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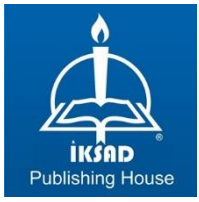

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CONTENTS

PREFACE

The scientists improves their experiences and their knowledges when increase their studies. They increase their experience by improving themselves by doing more work in some areas. Information that is not shared is not considered as an information. Fort his reason, it's among the main responsibilities of scientists tos hare their experience and knowledge. Only in this way, they can contribute to the improvement of science.

In this book, you will find reviews containing valuable information prepared by valuable scientists who continue their studies in different fields of medicine. These reviews have emerged as a result of a great accumulation of knowledge. We would like to thank all our professors who contributed to the development of this book and shared their valuable work with us, for their efforts and wish them success in their studies.

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CHAPTER 1

AN OVERVIEW OF LUNG HISTOLOGY AND STEM CELL TYPES IN THE LUNG

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INTRODUCTION

The lung is a gas exchange organ that provides oxygen and removes carbon dioxide from the blood so that every cell in the body can survive. Cells in the human body can survive for several weeks without energy components such as proteins, carbohydrates and fats, and for several days without water but without oxygen it can only survive for a few minutes (Dye, Miller, & Spence, 2016). In cases where the amount of oxygen needed by the body cannot be met, a metabolic crisis called hypoxia occurs, in which physiological functions and vitality are at risk (Araneda & Tuesta, 2012). For this reason, the transport and exchange of oxygen, unlike energy sources and water, which is the main source of circulation and cells, is critical and must be done uninterrupted (Dye et al., 2016). The lungs, the main organ of the respiratory system, are covered by the pleural membrane and are located in the thoracic cavity next to the mediastinum. The respiratory system functionally consists of two parts, the conductive part, which carries the air for gas exchange, and the respiratory part, where the exchange of carbon dioxide and oxygen between the respiratory air and blood takes place. Transmitting part; includes nasal cavities, larynx, trachea, bronchioles, and terminal bronchioles. Respiratory department; respiratory bronchioles include alveolar ducts and alveoli (Patwa & Shah, 2015). The conductive part cleans and humidifies the inhaled air and delivers it to the lungs. Smooth muscle, collagen and elastic fibers in its structure provide flexibility and structural support. Thus, uninterrupted gas exchange takes place (A. L. Mescher, (2013)).

Bronchial Tree;

The trachea branches together with veins, arteries, and lymphatic vessels to form two primary bronchi that enter the lung from the hilum. Supporting the pulmonary lobes as the bronchi enter the lung. The right lung divides into three secondary bronchi and the left lung into two secondary bronchi. Secondary bronchi branch to form tertiary bronchi. Each tertiary bronchus divides into smaller branches to form the bronchopulmonary segment. Tertiary bronchioles decrease in diameter, branch off, and their final branches are called bronchioles. Bronchioles enter the pulmonary lobule and branch into terminal bronchioles (A. L. Mescher, (2013)). Terminal

bronchioles branch into respiratory bronchioles, and respiratory bronchioles branch into alveolar ducts. The terminal portion of the alveolar ducts enlarges to form alveolar sacs (Michael H. Ross, (2020)).

When the general histological structure of the lung is examined; the lamina propria of the trachea contains mucus-producing seromucous glands. It is seen to be covered by a typical respiratory epithelium, which features a pseudostratified kinocilium columnar epithelium containing goblet cells. Hyaline cartilage rings supported by fibroelastic connective tissue in the trachea keep the lumen open. Although the diameter of the primary bronchi is smaller than that of the trachea, it is histologically similar. The hyaline in the bronchial wall prevents the collapse of the cartilage wall, and as the bronchi branch, the cartilage differentiates into irregular layers (Patrick C. Nahirney, (2013)). Intrapulmonary bronchi are differentiated by the hyaline cartilage plates in their walls. The bronchus is covered with typical respiratory epithelium. The bronchial wall consists of a thin lamina propria, a smooth muscle layer, a submucosa containing bronchial glands, and hyaline cartilage plates (Lumb & Thomas, 2020) When the diameter of the bronchi is approximately 1 mm or less, it is defined as a bronchiole and no more hyaline cartilage plaques are seen in its wall. The terminal bronchiolar epithelium is covered by a single layer of prismatic or cuboidal epithelium with kinocilium, and mucosal folds are formed by the contraction of the surrounding smooth muscle layer. A developed layer of smooth muscle, lamina propria, and advencia surrounds the terminal bronchiole (A. L. Mescher, (2013)). In terminal bronchioles, goblet cells are replaced by clara (club) cells, which lack cilia (Gartner, 2007). Club cells produce surfactant lipoprotein that covers the surface of the bronchial epithelium, proteolytic enzymes that destroy mucus produced in the bronchioles, leukocyte protease inhibitor and lysozymes to ensure the integrity of the bronchiolar epithelium. Club cells act as progenitor cells that regenerate damaged or absent ciliated or non-ciliated bronchial epithelial cells (Allen, 2008).

In addition to club cells, the terminal bronchiolar epithelium contains diffuse neuroendocrine system cells (DNES), chemoreceptor brush cells, and small granule cells. The mucous epithelium of the respiratory bronchioles is lined by club cells and a single-layered cuboidal epithelium. The part opening to the alveoli is covered with a single layer of squamous epithelium. Alveolar

sac structures are formed in each respiratory bronchiole wall. The walls of the alveolar ducts, which are branches of the respiratory bronchioles, contain the alveoli and are covered by a single layer of squamous epithelium. Alveolar canal and alveoli opening into the canal, it is surrounded by a matrix of elastic and collagen fibers. (A. L. Mescher, (2013)). An elastic and reticular network is seen at the entrance areas of the alveolar sacs and around each alveoli. There is also a capillary network surrounding each alveoli (Patrick C. Nahirney, (2013)). The epithelium of the alveoli is covered by a single layer of squamous epithelium. Among adjacent alveoli, there is a small amount of extracellular matrix of connective tissue rich in capillary networks, containing many elastic and reticular fibers, and an intraalveolar septum containing a small number of fibroblasts. When the septum is interrupted from place to place, these structures called Kohn's holes are formed (A. L. Mescher, (2013)).

Type 1 pneumocytes are the places where gas exchange takes place, and they constitute 40% of the cells lining the alveolar surface. Due to its fine structure, it covers 95% of the surface. There is no division ability. They are connected by tight junctions and participate in the structure of the blood air barrier (Michael H. Ross, (2020)). Type 2 pneumocytes or septal cells are found among type 1 pneumocytes. In addition, Type 2 pneumocytes are cubic shaped cells and are connected to each other by the zonula occludens and desmosomes. Type 2 pneumocytes have lamellar bodies in their apical cytoplasm. The lamellae are the distinguishing feature for septal cells. Type 2 pneumocytes secrete pulmonary surfactant from their apical surface. The released surfactant reduces the surface tension during gas exchange. In addition to secreting surfactant, type 2 pneumocytes can differentiate into type 1 cells. Alveolar macrophages, members of the mononuclear phagocytic system known as dust cells, are also found within the alveoli and in the alveolar septum. The main task of dust cells is to phagocytize microorganisms and airborne particles (A. Mescher, 2013).

Stem Cells In The Lung

The main function of the lung is to exchange carbon with oxygen in the air and carbon dioxide in the blood. This indicates that the lung can be exposed to pathogens and injuries from the external environment (Wu &

Tang, 2021). The prevalence of diseases involving lung injury, particularly asthma, emphysema, and Chronic Obstructive Pulmonary Disease (COPD), is increasing (Labaki & Han, 2020). Respiratory system diseases are very significant disease that cause of mortality and morbidity in the earth. The treatments applied aim to improve the quality of life of patients by reducing inflammation or inhibiting disease-specific mechanisms (Kiley & Senior, 2012).

The regeneration process after lung injury is carried out by pulmonary stem cells, which have self-renewal potential and inherent proliferative. Cellular therapies with pulmonary stem cells may ensure, significant therapeutic option in several lung diseases. For this reason, stem cell studies that contribute to the prevention, treatment and improvement of lung diseases are important. The respiratory epithelium provides an effective line of defense against inhaled substances and pathogens (Kotton & Morrisey, 2014). Cell types that are basically involved in the respiratory epithelium; secretory cells, multiciliary cells, and basal cells. Brush cells, goblet cells, and neuroendocrine cells, are rarely encountered (Montoro et al., 2018). The particular proximodistal location of cell types in the lung has a kind of physiological roles, including maintaining host defense, maintenance, and regeneration of respiratuar epithelial cells. The regeneration function provided by stem cells in the lung is very important (Rawlins & Hogan, 2008).

Lineage-tracing method is one of the widely used methods to study organ development and tissue regeneration. In studies using the lineage-tracing method, all surface epithelial hosts and some submucosal gland cells, which are accountable for alveolar homeostasis or epithelial, where damage to the respiratory tract epithelium and then the repair process begin, have been described (A. Tata et al., 2018). These studies demonstrate the capacity of the method.

Single cell RNA sequencing is a powerful method often used to determine the entire transcriptome profile of a single cell. Recent single-cell RNA sequencing studies of respiratory tract cells, has provided important insights into stem cell subsets, alteration express, and the complex pathways that enable a stem cell to differentiate or continue plasticity (Plasschaert et al., 2018; Ruiz García et al., 2019).

When the stem/Progenitor cells in the lung are examined;

Basal stem/Progenitor cells

Basal cells, defined as multipotent stem cells, are located in the pseudostratified epithelium of the tracheobronchial tree, close to the basal lamina. (Rock, Randell, & Hogan, 2010). Basal cells anchor the overlying cells to the underlying connective tissue at the site of their localization and play an important role in epithelial homeostasis because their self-renewal and differentiation into all luminal cell types (Pardo-Saganta et al., 2015). These functions of basal cells have been demonstrated after injury or by regeneration of the respiratory tract epithelium on a decellularized connective tissue (Ghosh et al., 2011; Musah, Chen, & Hoyle, 2012).

In the respiratory epithelium, basal cells are commonly defined based on their specific location in the basal lamina by statement of the cytoskeletal protein Keratin 5 (KRT5) and transcription factor p63 (TRP63) and the Nerve Growth Factor Receptor (Rock et al., 2009). Basal cells attach to the underlying basement membrane via α6β4 integrins and bind to cell types in the surface epithelium via cytokeratins (CRTs) to form a structural network (Knight & Holgate, 2003). KRT markers determine the functional features and determine of basal cells and it defines basal cells subtypes and differentiated luminal cells (Plasschaert et al., 2018).

In a study using multiple inducible Cre recombinase mouse lines, it was shown that TRP63+ basal cells form both proximal respiratory tract and alveolar epithelial lineages in the developing lung at the beginning of lung development and have polylinear differentiation ability (Y. Yang et al., 2018).

In a study examining spherical transcriptomes of basal cells, microarray analysis of refining basal cells, and single-cell RNA sequencing of proximal respiratory tract cells, including the transcription factor TRP63, the keratins KRT5 and KRT14, and the cell surface markers PDPN, NGFR, and GS1 lectin B4 a set of proteins selectively expressed in basal cells defined the marker (Plasschaert et al., 2018; Rock et al., 2009).

In the severe lung disease caused by influenza virus or bleomycin, express basal cell markers such as KRT5 and TRP63 after injury cells can be detected in the alveolar regions (W. Zuo et al., 2015). It has also been reported that the secretory cells (club cell) labeled as Scgb1a1 differentiate into TRP63/KRT5+ basal cells after selective depletion of basal cells in a study

conducted with lineage tracing method (P. R. Tata et al., 2013). Studies have revealed at minimum two different basal cell populations. One works as a self-renewing stem cell and the other is dependent on luminal differentiation and is characterized by poor expression of Krt8 (Watson et al., 2015). Another study showed that NOTCH2 signaling and activation of C-MYB expression among KRT8-expressing basal cells described differentiation into secretory or ciliary cells, respectively (Pardo-Saganta et al., 2015). In postnatal lung injury, secretion of basal cells Notch signal is essential for differentiation into cells (Rock et al., 2011). A significant small subset of the regenerative KRT14+ basal cells of the BC population was also found to adopt a multipotent appearance following acute injury (Ghosh et al., 2011).

