:

3.1. Primary Care Management

- First contact, open access, all health problems
- Care Coordination and Advocacy

Except in emergency conditions, family medicine should be the first point of contact in many common circumstances. In order for the individual to receive the best possible health care, a political and social interaction network coordinated if needed by the Family Physician should be organized.

3.2. Person Centred Care

- Doctor-patient relationship
- Centred on the patient and the situation
- Making a decision in collaboration with the patient
- Long-term continuity

A well-trained family physician who offers the best evidence available is mainly responsible for all of an individual's chronic illnesses and even his family's problems. This is the most critical aspect of a physician-patient

relationship, which is required for effective care. A family physician who seeks common ground with his patient is willing to collaborate. A family doctor provides continuity of care by following up on patients throughout their lifetimes.

3.3. Specific Problem Solving Abilities

- Undifferentiated diseases
- Decisions based on incidence and prevalence

A family physician can take immediate action when necessary by evaluating early or nonspecific complaint. The natural reflex of a family physician is to make specific decisions based on the incidence and prevalence of illnesses in the community.

3.4. Comprehensive Approach

- Acute and chronic diseases
- Health and well-being promotion

The family physician provides an integrated care that evaluates the patient's acute and chronic problems together. In addition, s/he provides health counseling for disease prevention, early diagnosis, and well-being improvement.

3.5. Community Orientation

- Being responsible for the community's health

Because the patient population is larger in Family practice, a population-based health survey is easier to conduct, and more effective to preventive public health.

3.6. Holistic Modelling

- Physical, psychological, social, cultural, and existential

The biopsychosocial approach proposed by psychiatrist George Engel in 1977 is essential in this modeling. A person's health or illness cannot be considered apart from biological, psychological, and social factors. Furthermore, patients' problems (not just symptoms) may not be explained purely scientifically. In this situation, the physician can understand the source of the problem (even findings of physical symptoms) through the dimension of existence.

A Family Medicine specialist is expected to realize these competencies in three areas.

- Clinical management
- Communication with patients
- Consulting room management

Furthermore, the implementation of these competences should be based on three characteristics.

- Contextual
- Attitude
- Scientific

CONCLUSION

A Family Physician who equipped with these principles establishes a individual's family, society, and cultural connection. When organizing treatment, the physician prioritizes evidence-based medical practices. He employs his professional talents in accordance with ethical standards while implementing all of these principles. As primary health care policies develop, the importance of Family Medicine will rise, and these principles will have a greater chance of being widely utilized.

REFERENCES

- Flexner, Abraham. (2002). Medical education in the United States and Canada. From the Carnegie Foundation for the Advancement of Teaching, Bulletin Number Four, 1910, Bulletin of the World Health Organization, 80 (7), 594–602. World Health Organization. <https://apps.who.int/iris/handle/10665/268537>
- F. Olesen, J. Dickinson, P. Hjortdahl. (2000). General practice: time for a new definition. *BMJ*, 320 (2000), pp 354–357
- Goroll AH, Mulley AG. (2006). Primary care medicine: Office evaluation and management of the adult patient, 5th ed., Philadelphia, PA: Wolters Kluwer, Chapter 1, The purpose and practice of primary care pp 1–6
- Leeuwenhorst Working Party. (1974). The General Practitioner in Europe, In: Second European Conference on the Teaching of General Practice, Leeuwenhorst, Netherlands, 1974. Available from: <https://euract.woncaeurope.org/sites/euractdev/files/documents/archive/publications/general-practitioner-europe-statement-working-party-appointed-2nd-european-conference-teaching.pdf>
- McWhinney IR. (1997). A textbook of family medicine, 2nd ed., New York, Oxford University Press, Chapter 1, The origins of family medicine, pp 3–12
- Metsemakers J. F. (2012). Wonca: World Organization of National Colleges, Academies and Academic Associations of General Practitioners/Family Physicians, *The European journal of general practice*, 18(2), pp 122–123 <https://doi.org/10.3109/13814788.2012.681364>
- Peabody FW. (1927). The care of the patient, *Journal of the American Medical Association*, 1927;88:877–82, <http://dx.doi.org/10.1001/jama.1927.02680380001001>
- Primary health care, WHO. (2021). <https://www.who.int/news-room/fact-sheets/detail/primary-health-care>. (Accessed 16.10.2022)
- Taylor RB. (2002). Family medicine: Now and future practice, In: Taylor RB, editor, David AK, Fields SA, Phillips DM, Scherger JE, associate editors, *Family medicine: Principles and practice*, 6th ed., New York: Springer, pp 3–9
- WONCA Europe. (2011). The European definition of general practice/family medicine [Internet]. WONCA Europe 2011 edition. Available from: <https://vdgmdev.woncaeurope.org/sites/default/files/documents/Defini>

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