Submucosal gland stem/Progenitor cells

Submucosal glands have long been studied as respiratory tract niches that house cells that can proliferate into respiratory epithelial cells (Borthwick, Shahbazian, Krantz, Dorin, & Randell, 2001). Submucosal gland epithelium is seen in all areas from the bronchioalveolar area to the proximal tracheal area in humans. However, it is found in the upper trachea and bundle of glands located between the cricoid cartilage and the first tracheal cartilage in mice (Hegab et al., 2011; Innes & Dorin, 2001) . This epithelium consists of goblet cells and serous cells and basally located myoepithelial cells (Alysandratos, Herriges, & Kotton, 2021).

Wnt signaling is required for description of origin of glandular stem cells during tracheal submucosal gland morphogenesis from surface respiratory tract epithelium. In mice (BAT-gal and TCF/Lef:H2B-GFP) two Wnt-reporters have been found to be actively co-expressed in cyclic glandular stem cells, primordial glandular placodes, and a small subunit of adult submucosal glands progenitor cells that enter the cell cycle 24 hours after respiratory tract injury (Lynch et al., 2016).

Another study in mice showed that submucosal gland myoepithelial cells can differentiate into both basal and luminal cells of the submucosal gland epithelium following severe respiratory epithelial damage (Lynch et al., 2018; A. Tata et al., 2018). In hypoxia-induced respiratory epithelial injury using lineage tracing, submucosal gland duct cells have been shown to contribute to respiratory epithelial regeneration (Hegab et al., 2011).

It is suggested that depletion of the submucosal glands stem cell niche and loss of multipotent submucosal glands stem cells in chronic lung allograft dysfunction may lead to the loss of respiratory epithelial basal cells and thus to fibrosis (Swatek et al., 2018). Myoepithelial cells are one of the progenitor subsets in the submucosal glands niche. It can differentiate into all subgroups of submucosal glands following respiratory tract injury (Lynch et al., 2016). Myoepithelial cells are located on the extraluminal surface of submucosal glands and express both smooth muscle actin (ACTA2 or-SMA) and smooth muscle myosin heavy chain 11 (K. R. Parekh et al., 2020).

In mouse models of respiratory tract damage caused by naphthalene and SO2, smooth muscle actin (SMA) + epithelial cells appeared on the surface respiratuar epithelium by the Lineage-traced method. In mouse models of respiratory tract damage caused by naphthalene and sulfur dioxide (SO2), smooth muscle actin + epithelial cells appeared on the surface respiratory tract epithelium by the Lineage-traced method (Lynch et al., 2018). This indicates that these cells are derived from glandular myoepithelial cells. Using lineage-traced and scRNA-seq methods, myoepithelial cells of submucosal glands proliferate and multiple injury models have been found to migrate to regenerate the respiratory surface epithelium. In the same study, it was described that the SOX9 transcription factor is required for MEC plasticity in respiratory epithelial regeneration (A. Tata et al., 2018).

Neuroendocrine stem/Progenitor cells

Pulmonary neuroendocrine cells, usually neuroendocrine concentrated in bodies (NEBs). The transcription factor ASCL1 is characterized by the expression of the calcitonin gene-related peptide and the ubiquitin carboxyl terminal enzyme L1 (H. J. Chen et al., 2019). Using the naphthalene damage model in which secretory cells are depleted, they described a subset of secretory cells, a group of cells called variant club cells. These cells, which are normally dormant, do not express the cytochrome P450 2F2 isoenzyme, which is responsible for the production of naphthalene metabolites that are toxic to other secretory cells (Guha et al., 2012).

These variants, which survive depletion of pulmonary neuroendocrine cells, following naphthalene injury, have been shown to rapidly reconstruct both secretory and ciliary cell populations of club cell-damaged respiratory

tracts (Giangreco, Reynolds, & Stripp, 2002). In a study in mice, Variant-club cells were found to be located in a region adjacent to NEBs and at the junction of the bronchoalveolar duct (Peake, Reynolds, Stripp, Stephens, & Pinkerton, 2000). Pulmonary neuroendocrine cells were also thought to supply a stem cell niche during injury. It was thought that there was evidence that they had the ability to act as a progenitor cell, and that the cells would differentiate into club and ciliary cells (Yao et al., 2018). Pulmonary neuroendocrine cells, can differentiate into other epithelial cells such as club cells, ciliary cells, and goblet cells, potentially via epigenetic regulation involving polycomb suppressor complex 2 and inflammatory responses involving the IL6-STAT3 pathway (Yao et al., 2017). In the study, Notch knows that the signaling pathway is a necessary and sufficient pathway for the reprogramming of pulmonary neuroendocrine cells. However, it has been stated that reprogramming requires additional signals (Ouadah et al., 2019).

In a recent study, it was stated that pulmonary neuroendocrine cells contribute little to epithelial regeneration in the current environment, even though they act as stem cells in the regeneration process in a mouse model of respiratory injury, and genetic loss of pulmonary neuroendocrine cells in mice may not affect respiratory tract homeostasis or regeneration. Because it was concluded that alternative stem cell populations in the respiratory tract epithelium can induce cell regeneration such as club and basal cells (Noguchi, Furukawa, & Morimoto, 2020).

Alveolar stem/Progenitor cells

Type-1 alveoli (AT1) and type-2 alveoli (AT2) cells are the two main cell types that make up the alveolar epithelium (Yanjie Wang et al., 2018). AT1 cells are morphologically slender and characterized by the expression of Homeobox-only protein x (HOPX) and Podoplanin (PDPN) specialized for gas exchange (J. Yang & Chen, 2014). Approximately 95% of the alveolar surface is covered by AT1 cells (Weibel, 2015). AT2 cells secrete surfactant protein C (SFTPC), as well as bifunctional alveolar progenitor cells that act as stem cells (Katsura, Kobayashi, Tata, & Hogan, 2019). In the study using the lineage-tracing method, it was stated that AT2 cells can differentiate into AT1 cells in a steady state and the process takes about a few months. In the same study, the self-renewal and differentiation capacity of mature AT2s was

approved by in vivo lineage tracing studies using Cre recombinase induced by genes associated with differentiated functions such as Sftpc and Lyz2 (LysM) (Barkauskas et al., 2013).

In another study, various factors including yes-associated protein (YAP) activation and stromal cell-derived factor 1 (SDF1) signaling, which lead to the production of growth factors such as epithelial growth factor-(EGF) in the self-regeneration of AT2s after distal airway epithelial damage, and signaling pathways have been identified.(Chung, Bujnis, Barkauskas, Kobayashi, & Hogan, 2018) Several factors and signaling pathways have been described as involved, including paracrine signals released AT2 cells are described by surfactant-c SFTPC, (Lysosomal Associated Membrane Protein 3), and ATP Binding Cassette Subfamily A Member 3, specific markers (Treutlein et al., 2014). A subpopulation of AT2 cells was found to express transmembrane 4L (TM4SF1) in reply to acute lung injury (Zacharias et al., 2018). A study in postnatal mice showed that AT2s can self-renew and transform into AT1s following aging or hyperoxic injury via epidermal growth factor receptor (EGFR)-KRAS-mediated signaling (Desai, Brownfield, & Krasnow, 2014).

In another study, groups of cells expressing TRP63 and KRT5, termed distal respiratory tract stem cells (DASCs), were described in mouse and human distal respiratory tract epithelium, which can self-renew and alteration into alveolar and bronchial epithelial cells (Ma et al., 2018). TRP63(4) and KRT5(+) cells have been reported to be a potential therapeutic modality to treat COPD and acute lung injury (Yujia Wang et al., 2019).

Secretory stem/Progenitor cells

Secretory cells in the respiratory epithelium have been classified according to their morphological features and location as goblet cells , club cells, and serous cells (Jeffery, Gaillard, & Moret, 1992). Club cells are found in the trachea facing the bronchial lumen, the proximal bronchus, and the distal respiratory tract. Club cells are cells restricted to the terminal and respiratory bronchioles (P. R. Tata et al., 2013). Club cells are defined by the expression of SCGB1A1 and show PAS negative effect. Also, SCGB3A1 and SCGB3A2 are expressed in clup cell populations. Recent studies have shown

that KRT17 and KRT19 are strongly increased in Club cells (Kalpaj R. Parekh et al., 2020).

The main secretory product of Club cells is mucin glycoprotein. In addition, MUC1 produces KL-6 proteins, glycoproteins and lipids (Rokicki, Rokicki, Wojtacha, & Dżeljijli, 2016). In the study, it was described that SCGB1A1+ club cells can differentiate after severe lung injury and contribute to the basal stem cell pool. In response to pulmonary allergen-induced lung injury, club cells have been reported to differentiate into goblet cells when physiological conditions are met (G. Chen et al., 2009). In a SCGB1A1-CreER lineage-traced model for Club cells, Following damage to the mice respiratory epithelium with bleomycin, or influenza virus infection, grafts of novel SCGB1A1, TRP63 cells were detected to be organized into luminal-like structures, in which the epithelial structure was similar to bronchioles. In the study, it was stated that these cells expressed the immature club cell marker, cytochrome P-450 2F2 (CYP2f2). It has been stated that TRP63 marker is not seen after regeneration and the bronchioles become monolayered (Zheng, Liu, Lin, Guo, & Zheng, 2019).

Differentiation of basal cells to secretory cells is sustained and regulated by the Notch pathway and the Nuclear factor 1 A-type protein. In addition, club cells are primary progenitor cells for both multiciliary and goblet cells (Yujia Wang et al., 2019; W. L. Zuo et al., 2018). Differentiation of multiciliary cells is regulated by the chromatin-binding protein, which controls the transcriptional activity and positioning of ciliary proteins (Zhou et al., 2015).

REFERENCES

Allen, W. E. (2008). diFiore's Atlas of Histology with Functional Correlations (11th Edition). Journal of Anatomy, 213(3), 357-358. doi:https://doi.org/10.1111/j.1469-7580.2008.00956.x

Alysandratos, K. D., Herriges, M. J., & Kotton, D. N. (2021). Epithelial Stem and Progenitor Cells in Lung Repair and Regeneration. Annu Rev Physiol, 83, 529-550. doi:10.1146/annurev-physiol-041520-092904

Araneda, O. F., & Tuesta, M. (2012). Lung oxidative damage by hypoxia. Oxid Med Cell Longev, 2012, 856918. doi:10.1155/2012/856918

Barkauskas, C. E., Cronce, M. J., Rackley, C. R., Bowie, E. J., Keene, D. R., Stripp, B. R., . . . Hogan, B. L. (2013). Type 2 alveolar cells are stem cells in adult lung. J Clin Invest, 123(7), 3025-3036. doi:10.1172/jci68782

Borthwick, D. W., Shahbazian, M., Krantz, Q. T., Dorin, J. R., & Randell, S. H. (2001). Evidence for stem-cell niches in the tracheal epithelium. Am J Respir Cell Mol Biol, 24(6), 662-670. doi:10.1165/ajrcmb.24.6.4217

Chen, G., Korfhagen, T. R., Xu, Y., Kitzmiller, J., Wert, S. E., Maeda, Y., . . . Whitsett, J. A. (2009). SPDEF is required for mouse pulmonary goblet cell differentiation and regulates a network of genes associated with mucus production. J Clin Invest, 119(10), 2914-2924. doi:10.1172/jci39731

Chen, H. J., Poran, A., Unni, A. M., Huang, S. X., Elemento, O., Snoeck, H. W., & Varmus, H. (2019). Generation of pulmonary neuroendocrine cells and SCLC-like tumors from human embryonic stem cells. J Exp Med, 216(3), 674-687. doi:10.1084/jem.20181155

Chung, M. I., Bujnis, M., Barkauskas, C. E., Kobayashi, Y., & Hogan, B. L. M. (2018). Niche-mediated BMP/SMAD signaling regulates lung alveolar stem cell proliferation and differentiation. Development, 145(9). doi:10.1242/dev.163014

Desai, T. J., Brownfield, D. G., & Krasnow, M. A. (2014). Alveolar progenitor and stem cells in lung development, renewal and cancer. Nature, 507(7491), 190-194. doi:10.1038/nature12930

Dye, B. R., Miller, A. J., & Spence, J. R. (2016). How to Grow a Lung: Applying Principles of Developmental Biology to Generate Lung Lineages from Human Pluripotent Stem Cells. Curr Pathobiol Rep, 4, 47-57. doi:10.1007/s40139-016-0102-x

Gartner, L. P. H. J. L. (2007). Color textbook of histology. Edinburgh: Elsevier Saunders.

Ghosh, M., Brechbuhl, H. M., Smith, R. W., Li, B., Hicks, D. A., Titchner, T., . . . Reynolds, S. D. (2011). Context-dependent differentiation of multipotential keratin 14-expressing tracheal basal cells. Am J Respir Cell Mol Biol, 45(2), 403-410. doi:10.1165/rcmb.2010-0283OC

Giangreco, A., Reynolds, S. D., & Stripp, B. R. (2002). Terminal bronchioles harbor a unique airway stem cell population that localizes to the bronchoalveolar duct junction. Am J Pathol, 161(1), 173-182. doi:10.1016/s0002-9440(10)64169-7

Guha, A., Vasconcelos, M., Cai, Y., Yoneda, M., Hinds, A., Qian, J., . . . Cardoso, W. V. (2012). Neuroepithelial body microenvironment is a niche for a distinct subset of Clara-like precursors in the developing airways. Proc Natl Acad Sci U S A, 109(31), 12592-12597. doi:10.1073/pnas.1204710109

Hegab, A. E., Ha, V. L., Gilbert, J. L., Zhang, K. X., Malkoski, S. P., Chon, A. T., . . . Gomperts, B. N. (2011). Novel stem/progenitor cell population from murine tracheal submucosal gland ducts with multipotent regenerative potential. Stem Cells, 29(8), 1283-1293. doi:10.1002/stem.680

Innes, B. A., & Dorin, J. R. (2001). Submucosal gland distribution in the mouse has a genetic determination localized on chromosome 9. Mamm Genome, 12(2), 124-128. doi:10.1007/s003350010244

Jeffery, P. K., Gaillard, D., & Moret, S. (1992). Human airway secretory cells during development and in mature airway epithelium. Eur Respir J, 5(1), 93-104.

Katsura, H., Kobayashi, Y., Tata, P. R., & Hogan, B. L. M. (2019). IL-1 and TNFα Contribute to the Inflammatory Niche to Enhance Alveolar Regeneration. Stem Cell Reports, 12(4), 657-666. doi:10.1016/j.stemcr.2019.02.013

Kiley, J. P., & Senior, R. M. (2012). Pulmonary research in 2013 and beyond: a National Heart, Lung, and Blood Institute perspective. American Journal of Physiology-Lung Cellular and Molecular Physiology, 303(9), L729-L732. doi:10.1152/ajplung.00305.2012

Knight, D. A., & Holgate, S. T. (2003). The airway epithelium: structural and functional properties in health and disease. Respirology, 8(4), 432-446. doi:10.1046/j.1440-1843.2003.00493.x

Kotton, D. N., & Morrisey, E. E. (2014). Lung regeneration: mechanisms, applications and emerging stem cell populations. Nat Med, 20(8), 822-832. doi:10.1038/nm.3642

Labaki, W. W., & Han, M. K. (2020). Chronic respiratory diseases: a global view. Lancet Respir Med, 8(6), 531-533. doi:10.1016/s2213-2600(20)30157-0

Lumb, A. B., & Thomas, C. R. (2020). Nunn's applied respiratory physiology eBook: Elsevier Health Sciences.

Lynch, T. J., Anderson, P. J., Rotti, P. G., Tyler, S. R., Crooke, A. K., Choi, S. H., . . . Engelhardt, J. F. (2018). Submucosal Gland Myoepithelial Cells Are Reserve Stem Cells That Can Regenerate Mouse Tracheal Epithelium. Cell Stem Cell, 22(5), 653-667.e655. doi:10.1016/j.stem.2018.03.017

Lynch, T. J., Anderson, P. J., Xie, W., Crooke, A. K., Liu, X., Tyler, S. R., . . . Engelhardt, J. F. (2016). Wnt Signaling Regulates Airway Epithelial Stem Cells in Adult Murine Submucosal Glands. Stem Cells, 34(11), 2758-2771. doi:10.1002/stem.2443

Ma, Q., Ma, Y., Dai, X., Ren, T., Fu, Y., Liu, W., . . . Zuo, W. (2018). Regeneration of functional alveoli by adult human SOX9+ airway basal cell transplantation. Protein & Cell, 9(3), 267-282. doi:10.1007/s13238-018-0506-y

Mescher, A. (2013). Junqueira's Basic Histology: Text & Atlas (13th ed.), by Anthony L. Mescher.

Mescher, A. L. ((2013)). Junqueira's basic histology : text and atlas. .

Michael H. Ross, W. P. ((2020)). Histology : a text and atlas : with correlated cell and molecular biology.

Montoro, D. T., Haber, A. L., Biton, M., Vinarsky, V., Lin, B., Birket, S. E., . . . Rajagopal, J. (2018). A revised airway epithelial hierarchy includes CFTR-expressing ionocytes. Nature, 560(7718), 319-324. doi:10.1038/s41586-018-0393-7

Musah, S., Chen, J., & Hoyle, G. W. (2012). Repair of tracheal epithelium by basal cells after chlorine-induced injury. Respiratory Research, 13(1), 107. doi:10.1186/1465-9921-13-107

Noguchi, M., Furukawa, K. T., & Morimoto, M. (2020). Pulmonary neuroendocrine cells: physiology, tissue homeostasis and disease. Dis Model Mech, 13(12). doi:10.1242/dmm.046920

Ouadah, Y., Rojas, E. R., Riordan, D. P., Capostagno, S., Kuo, C. S., & Krasnow, M. A. (2019). Rare Pulmonary Neuroendocrine Cells Are Stem Cells Regulated by Rb, p53, and Notch. Cell, 179(2), 403-416.e423. doi:https://doi.org/10.1016/j.cell.2019.09.010

Pardo-Saganta, A., Law, B. M., Tata, P. R., Villoria, J., Saez, B., Mou, H., . . . Rajagopal, J. (2015). Injury induces direct lineage segregation of functionally distinct airway basal stem/progenitor cell subpopulations. Cell Stem Cell, 16(2), 184-197. doi:10.1016/j.stem.2015.01.002

Parekh, K. R., Nawroth, J., Pai, A., Busch, S. M., Senger, C. N., & Ryan, A. L. (2020). Stem cells and lung regeneration. Am J Physiol Cell Physiol, 319(4), C675-c693. doi:10.1152/ajpcell.00036.2020

Parekh, K. R., Nawroth, J., Pai, A., Busch, S. M., Senger, C. N., & Ryan, A. L. (2020). Stem cells and lung regeneration. American Journal of Physiology-Cell Physiology, 319(4), C675-C693. doi:10.1152/ajpcell.00036.2020

Patrick C. Nahirney, W. K. O. ((2013)). Netter's essential histology. Elsevier/Saunders. .

Patwa, A., & Shah, A. (2015). Anatomy and physiology of respiratory system relevant to anaesthesia. Indian J Anaesth, 59(9), 533-541. doi:10.4103/0019-5049.165849

Peake, J. L., Reynolds, S. D., Stripp, B. R., Stephens, K. E., & Pinkerton, K. E. (2000). Alteration of pulmonary neuroendocrine cells during epithelial repair of naphthalene-induced airway injury. Am J Pathol, 156(1), 279-286. doi:10.1016/s0002-9440(10)64728-1

Plasschaert, L. W., Žilionis, R., Choo-Wing, R., Savova, V., Knehr, J., Roma, G., . . . Jaffe, A. B. (2018). A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte. Nature, 560(7718), 377-381. doi:10.1038/s41586-018-0394-6

Rawlins, E. L., & Hogan, B. L. M. (2008). Ciliated epithelial cell lifespan in the mouse trachea and lung. American Journal of Physiology-Lung Cellular and Molecular Physiology, 295(1), L231-L234. doi:10.1152/ajplung.90209.2008

Rock, J. R., Gao, X., Xue, Y., Randell, S. H., Kong, Y. Y., & Hogan, B. L. (2011). Notch-dependent differentiation of adult airway basal stem cells. Cell Stem Cell, 8(6), 639-648. doi:10.1016/j.stem.2011.04.003

Rock, J. R., Onaitis, M. W., Rawlins, E. L., Lu, Y., Clark, C. P., Xue, Y., . . . Hogan, B. L. (2009). Basal cells as stem cells of the mouse trachea and human airway epithelium. Proc Natl Acad Sci U S A, 106(31), 12771-12775. doi:10.1073/pnas.0906850106

Rock, J. R., Randell, S. H., & Hogan, B. L. (2010). Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. Dis Model Mech, 3(9-10), 545-556. doi:10.1242/dmm.006031

Rokicki, W., Rokicki, M., Wojtacha, J., & Dżeljijli, A. (2016). The role and importance of club cells (Clara cells) in the pathogenesis of some respiratory diseases. Kardiochir Torakochirurgia Pol, 13(1), 26-30. doi:10.5114/kitp.2016.58961

Ruiz García, S., Deprez, M., Lebrigand, K., Cavard, A., Paquet, A., Arguel, M. J., . . . Zaragosi, L. E. (2019). Novel dynamics of human mucociliary differentiation revealed by single-cell RNA sequencing of nasal epithelial cultures. Development, 146(20). doi:10.1242/dev.177428

Swatek, A. M., Lynch, T. J., Crooke, A. K., Anderson, P. J., Tyler, S. R., Brooks, L., . . . Parekh, K. R. (2018). Depletion of Airway Submucosal Glands and TP63(+)KRT5(+) Basal Cells in Obliterative Bronchiolitis. Am J Respir Crit Care Med, 197(8), 1045-1057. doi:10.1164/rccm.201707-1368OC

Tata, A., Kobayashi, Y., Chow, R. D., Tran, J., Desai, A., Massri, A. J., . . . Tata, P. R. (2018). Myoepithelial Cells of Submucosal Glands Can Function as Reserve Stem Cells to Regenerate Airways after Injury. Cell Stem Cell, 22(5), 668-683.e666. doi:10.1016/j.stem.2018.03.018

Tata, P. R., Mou, H., Pardo-Saganta, A., Zhao, R., Prabhu, M., Law, B. M., . . . Rajagopal, J. (2013). Dedifferentiation of committed epithelial cells into stem cells in vivo. Nature, 503(7475), 218-223. doi:10.1038/nature12777

Treutlein, B., Brownfield, D. G., Wu, A. R., Neff, N. F., Mantalas, G. L., Espinoza, F. H., . . . Quake, S. R. (2014). Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature, 509(7500), 371-375. doi:10.1038/nature13173

Wang, Y., Lu, Y., Wu, Y., Sun, Y., Zhou, Y., Ma, Q., . . . Zuo, W. (2019). Alveolar Differentiation Potency of Human Distal Airway Stem Cells Is Associated with Pulmonary Pathological Conditions. Stem Cells International, 2019, 7123078. doi:10.1155/2019/7123078

Wang, Y., Tang, Z., Huang, H., Li, J., Wang, Z., Yu, Y., . . . Tang, N. (2018). Pulmonary alveolar type I cell population consists of two distinct subtypes that differ in cell fate. Proceedings of the National Academy of Sciences, 115(10), 2407-2412. doi:10.1073/pnas.1719474115

Watson, J. K., Rulands, S., Wilkinson, A. C., Wuidart, A., Ousset, M., Van Keymeulen, A., . . . Rawlins, E. L. (2015). Clonal Dynamics Reveal Two Distinct Populations of Basal Cells in Slow-Turnover Airway Epithelium. Cell Rep, 12(1), 90-101. doi:10.1016/j.celrep.2015.06.011

Weibel, E. R. (2015). On the tricks alveolar epithelial cells play to make a good lung. Am J Respir Crit Care Med, 191(5), 504-513. doi:10.1164/rccm.201409-1663OE

Wu, H., & Tang, N. (2021). Stem cells in pulmonary alveolar regeneration. Development, 148(2). doi:10.1242/dev.193458

Yang, J., & Chen, J. (2014). Developmental programs of lung epithelial progenitors: a balanced progenitor model. WIREs Developmental Biology, 3(5), 331-347. doi:https://doi.org/10.1002/wdev.141

Yang, Y., Riccio, P., Schotsaert, M., Mori, M., Lu, J., Lee, D. K., . . . Cardoso, W. V. (2018). Spatial-Temporal Lineage Restrictions of Embryonic p63(+)

Progenitors Establish Distinct Stem Cell Pools in Adult Airways. Dev Cell, 44(6), 752-761.e754. doi:10.1016/j.devcel.2018.03.001

Yao, E., Lin, C., Wu, Q., Zhang, K., Song, H., & Chuang, P.-T. (2017). Notch Signaling Controls Transdifferentiation of Pulmonary Neuroendocrine Cells in Response to Lung Injury. Stem Cells, 36(3), 377-391. doi:10.1002/stem.2744

Yao, E., Lin, C., Wu, Q., Zhang, K., Song, H., & Chuang, P.-T. (2018). Notch Signaling Controls Transdifferentiation of Pulmonary Neuroendocrine Cells in Response to Lung Injury. Stem Cells, 36(3), 377-391. doi:https://doi.org/10.1002/stem.2744

Zacharias, W. J., Frank, D. B., Zepp, J. A., Morley, M. P., Alkhaleel, F. A., Kong, J., . . . Morrisey, E. E. (2018). Regeneration of the lung alveolus by an evolutionarily conserved epithelial progenitor. Nature, 555(7695), 251-255. doi:10.1038/nature25786

Zheng, B. N., Liu, H. T., Lin, Q. H., Guo, J. Y., & Zheng, C. L. (2019). [To discuss the problems existing in the labor ability appraisal of an occupational chronic benzene poisoning incidence]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi, 37(5), 379-381. doi:10.3760/cma.j.issn.1001-9391.2019.05.014

Zhou, F., Narasimhan, V., Shboul, M., Chong, Y. L., Reversade, B., & Roy, S. (2015). Gmnc Is a Master Regulator of the Multiciliated Cell Differentiation Program. Current Biology, 25(24), 3267-3273. doi:10.1016/j.cub.2015.10.062

Zuo, W., Zhang, T., Wu, D. Z., Guan, S. P., Liew, A. A., Yamamoto, Y., . . . McKeon, F. (2015). p63(+)Krt5(+) distal airway stem cells are essential for lung regeneration. Nature, 517(7536), 616-620. doi:10.1038/nature13903

Zuo, W. L., Shenoy, S. A., Li, S., O'Beirne, S. L., Strulovici-Barel, Y., Leopold, P. L., . . . Crystal, R. G. (2018). Ontogeny and Biology of Human Small Airway Epithelial Club Cells. Am J Respir Crit Care Med, 198(11), 1375-1388. doi:10.1164/rccm.201710-2107OC

CHAPTER 2

CANCER-ASSOCIATED CACHEXIA

AND IRISIN RELATION

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INTRODUCTION

Cancer cachexia is a crucial disease characterized by skeletal muscle wasting (de Castro et al ., 2021). Cachexia is difficult to treat due to the lack of drugs approved by the Federal Drug Administration (FDA) and does not respond to traditional cancer treatments. For patients with cancer, there is a 30-80% chance of developing cachexia with a higher incidence of cachexia in certain cancers such as stomach, prostate, pancreas, colon, and lung cancer. Some cancer patients with the same tumor type and burden become cachectic, while others do not. This situation is closely related to the immune system and genetic variations of cancer patients (Thair et al., 2011). Cachexia accounts for nearly 20-40 % of cancer-related deaths (Devine et al., 2017). Cancer cachexia is also closely related to systemic inflammation (Zhou et al., 2010). Surprisingly, stopping muscle wasting triggers prolongation of survival in cancer cachexia. Precisely for this reason, detecting cachexia and preserving muscle mass is of great importance for patients with cancer cachexia but early detection of cancer cachexia is limited for several reasons (Loumaye &Thissen., 2017).

Irisin is a newly identified myokine that is regulated by Fibronectin Type III domain-containing protein-5 (FNDC5) (Bostrom et al., 2012). FNDC5 is a kind of type 1 membrane glycoprotein and proteolytically cleaved from the N-terminal domain by an unknown enzyme and 112 aa irisin is released into the bloodstream. Irisin is present in the blood at the basal level, and it has been reported that its releases from skeletal muscle to the blood increases with acute exercise (short and intense exercise), and it binds to its yet unidentified receptor on adipose tissue, causing a significant increase in total body energy expenditure and slimming (Huh et al., 2012, Castillo-Quan., 2012). Recently, the anti-inflammatory, anti-apoptotic, anti-cancer, and anti-oxidative properties of irisin have received a great deal of attention from the scientific society. Inflammation is closely related to diseases such as obesity, T2DM, and various cancers (Askari et al., 2018). This article, it is aimed to reveal the relationship of irisin with cancer cachexia.

Cancer Cachectic Factors

In recent years, researchers have been searching for new mediators in cancer cachexia to prevent and treat tumor-induced fat and muscle wasting. Although the mechanism of weight loss in cancer cachexia has not been fully

elucidated, cachectic factors such as proteolysis-inducing factor (PIF),a lipid mobilizing factor (LMF)/Zinc-α-2-glycoprotein (ZAG) released from tumor cells in cancer, and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6), which are over-synthesized by the body is important and well-known molecules. But precise agents for the treatment of cachexia continue to be investigated.

1. PIF (Proteolysis Inducing Factor)

Dermicidin (DCD) protein consists of 19 aa signal peptide, 30 aa proteolysis-inducing factor-central peptide (PIF-CP) (neuronal life stimulating peptide), 13 aa propeptide, 48 aa dermicidin-1 (DCD-1) polypeptide. PIF, identified as a cachectic factor, was first isolated from the serum of rodents with MAC16 tumors. Cachexia was induced in mice by the implantation of the MAC16 tumor and PIF was purified from this serum by binding to the antibody added to mouse serum, but PIF was not found in mouse sera with MAC13 tumor (non-cachexia-induced) (Cariuk et al., 1997). In another study on mice, PIF was shown to cause weight loss by stimulating protein breakdown without reducing appetite, and it was elucidated in the circulation of mice with cachexia-induced tumors. The positive correlation between weight loss and PIF led to the elucidation of this protein as a new cachectic factor. PIF found in humans is localized at 13.1 on chromosome 12. It consists of 523 base pairs and has 6 exons. Purified PIF weighs approximately 24 kDa and contains a short polypeptide chain of N-(10kDa) and O-linked (6kDa) sulfated oligosaccharides. PIF is not found in cancer patients without weight loss and in patients with the benign disease who have weight loss (Wieland et al., 2007). PIF expression has also been found in tumors where weight loss occurs, such as prostate, colon, liver, esophagus, lung, and pancreas. Its expression was not found in normal tissues (Smith et al., 2004). Its contribution to the reduction in body weight is due to the fact that it reduces protein synthesis by 50% and increases its breakdown by 50%. Treatment of skeletal muscle and rodent myotubes with PIF increases the expression and activity of key molecules involved in the ubiquitin-proteosome proteolytic pathway 8Loriette et al., 2001). As a result, it can be stated that LMF/ZAG secreted from tumor tissue causes atrophy of adipose tissue and PIF causes

atrophy of skeletal muscle, leading to the development of cachexia in cancer patients.

2. LMF (Lipid Mobilizing Factor) /ZAG (Zinc-α-2-Glycoprotein)

Numerous factors originating from tumors and hosts cause lipid degradation in cachexia. These factors include lipolytic factors such as inflammatory cytokines (TNF, IL-1, IL-6) and LMF/ZAG. The tumor product LMF, which has lipid mobilizing activity, was purified as a 41 kDa glycoprotein from the urine of patients with cancer cachexia (Todorov et al., 1998; Todorov et al., 1996). This glycoprotein is the plasma protein Zinc-α-2-glycoprotein (ZAG, AZGP1) with amino acid sequence, electrophoretic mobility, and immunoreactivity. ZAG is a 41 kDa secretory protein discovered in human plasma in 196 (Burgi and Schmid., 1961). ZAG derives its name from its tendency to precipitate with zinc salts and its similar electrophoretic mobility with plasma α-2 globulins (Hale et al., 2001). According to the results of immunohistochemical studies, it is mainly found in epithelial cells of the breast, prostate, liver, and other gastrointestinal organs, it is highly expressed in many malignant tumors such as breast, prostate, and lung cancer, and it is thought that it can be used as a cancer biomarker accordingly (Mrack et al., 2011). In an in vitro study, ZAG isolated from human and rodent adipocytes was shown to induce lipolysis, and it was demonstrated that it did so through the β3 adrenergic receptor (β3-AR).

3. Tumor Necrosis Factor- α (TNF-α)

TNF-α is a cytokine synthesized as a 26 kDa transmembrane monomer (mTNFα). mTNFα is proteolytically degraded by TNF-α converting enzyme (TACE) to form a 17 kDa soluble TNF-α (sTNFα) molecule 8Black et al., 1997). TNF-α is an inflammatory cytokine produced by macrophages and monocytes in acute inflammation and causes apoptosis and necrosis by affecting intracellular signaling pathways. Also resists cancer and infection, it has many biological functions, but its mechanisms are complex. While this protein provides resistance against some infections, it also plays opposing roles by mediating some pathological complications. Receptors of TNF-α, the main inflammatory cytokine responsible for weight loss are found in muscle,

liver, and adipose tissues. It increases protein breakdown in muscle, protein synthesis in the liver, and glucose production. It plays a role in the development of cancer cachexia by inhibiting the lipoprotein lipase (LPL) enzyme in adipose tissue. (Camps et al., 2006; Tisdale, 2009) The short half-life of biologically active TNF-α and its complex formation with soluble receptors cause uncertainty in its determination.

4. IL-1 (Interleukin-1)

Interleukin-1 (IL-1) is a 15 kDa prototypical proinflammatory cytokine secreted from macrophages, endothelial, and some epithelial cells. It has two forms, IL-1α and IL-1β. In the studies, the biological activities of these two forms could not be distinguished. IL-1, in concert with another proinflammatory cytokine, TNF-α, affects almost every cell type and functions as an immunoadjuvant. In particular, the synthesis, secretion, and activity of IL-1β are regulated by tight control (Dinarello., 1997) Due to infection, trauma, and cancer IL-1 releases increases. It has been reported by many studies that it increases the release of TNF-α, which is the main mediator of weight loss, also known as cachectin (Tisdale., 2009).

5. IL-6 (Interleukin-6)

Interleukin-6 (IL-6) is a protein consisting of 212 amino acids in its signal sequence in humans and 183 amino acids in its mature form. IL-6 exerts its biological effects by binding to the IL-6 receptor. Janus Activated Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) activate the signaling pathway. Its mechanism is similar to that of the hormone leptin (Ernst et al., 2004). It is a multifunctional cytokine that plays a central role in cell defense and stimulates the synthesis of acute-phase proteins in the liver and triggers tissue catabolism. Although it has a strong effect on creating the acute phase response, its overexpression is associated with the pathology of some diseases such as multiple myeloma, rheumatoid arthritis, psoriasis, and postmenopausal osteoporosis. (Simpson et al., 1997). While the IL-6 level increases slowly in the early stages of cachexia, according to the results of the study, it has been shown that IL-6 increases suddenly and rapidly just before death. Figure 1 summarizes the metabolic

picture related to increased cytokine synthesis and severe weight loss resulting from acute phase response in cancer cachexia.

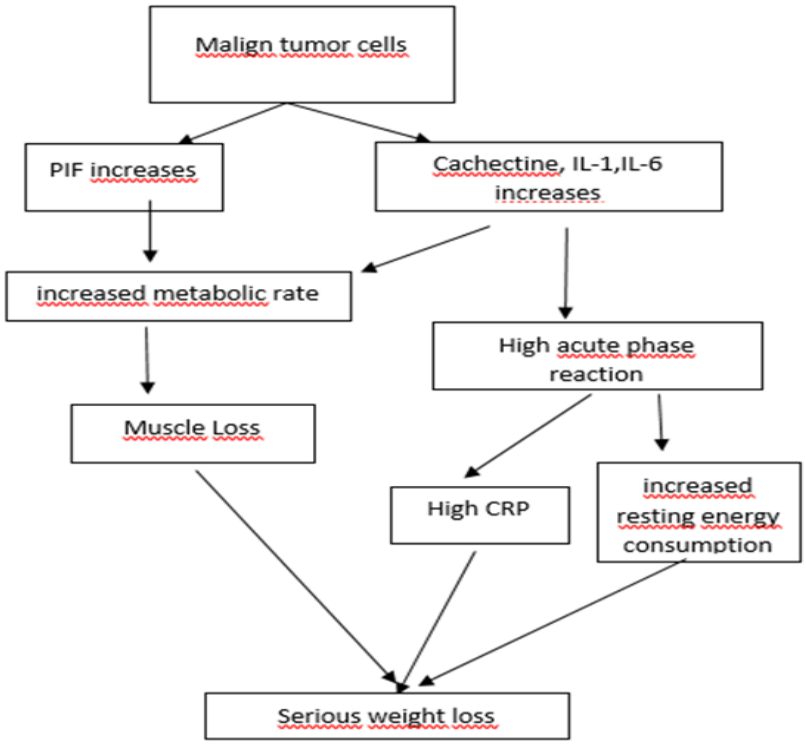

Figure 1. Increased cytokine synthesis and acute phase response in cancer cachexia Saka (2011)

6. Irisin Structure

Irisin is mainly released from skeletal muscle. In later studies, it has been found that it is released in many tissues (such as the pancreas, testis, liver, and stomach) (Korta et al., 2019**).**

As shown in Figure 2, PGC-1α is a coactivator of FNDC5 and FNDC5 expression is increased after exercise, and PGC-1α increases the expression of several muscle gene products, including FNDC5. FNDC5 is a Type-1 membrane protein and is cleaved from its N-terminal domain by an unknown protease and released into the bloodstream of the iris (Waseem et al., 2021).

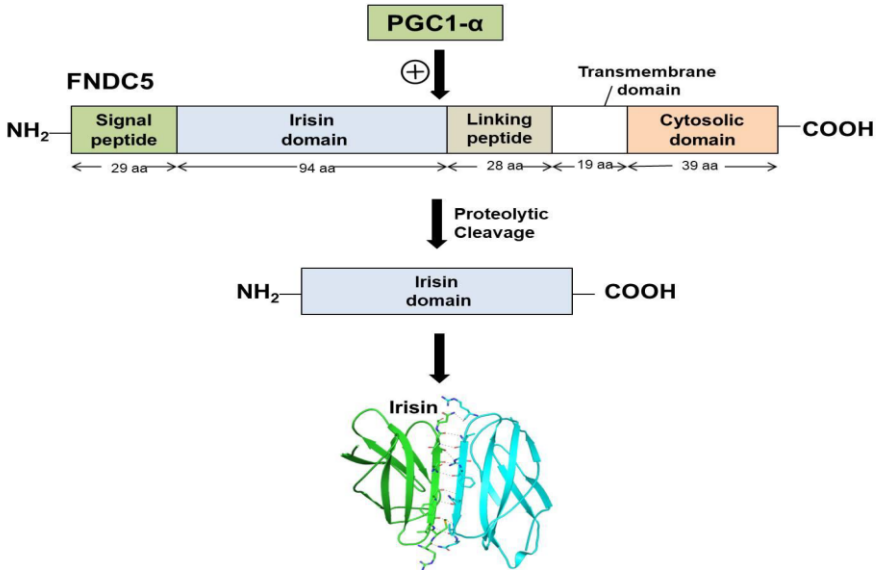

Figure 2. Schematic representation of FNDC5 structure and formation of irisin (Waseem, et al., 2022)

As described before irisin is a newly identified exercise induced adipomiyokine and its level increases after exercise-induced activities and decreases in sedentary and less active peoples (Clin., 2018). Further, the incidence of cancer is 30 to 50% lower in individuals who exercise. In that case, one possible explanation why cancer is less common in individuals who exercise may be the prevention of the reproduction of cancer cells by elevated irisin after exercise (Aydın., 2016). Since cancer cells are not heat resistant, irisin may also be preventing cancer cell growth by producing heat. After acute and intense exercise; irisin level and UCP1 expression increases and causes the release of energy as heat, not ATP (Aydın., 2016).

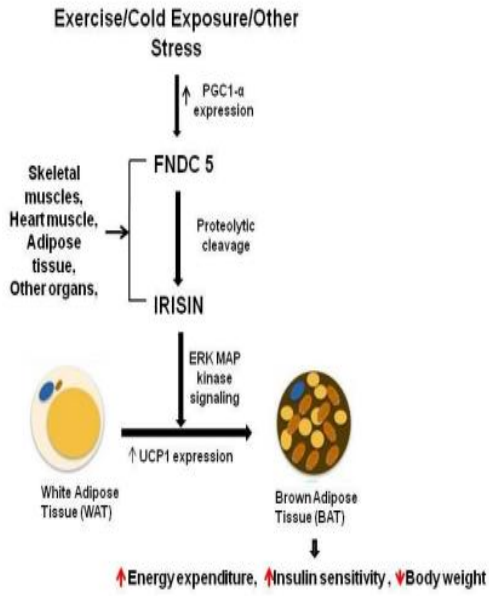

Figure 3. Secretion of irisin and its role of browning of WAT (Waseem, et al., 2022).

There was an alarming situation in which the specificity of anti-irisin antibodies was lacking in many studies. Many experimental human and animal studies have produced worrying results about the presence of irisin. But ELISA tests and quantitative mass spectrometry (MS) etc. are successful methods used to confirm the presence of irisin and to measure circulating irisin levels in humans and animals (Albrecht et al., 2015). In a mouse model with muscle damage, the amount of skeletal muscle was increased, necrosis in muscle tissue was reduced, and muscle strength was increased with irisin injection. These results demonstrate the potential therapeutic value of irisin in muscle injury. (Reza et al., 2017).Recombinant irisin potentially induce skeletal muscle hypertrophy and has therapeutic benefit in overcoming atrophy(Reza et al., 2017). Preserving muscle mass, which is the primary goal of cancer cachexia treatment, as irisin injection will increase muscle hypertrophy. Focusing on studies in this area will be an important step in the treatment of cancer cachexia.

REFERENCES

Albrecht, E.; Norheim, F.; Thiede, B.; Holen, T.; Ohashi, T.; Schering, L.; Lee, S.; Brenmoehl, J.; Thomas, S.; Drevon, C.A. Irisin–a myth rather than an exercise-inducible myokine. Sci. Rep. 2015, 5, 8889.

Askari H, Rajani SF, Poorebrahim M, Haghi-Aminjan H, Raeis-Abdollahi E et al. A glance at the therapeutic potential of irisin against diseases involving inflammation, oxidative stress, and apoptosis: An introductory review. Pharmacol Res. 2018; 129: 44-55.

Aydin S. Is irisin a decisive protein in cancer cachexia and death of cancer cells? Eur Rev Med Pharmacol Sci. 2016 ;20(18):3727-3729.

Black, R,A., Rauch, C,T., Kozlosky, C.J., Peschon, J.J., Slack, J.L., Wolfon, M.F., Castner, B.J., Stocking, K.L., Reddy, P., Srinivason, S., Nelson, N., Bolani, N., Schooley, K.A., Gerthart, M., Dauis, R., Fitzner, J.N., Johnson, R.S, Paxton, R.J., March, C.J., Cerretti, D.P. 1997. ‘’A metalloproteinase disintegrin that releases tumor necrosis factor-alpha from cells’’, Nature, 385, 729-733

Boström, P.; Wu, J.; Jedrychowski, M.P.; Korde, A.; Ye, L.; Lo, J.C.; Rasbach, K.A.; Boström, E.A.; Choi, J.H.; Long, J.Z.; et al.A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481:463–468.

Burgi, W., Schmıd, K. 1961. ‘’Preparation and Properties of Zinc-alpha-2 glycoprotein of normal human plasma’’, J Biol C, 236 (4), 1066-1074.

Camps, C., Iranzo, V., Bremnes, R.M., Sirera, R. 2006. ‘’Anorexia–Cachexia syndrome in cancer: İmplications of the ubiquitin–proteasome pathway’’, Support Care Cancer, 14, 1173–1183.

Castillo-Quan JI. From White to brown fat through the PGC-1α-dependent myokine irisin: implications for diabetes and obesity. Dis Model Mec 2012; 5: 293-295.

Cariuk, P., Lorite, M.J., Todorov, P.T., Field, W.N., Wigmore, S.J., Tisdale, M.J. 1997. ‘’Induction of cachexia in mice by a product isolated fromthe urine of cachectic cancer patients’’, Br J Cancer, 76(5), 606-613.

Clin, A. The Relationship between High-Fat Diet and Fibronectin Type-III Domain-Containing Protein 5 mRNA Expression. Anatol. Clin. J. Med. Sci. 2018, 23, 1–5.

de Castro, G.S., Correia-Lima, J., Simoes, E., Orsso, C.E., Xiao, J., Gama, L.R., Gomes, S.P., . . . Seelaender, M. (2021). Myokines in treatment-naïve

patients with cancer-associated cachexia, Clinical Nutrition. 40(4):2443-2455.

Devine, RD., Bicer, S., Reiser, P.J., Wold, L.E. Increased hypoxia-inducible factor-1α in striated muscle of tumor-bearing mice. Am J Physiol Heart Circ Physiol. 2017 Jun 1;312(6): H1154-H1162.

Dinarello, C.A. 1997. ‘’Interleukin-1. Cytokine Growth Factor Rev’’ 8(4), 253 – 26.

Ernst, M, Jenkins, B.J. 2004. ‘’Acquiring signalling specificity from the cytokine receptor gp130’’, Trends Genet, 20(1), 23-32.

Hale, L.P., Price, D.T., Sanchez, L.M., Demark-Wahnefried, W., Madden, J. 2001. ‘’Zinc alpha-2-Glycoprotein is Expressed by Malignant Prostatic Epithelium and May Serve as a Potential Serum Marker for Prostate Cancer’’ Clin Cancer Res, 7, 846–853.

Huh JY, Panagiotou G, Mougios V et al. FNDC5 and irisin in humans. I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism 2012; 61:1725–38.

Korta, P.; PPoche’s E.; Mazur-Biały, A. Irisin is a multifunctional protein: Implications for health and certain diseases. Medicine 2019, 55, 485

Loumaye A, Thissen JP. Biomarkers of cancer cachexia. Clin Biochem. 2017;50(18):1281-1288.

Lorite, M.J., Smith, H.J., Arnold, J.A., Morris, A., Thompson, M.G., Tisdale, M.J. 2001. ‘’Activation of ATP-ubiquitin-dependent proteolysis in skeletal muscle in vivo and murine myoblasts in vitro by a proteolysis-inducing factor (PIF)’’, Br J Cancer, 85, 297–302.

Mracek, T., Stephens, N.A., Gao, D., Bao, Y., Ross, J.A., Ryden, M., Arner, P., Trayhurn, P., Fearon, KCH. 2011. ‘’Enhanced ZAG production by subcutaneous adipose tissue is Linked to weight loss in gastrointestinal cancer patients’’, Br J Cancer, 104, 441–447.

Reza MM, Subramaniyam N, Sim CM, Ge X, Sathiakumar D, McFarlane C, Sharma M, Kambadur R. Irisin is a pro-myogenic factor that induces skeletal muscle hypertrophy and rescues denervation-induced atrophy. Nat Commun. 2017 24;8(1):1104

Reza, M.M., Sim, C.M., Subramaniyam, Ge, X., Sharma, M., Kambadur, M. ve Mcfarlane, C. Irisin treatment improves healing of dystrophic skeletal muscle, Oncotarget, 2017;8(58):98553–98566.

Saka, B. 2011. Beslenme ve Kanser. İstanbul Üniversitesi İstanbul Tıp Fakültesi, Genel Dahiliye Bilim Dalı, İstanbul XXXVII. Ulusal Hematoloji Kongresi, 19-22 Ekim 2011.

Simpson, R.J., Hammacher, A., Smith, D.K., Matthews, J.M., Ward, L.D. 1997. ‘’Interleukin-6: structure-function relationships’’, Protein Sci 6,929-955.

Smith, H.J, Wyke, S.M., Tisdale, M.J. 2004. ‘’Mechanism of the Attenuation of Proteolysis-Inducing Factor Stimulated Protein Degradation in Muscle by Hydroxy Methyl butyrate.’’ Cancer Res, 64, 8731–8735.

Thair, S.A., Walley, K.R., Nakada, T.A., McConechy, M.K., Boyd, J.H., Wellman, H ., and Russell, J.A. (2011). Single nucleotide polymorphism in NFkB inducing kinase is associated with mortality in septic shock. J. Immunol.186, 2321–2328.

Tisdale, M.J. 2009. ‘’Mechanisms of Cancer Cachexia’’, Physiol Rev, 89, 381–410.

Todorov, P., Cariuk, P., McDevitt, T., Coles, B., Fearon, K., Tisdale, M. 1996. ‘’Characterization of a cancer cachectic factor’’, Nature, 379, 739–742.

Todorov, P.T., McDevitt, T.M., Meyer, D.J., Ueyama, H., Ohkubo, I., Tisdale, M.J. 1998. ‘’Purification and characterization of a tumor lipid-mobilizing factor’’, Cancer Res, 58, 2353–2355.

Waseem, R.; Shamsi, A.; Mohammad, T.; Alhumaydhi, F.A.; Kazim, S.N.; Hassan, M.I.; Ahmad, F.; Islam, A. Multispectroscopic and Molecular Docking Insight into Elucidating the Interaction of Irisin with Rivastigmine Tartrate: A Combinational TherapyApproach to Fight Alzheimer’s Disease. ACS Omega 2021, 6, 7910–7921

Waseem, R.; Shamsi, A.; Mohammad, T.; Hassan, M.I.; Kazim, S.N.; Chaudhary, A.A.; Rudayni, H.A.; Al-Zharani, M.; Ahmad, F.; Islam, A. FNDC5/Irisin: Physiology and Pathophysiology. Molecules 2022,27, 1118.

Wieland, B.M., Stewart, G.D., Skipworth, R.J.E., Sangster, K., Fearon, K.C.H., Ross, J.A., Reiman, T.J., Easaw, J., Mourtzakis, M., Kumar, V., Pak, B.J., Calder, K., Filippatos, G., Kremastinos, D.T., Palcic, M., Baracos, V.E. 2007. ‘’Is There a Human Homologue to the Murine Proteolysis- Inducing Factor?’’, Clin Cancer Res, 13(17), 4984-4992.

Zhou X, Wang JL, Lu J, Song Y, Kwak KS, Jiao Q, Rosenfeld R, Chen Q, Boone T, Simonet WS, Lacey DL, Goldberg AL, Han HQ. Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. Cell. 2010; 20;142(4):531-43.

CHAPTER 3

EFFECTS OF LYCOPENE

ON THE HUMAN BODY

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1. INTRODUCTION

It is stated that antioxidant compounds are effective in the prevention of some heart and cancer diseases. For this reason, studies in recent years have focused on antioxidant compounds. Antioxidant compounds prevent the oxidation of oils by binding oxygen to free radical groups. For this reason, they prevent diseases caused by the oxidation of fats (Bruckdorfer, 1990, Sies, 1991).

Tomato (Lycopersicun esculentum) is an annual plant variety and is native to Mexico and Peru. Starting from Peru, it has spread almost all over the world and has been widely grown in our country since the early 1900s. It contains vitamins A, B1, B2, C, K, niacin, protein, fat, carbohydrates, potassium, calcium and iron (Aybak and Kaygısız, 2004). It is lycopene, a carotenoid with very strong antioxidant properties, that gives the red pigment to tomatoes. Lycopene cannot be produced in the body. Carotenoids are the starting materials of vitamin A and are natural fat-soluble pigments. Although the number of known carotenoids is about six hundred, lycopene is the most important in terms of health. The best known sources of lycopene are; 85% of lycopene is found in tomatoes and tomato products, although tomatoes, processed tomato products, watermelon, pink grapefruit and apricots (Giovannucci, 2002, Agarwal and Rao, 2000). (Table 1).

Chemically, lycopene; It contains 13 double bonds, 11 conjugated and 2 unconjugated. It is a carotenoid that is non-cyclic and lacks vitamin A activity because it does not contain a beta ion ring. Due to the linear hydrocarbon structure in its structure, the chance of being found in oily areas also increases. Therefore, lycopene is most abundant in the human body in the low-density (LDL) and very low-density (VLDL) lipoprotein fractions of serum and in the adrenal glands, testicles, liver, and prostate gland (Agarwal and Rao, 2000).

Table 1. Sources of lycopene

Foods Containing Lycopene		Content of Lycopene (mg/kg)
Tomatoes	Fresh, red Peeled, processed Processed juice Tomato paste Ketchup	31-77.4 112.1 78.3 300.7 166
Apricot	Fresh Preserves Dried	0.05 0.65 8.6
Red pepper	Finished	10.8-26.2
Grapefruit	Fresh, pink	33.6
Watermelon	Red	41

The positive effects of lycopene on human health have also been proven by studies. Lycopene shows antioxidant activity due to the conjugated double bond in its structure. The protective effect of lycopene is also due to this antioxidant property. Antioxidant effect of lycopene; because they bind free radicals and active forms of oxygen (Bruckdorfer, 1990). In a recent study, it was determined that lycopene showed the highest antioxidant activity during lipid peroxidation. Free radicals are atoms or molecules that carry at least one unshared electron. They are very aggressive and destructive due to their unpaired electrons. Free radicals occur during cell metabolism as a result of various factors and take part in intermediate steps in chemical reactions, they can react with cell components and cause permanent damage. This is a chain reaction triggered by radicals and is stated to play a role in aging and various diseases (cancer, atherosclerosis, inflammatory joint disease). The harmful rays of the sun, toxins, smoking, environmental pollution and some factors cause oxidative stress, activating the defense mechanism of the immune system. Normally, free radicals are destroyed by leukocytes in the immune system. Antioxidants, on the other hand, bind to the unpaired electrons of free radicals, allowing them to be easily removed from the body. Thus, cell damage caused by free radicals is reduced and the immune system is supported. Although free radicals affect the proteins, DNA, carbohydrates and enzymes of cells, they show their main effects on membrane lipids (lipid peroxidation). Free radicals, the unsaturated bonds of fatty acids in the

membrane, easily react with free radicals to form peroxidation products. The oxidative breakdown of polyunsaturated fatty acids is known as lipid peroxidation and is extremely harmful. Lipid peroxidation can cause damage to membrane proteins, membrane leakage, and ultimately complete membrane destruction. This continues as a self-sustaining chain reaction and the resulting membrane damage is irreversible (Agarwal and Rao, 2000). (Figure 1).

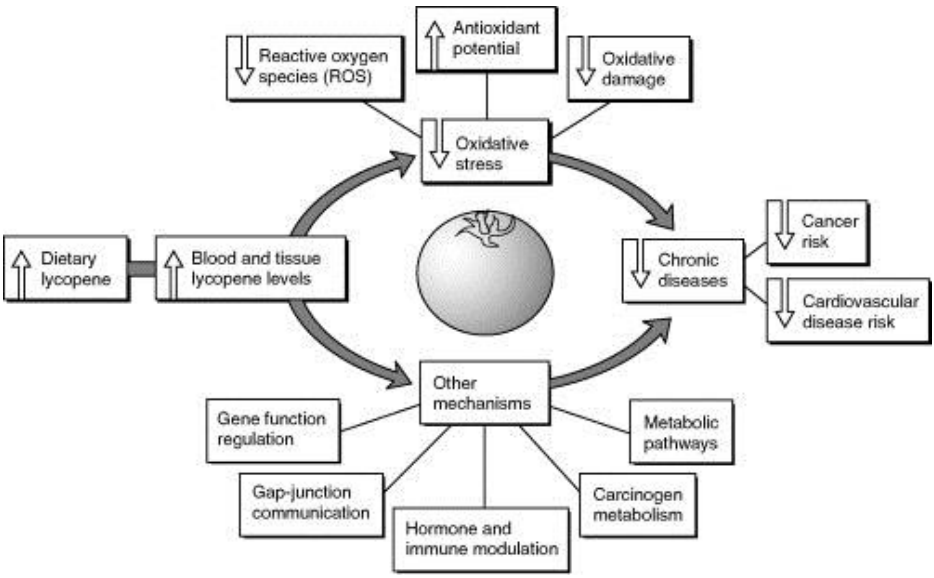

Figure 1. Mechanisms of Action of Lycopene

Oxidized lipids for different reasons can cause a series of events such as cholesterol accumulation on the vessel walls and the formation of atherosclerotic plaques in the vessels. Lycopene significantly reduces the risk of atherosclerosis and coronary heart diseases by inhibiting the oxidation of lipids and low-density lycoprotein. It has been suggested that lycopene is the only carotenoid that can protect overweight people from heart attacks. Lycopene is twice as effective as beta carotene and 100 times more effective than vitamin E in preventing harmful oxygen radicals (Bruckdorfer, 1990, Agarwal and Rao, 2000).

Increased oxidative stress causes the formation of abnormal spermatozoa, resulting in idiopathic male-induced infertility. The cell membrane of spermatozoa is rich in polyunsaturated fatty acids, which creates

a highly sensitive feature that is affected much more quickly by oxidative damage. As a result, when the sperm cell membrane is damaged, sperm motility and viability will be adversely affected, leading to a chain of events leading to serious disorders in sperm oocyte fusion, especially abnormal acrosome reaction (Said, Agarwal, Sharma, Mascha, and Sikka, 2004).

In a prospective cohort study, they compared the consumption of different vegetable varieties in 42 cases with cancer-related death among 1271 elderly people and found that tomato consumption was associated with a 50% reduction in cancer-related mortality in all regions (Colditz, Branch, Lipnick, Willett, Rosner, and Posner, 1985).

In a study conducted in mouse species with a high incidence of breast cancer, it was shown that regular dietary lycopene intake delays the onset of breast cancer and slows its development. This effect was associated with decreased enzyme activity in the mammary gland and decreased serum fatty acid and prolactin levels (Nagasawa, Mitamura, Sakamoto, ve Yamamoto, 1995).

A study by a group of researchers from Harvard University examined the relationship between carotenes and prostate cancer risk. In this review, only the protective feature of lycopene against this cancer risk was clearly identified. It has been shown that men who ate a high amount of lycopene in food daily (6.5 mg/day or more) had a 21% reduced risk of prostate cancer compared to those who ate less lycopene. This research shows that lycopene is an important substance to prevent prostate cancer. This study also reported that people who ate 10 or more tomatoes or tomato-derived foods per week had a 35% reduced risk of prostate cancer compared to those who ate an average of 1.5 times per week. (Giovannucci, Ascherio, Rimm, Stampfer, Colditz, and Willett, 1995).

The mechanism of action of lycopene on prostate health is not fully known. The first thing that comes to mind is the relationship between lycopene and insulin-like growth factor. High levels of insulin growth factor increase the risk of prostate cancer. Thus, an increase in lycopene consumption will decrease the level of insulin growth factor. The other mechanism is its activity, which includes both the cessation of tumor growth and increased differentiation of normal cells. Lycopene and other carotenoids inhibit tumor growth by increasing communication between healthy prostate

cells. Another and most widely accepted theory is the antioxidant effect of lycopene. Lycopene works like a scavenger for DNA damage and free oxygen radicals theorized to be the cause of cancer. Lycopene is found in high amounts in prostate cells. (Everson and McQueen, 2004, Wei and Giovannucci, 2012).

The effects of lycopene on the growth rate of DU145 tumor xenografts with human prostate cancer cells were studied in BALB/c mice. In this study, it was shown that tumor growth in mice administered 100-300 mg/kg lycopene was reduced by 55.6% and 75.8% compared to the control group. It was determined that lycopene administration disrupted the G0/G1 phase in these cells. Lycopene administration has been shown to lead to apoptosis in a dose-dependent manner (Tang, Jin, Zeng, and Wang, 2005).

In an ecological study conducted in Japan, which has a high incidence of digestive system cancer, plasma levels of various foods were investigated in population groups living in various regions. As a result of the research, it was found that the rate of gastric cancer was lower in regions with high plasma lycopene levels, while this rate was higher in regions with low lycopene levels. It was determined that the inverse relationship between tomato and gastric cancers was the most consistent and strongest when compared to other vegetables and fruits (Tsugane, Tsuda, Gey, and Watanabe, 1992). Ultraviolet rays cause the formation of free radicals. Free radicals can cause sunburns, skin aging and skin cancers. Tomato-based foods provide great protection against sunburns caused by ultraviolet rays.

Although not possible for all cancers, epidemiological data show that those who consume tomatoes and tomato products have a reduced risk of many types of cancer. The evidence is stronger for lung, stomach, prostate, glandular cancers and is also significant for cancers of the cervix, breast, oral cavity, pancreas, colorectal, and esophagus. Much of the evidence also indicates that other vegetables and fruits have additional or complementary benefits. Data from various studies conducted with various methods further support current dietary recommendations to increase fruit and vegetable consumption to reduce cancer risk (Giovannucci, 1999).

In addition, antiproliferative effects of lycopene on rat prostate cancer AT3 cells (Gunasekera, Sewgobind, Desai, Dunn, Black, McKeehan, and Patil, 2007), human breast cancer MCF cells (Fornelli, Leone, Verdesca,

Minervini, and Zacheo, 2007), primary human prostate epithelial cells (Barber, Zhang, and Zhu, 2006), human erythroleukemia K562 cells, Raji cells and human colon cancer HuCC cells (Salman, Bergman, Djaldetti, and Bessler, 2007) are available.

2. CONCLUSION

The results of epidemiological studies on lycopene or tomato consumption have shown that these products can reduce the risk of various types of cancer. Many mechanisms are responsible for these beneficial effects of lycopene, but since the exact mechanisms have not been determined yet, long-term clinical studies are required to support the clinical use of lycopene and confirm these results.

REFERENCES

Agarwal S, Rao AV. (2000). Tomato lycopene and its role in human health and chronic diseases. CMAJ. 163(6):739-744.

Agarwal S, Rao AV. 2000. Tomato lycopene and its role in human health and chronic diseases. CMAJ. 163(6):739-44.

Aybak K ve Kaygısız H. 2004. Domates Yetiştiriciliği. Hasad Yayınları, pp 3-8.

Basaga HS. (1990). Biochemical aspect of free radicals. BiochemCell Biol. 68: 989-998.

Bruckdorfer KR. (1990). Free radical lipid peroxidation and atherosclerosisi Curr. Opin. Lipidol, 1, 529-535.

Barber NJ, Zhang X, Zhu G. (2006). Lycopene inhibits DNA synthesis in primary prostate epithelial cells in vitro and its administration is associated with a reduced prostatespecific antigen velocity in a phase II clinical study. Prostate Cancer Prostatic Dis; 9: 407–413. doi: 10.1038/sj.pcan.4500895.

Colditz GA, Branch LG, Lipnick RJ, Willett WC, Rosner B, Posner BM, et al. (1985).Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. Am J Clin Nutr. 41(1):32-36.

Everson KM, McQueen CE. (2004). Lycopene for prevention and treatment of prostate cancer. Am J Health Syst Pharm. 61(15):1562-1566. doi: 10.1093/ajhp/61.15.1562.

Fornelli F, Leone A, Verdesca I, Minervini F, Zacheo G. (2007). The influence of lycopene on the proliferation of human breast cell line (MCF-7). Toxicol In Vitro. 21: 217-223. doi: 10.1016/j.tiv.2006.09.024.

Giovannucci E. (1999). Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer Inst. 91(4):317-331.

Giovannucci E. (2002). A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. Experimental Biology and Medicine; 227:852-859.

Gunasekera RS, Sewgobind K, Desai S, Dunn L, Black HS, McKeehan WL, Patil B. (2007). Lycopene and lutein inhibitproliferation in rat prostate carcinoma cells. Nutr Cancer. 58:171-177. doi: 10.1080/01635580701328339.

Giovannucci E. (2002). A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. Experimental Biology and Medicine; 227:852-859.

Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. (1995). Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst. 87(23):1767-1776.

Nagasawa H, Mitamura T, Sakamoto S, Yamamoto K. (1995). Effects of lycopene on spontaneous mammary tumour development in SHN virgin mice. Anticancer Res. 15(4):1173-1178.

Rao AV, Ray MR, Rao LG. (2006). Lycopene. Adv Food Nutr Res. 51:99-164.

Said TM, Agarwal A, Sharma RK, Mascha E, Sikka SC. (2004). Human sperm superoxide anion generation and correlation with semen quality in patients with male infertility. Fertil Steril. 82: 871–877. doi: 10.1016/j.fertnstert.2004.02.132.

Salman H, Bergman M, Djaldetti M, Bessler H. (2007). Lycopene affects proliferation and apoptosis of four malignant cell lines. Biomed Pharmacother. 61: 366-369. doi: 10.1016/j.biopha.2007.02.015.

Sies H. (1991). Oxidative Stres: Oxidant and Antioxidant, Academic Pres, London.

Slater TF. (1984). Free radical mechanism in tissue injury. Biochem. J 222:1-5.

Tang L, Jin T, Zeng X, Wang JS. (2005). Lycopene inhibits the growth of human androgen-independent prostate cancer cells in vitro and in BALB/c nude mice. Journal of Nutrition. 135: 287-290. doi: 10.1093/jn/135.2.287.

Tsugane S, Tsuda M, Gey F, Watanabe S. (1992). Cross-sectional study with multiple measurements of biological markers for assessing stomach cancer risks at the population level. Environ Health Perspect. 98:207-210.

Wei MY, Giovannucci EL (2012) Lycopene, tomato products, and prostate cancer incidence: a review and reassessment in the PSA screening era. J Oncol 2012:271063. doi: 10.1155/2012/271063.

CHAPTER 4

SNARE PROTEINS:

Effects on Synaptic Transmission and Memory Functions

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INTRODUCTION

The word of synapse was first used to describe communication between neurons by Foster and Sherrington in 1897. Synaptic transmission is the neural interaction of a neuron with another neuron, muscle or gland cell. This interaction takes place in two main ways as chemical and electrical. Chemical transmission involves release of neurotransmitters stored at the presynaptic end of a neuron into synaptic cleft and binding to their specific receptor at the postsynaptic membrane. On the other hand, electrical synapses, which take an active role in the neural innervation of the heart and smooth muscles, provide signaling by interacting with the cytoplasm of neighboring cells via slit connections. Although synapses are structurally evaluated under two headings, chemical synapses are commonly found in the central nervous system (CNS). A chemical synapse basically consists of the presynaptic terminal, synaptic gap, and postsynaptic membrane. The presynaptic terminal contains a large number of neurotransmitters within vesicles at the axon terminal. The electrical signal reaching the axon terminal causes calcium to pass to the presynaptic terminal by opening voltage-gated calcium channels. This situation results in the release of neurotransmitters into the synaptic cleft as triggers of vesicle exocytosis. The approach of the vesicle to the membrane and the release of neurotransmitters are provided by activation of a series of proteins. Calcium activates the synapsin kinase enzyme and phosphorylates synapsin, a cytoskeletal protein. The vesicle, released by synapsin activation, moves to the cell membrane with the Rab3 protein. At this stage, the vesicle is positioned to the appropriate region in the membrane by interaction of the soluble N-ethylmaleimide sensitive factor activating protein receptor (SNARE) in both the vesicle wall and the cell membrane. The positioning protein located at the vesicle membrane is called v-SNARE, and the one located at the axon terminal is called t-SNARE. More than 38 members of the SNARE family have been described. Briefly; synaptic transmission is triggered by the activation of voltage-gated calcium channels in response to an action potential and the transition of calcium to the presynaptic terminal. This process results in rapid and synchronized fusion of neurotransmitter-containing synaptic vesicles to the presynaptic plasma membrane.

Membrane Fusion

Membrane fusion forms the basis of performing many functions such as neurotransmission, enzyme release, protein maturation and hormone secretion. Membrane fusion is a ubiquitous process. It involves joining two discrete lipid membranes to provide molecules to spill into synaptic cleft. SNARE proteins are structures that allow synaptic vesicles to associate with the presynaptic membrane. Synaptobrevin, syntaxin, and synaptosome-associated protein 25 (SNAP-25) were among the first SNAREs to be identified. Synaptobrevin 2 and syntaxin 1A are fixed by transmembrane domains in the synaptic vesicle and plasma membrane. SNAP-25 is palmitoylated on the target membrane via 4 cysteine residues in the center of the protein (Kumar etal., 2015). In this review, the role of SNARE proteins in vesicle fusion will be detailed.

1. SNARE proteins

The most distinctive feature of eukaryotic cells is that they have organelles. These organelles play important roles in various physiological functions, including secretory and endocytic functions. Vesicles and tubules are needed for the secretory function to take place. The transfer of the molecule to be transported to target site occurs via SNARE proteins. There are a variety of molecules within the vesicles, including lipid, signaling molecules, membrane proteins, biosynthetic and hydrolytic enzymes (Yoon & Munson, 2018). These proteins play an important role in many functions such as cell division and differentiation, and especially intercellular communication. Therefore, structural defects in SNARE proteins result in disruption of cellular transport. Defects in vesicle fusion are associated with many pathologies such as diabetes and cancer, especially neurodegenerative diseases. Moreover, suppression of SNARE complex formation attenuates neurotransmitter release. The release of neurotransmitter from presynaptic terminals and its modulation through synaptic plasticity are cornerstones of directed information flow within the neuronal circuits of the CNS. Therefore, in cases where vesicle fusion cannot occur, physiological functions related to nerve conduction and muscle contraction are impaired.

SNARE proteins are a large protein family of more than 60 members in both mammalian and yeast cells. They have a helix-helix extension

containing 60-70 amino acids called the SNARE motif. Syntaxin and synaptobrevin within the SNARE motifs bind to peptidic transmembrane domains at the C-terminus via a short linker site. These two SNARE proteins are embedded in their respective membranes through their transmembrane domains. A third protein is SNAP-25. SNAP-25 consists of two SNARE motifs attached to the plasma membrane by multiple palmitoyl tails (Han et al., 2017). Formation of the SNARE complex carries out positioning, preparation and fusion of the vesicle with a certain synchronization (Figure 1). This complex essentially constitutes the main component of the release of neurotransmitters into the synaptic cleft. SNAREs can basically be divided into two categories: 1) Vesicle proteins, namely v-SNAREs, which are incorporated into the membranes of their vesicles during maturation. 2) Target or t-SNAREs associated with nerve terminal membranes. It has been stated that t-SNAREs are stable subcomplexes that act as guides for v-SNAREs. (Malsam & Söllner, 2011).

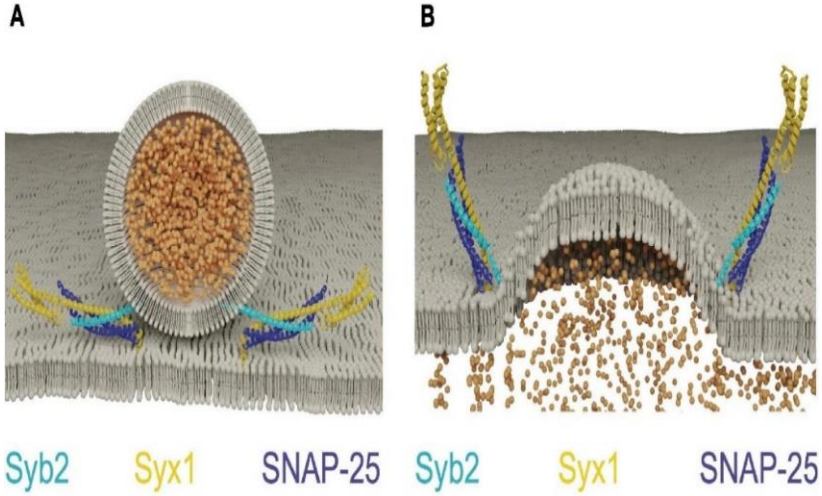

Figure 1. SNAREs assemble between opposing membranes to induce membrane fusion and neurotransmitter release (Sauvola & Littleton, 2021).

1.1. v-SNARE proteins

v-SNAREs form one of two main components of the SNARE complex. v-SNAREs located on the vesicle surface are fusion proteins. Synaptobrevin (also known as vesicle-associated membrane protein (VAMP))

is the most important type of v-SNARE that plays an active role in vesicle fusion.

1.1.1. Synaptobrevin

Synaptobrevin is among the first identified synaptic vesicle proteins (Jahn et al., 1989). Synaptobrevin is a small 18 kDa protein group that integrates into the vesicle membrane and plays a pivotal role in calcium-induced vesicle fusion. Synaptobrevin on the synaptic vesicle directs vesicle fusion by interacting with the cognate SNARE proteins on the presynaptic plasma membrane. Two different isoforms of synaptobrevin are involved in calcium-dependent exocytosis. These are synaptobrevin/VAMP1 and synaptobrevin/VAMP2. The physiological significance of these two isoforms has not been adequately clarified. In a comprehensive study, the anatomical localization of VAMP1 and VAMP2 was investigated in the rat brain. Accordingly, although VAMP2 is the most common form in the rat brain, VAMP1 has been found to be the major isoform in certain brain areas (for example, nerve terminals surrounding the thalamic neurons) (Raptis et al., 2005). SNARE protein consisting only of a short NH2-terminal sequence, a SNARE motif, and a COOH-terminal transmembrane domain.

Preliminary data point to a prominent role for synaptophysins in the mechanism of action of synaptobrevin. Synaptophysin has four transmembrane domains. Moreover, it has been stated that there is a direct interaction between VAMP and synaptophysin. Moreover, they showed that a distinct 56 kDa complex consisting of VAMP and synaptophysin was formed and enriched in the synaptic vesicle fraction of the rat brain (Calakos & Scheller, 1994). Absence of synaptophysin has been shown to cause mislocalization of synaptobrevin and accumulation of synaptobrevin 2 on nerve terminals (Gordon et al., 2011). Spontaneous synaptic vesicle fusion caused by hypertonic sucrose have been shown to reduce approximately 10-fold on knockout mice lacking VAMP2, the SNARE protein responsible for synaptic vesicle fusion in forebrain synapses. This result indicates that synaptobrevin 2 may be functional in catalyzing fusion reactions and stabilizing fusion intermediates (Schoch et al., 2001). In a different study, while homozygous VAMP2 knockout mice died shortly after birth, heterozygous mice were shown to survive. Moreover, these mice have been

associated with dysfunctions such as delayed postnatal development, reduced anxiety-related behaviors, and weakened nerve conduction (Koo et al., 2015). These results suggest that genetic defects or structural defects in proteins that perform membrane fusion may be a potential risk factor for neurodevelopmental diseases.

1.2. t-SNARE proteins

SNARE proteins are classified mainly as v- and t-SNARE, localized in vesicle and target membranes. These proteins form a stable complex that binds the membranes together and ultimately fuses them. t-SNAREs consist primarily of SNAP-25 and syntaxin. These proteins are arranged in dense clusters with a diameter of 50-60 nm in the plasma membrane. In a previous study, it was stated that each of these clusters contains about 35-70 t-SNARE molecules (Dun et al.,2010).

1.2.1. SNAP 25

SNAP-25 is an important t-SNARE protein that plays an active role in regulation of exocytosine. This molecule is anchored to the cytosolic surface of membranes via palmitoyl side chains that are covalently linked to cysteine amino acid residues. It is localized on chromosome 20p 12.2 in humans and contains 17 exons. SNAP-25 has been extensively studied for its basic role in neurotransmitter release, and there are three other SNAP protein isoforms named according to their molecular weights (Figure 2). These are: SNAP-23, SNAP-29 and SNAP-47. SNAP-25 and SNAP-23 are two highly important isoforms for driving regulated exocytosis (Kádková et al., 2019). On the other hand, SNAP-25 is also divided into two different sub-isoforms as SNAP-25a and SNAP-25b. While SNAP-25a plays a role in the embryonic and early fetal stages, SNAP-25b is the active form in the postnatal period (Prescott & Chamberlain, 2011).

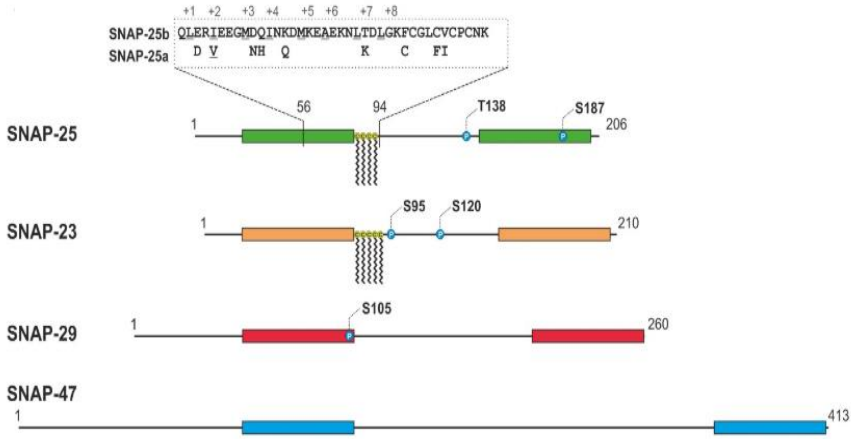

Figure 2. Members of the SNAP-25 protein subfamily in vertebrates (Kádková et al., 2019)**.**

While SNAP-25 is primarily involved in the fusion of synaptic vesicles and the release of neurotransmitters into the synaptic cleft, it also plays a prominent role in the regulation of insulin release, calcium homeostasis and synaptic plasticity (Najera et al., 2019). Besides, SNAP-25 is expressed in most neuronal areas of the brain (Boschert et al., 1996), it has also been identified in the endocrine anterior pituitary cells, chromaffin cells of the adrenal medulla, alpha and beta cells of the pancreatic islets of Langerhans, and enteroendocrine cells. These cells, which have intense neuronal activity and secretory function, secrete calcium-triggered neurotransmitter or hormone-like molecules following an excitatory potential with other SNARE proteins such as syntaxin 1 and synaptobrevin. As a result, SNAP-25 realizes neurotransmitter release with synaptobrevin 2 and syntaxin 1A to form a triplet SNARE complex (Washbourne et al., 2002). This complex leads to exocytosis by controlling the fusion process of presynaptic vesicles with the plasma membrane. In the absence of SNAP-25, it has been observed that vesicle clamping continues in the active regions at the presynaptic ends, but the vesicle pool, which is about to be released, is empty. Moreover, it has been reported that calcium-induced exocytosis does not

occur (Sørensen et al., 2003). It has been reported that botulinum neurotoxin A inhibits axon development, dendritic branching and synapse formation by cleaving SNAP 25 in rat cortical or mouse hippocampal neurons (Grosse et al., 1999).

The anatomical localization of the SNAP-25 gene in the mammalian brain in the neocortex, hippocampus, anterior thalamic nuclei, substatia nigra and cerebellar granular cells reinforces its potential role in the modulation of learning and memory (Gosso et al., 2006). Thus, Hou Q et al. showed that inhibition of SNAP-25 in adult male rats causes a deficit in long-term memory by affecting the hippocampal CA1 region. Moreover, SNAP-25 inhibition also impaired long-term contextual fear memory and spatial memory (Hou et al., 2004). Indeed, it has been shown that the level of SNAP-25 mRNA increases two hours after induction of long-term potentiation (LTP) in granular cells of the dentate gyrus (Roberts et al., 1998). Recent evidence has reported different physiological effects of two distinct isoforms of SNAP-25 on activity-related short- and long-term plasticity at hippocampal Schaffer collateral-CA1 synapses (Irfan et al., 2019). In a similar study, SNAP-25a was shown to play a prominent role in the regulation of both long-term depression (LTD) and LTP in the hippocampal Schaffer collateral-CA1 synapses (Gopaul et al., 2020). These results point to the key role of SNAP-25 in learning and memory consolidation.

1.2.2. Syntaxins

Syntaxins are a family of membrane proteins localized in the active sites of presynaptic sites. Syntaxins have a single C-terminal transmembrane domain, a SNARE domain (known as H3) and an N-terminal regulatory domain. There are 15 members of the syntaxin family in the human genome. Four of this family are localized to the plasma membrane. Syntaxin 1 is mainly located in neurons and plays a role in secretion and exocytosis (Bennett et al., 1992). On the other hand, while syntaxin 2 and syntaxin 3 take an active role in exocytosis, syntaxin 4 is active in glucose transporter traffic in adipocytes. In this family, syntaxin 1 is the most active form involved in vesicle fusion. Syntaxin 1 brings the vesicle and plasma membrane closer together by forming a triple complex with synaptobrevin 2 and SNAP25. Thus, vesicle fusion is catalyzed (Rizo & Rosenmund, 2008; Rizo & Südhof,

2012). Structurally, syntaxin 1 has two isoforms, A and B, specific to the nervous system, which play an active role in the docking of synaptic vesicles with the presynaptic plasma membrane. Syntaxin 1A consists of 288 amino acids. Syntaxin 1A is most commonly found in neurons and neuroendocrine cells, accounting for approximately 1% of the total amount of brain proteins (Lang & Jahn, 2008). A recent experimental study has associated deletion of syntaxin 1 in mature neurons with a significant reduction in neuronal viability. The same study reported that loss of syntaxin 1 completely suppressed vesicle priming and fusion, while also causing a significant reduction in the docking of vesicles (Vardar et al., 2016). All these data point to the pivotal role of syntaxin 1 in all stages of neuronal survival and neurotransmitter release from the presynaptic terminal. In contrast, it has also been demonstrated that syntaxin 1A mutant mice can develop normally and do not show abnormalities in rapid synaptic transmission. However, impaired monoaminergic transmissions in syntaxin 1A mutant mice were observed (Mishima et al., 2014).

Cav 2.1 (P/Q voltage-gated calcium channel), a calcium channel, is found specifically at the presynaptic terminals of neurons in the brain and cerebellum. These channels have a prominent role in the control of neurotransmitter release (Sheng & Leenders, 2009). Syntaxin 1A causes a decrease in channel expression levels and a hyperpolarizing shift in the steady-state inactivation curve. This suggests that syntaxin 1A is a membrane-associated protein that limits the functional availability of Cav2.2 (Lee, 2009). Taken together, it seems that syntaxin, one of the target proteins in the plasma membrane, may play an active role in vesicle aggregation and complex formation.

2. Integral Synaptic Vesicle Proteins: Synaptophysin and Synapsins

2.1. Synaptophysin

Synaptophysin is a 38 kDa integral tetra span transmembrane protein (cytosolic C and N terminals) and approximately 10% of total synaptic vesicle proteins (Takamori et al., 2006). Synaptophysins are found in neuroendocrine cells and nearly all neurons involved in synaptic transmission in the brain and spinal cord (Redecker & Grube, 1992). However, the role of synaptophysin, a

glycoprotein, is not entirely clear in synaptic transmission. In a previous study, it was reported that inactivation of the synaptophysin gene experimentally did not adversely affect synaptic functions (Mcmahon et al., 1996). On the other hand, it has been stated that synaptophysin may play a prominent role in different steps of synaptic biogenesis, vesicle protein classification, vesicle preparation, synapse formation, exocytosis and endocytosis (Gudi et al., 2017). Recent evidence has shown that synaptophysin is required for the uptake of synaptobrevinin, but not other synaptic vesicle protein transporters, from the nerve terminal plasma membrane during endocytosis (Gordon, Leube, & Cousin, 2011).

Synaptophysin has been used as a presynaptic marker to describe synapses in the hippocampus (Calhoun et al., 1996). In an experimental study, it was reported that synaptophysin immunoreactivity in the hippocampus decreases with aging (Xu et al., 2019). Considering the role of synaptophysin in synaptic neurotransmission, any decrease in synaptophysin level strengthens the potential to negatively affect learning and memory functions. In this context, effects of environmental enrichment on memory and presynaptic synaptophysin levels were investigated in young and old mice. The results showed that hippocampal and cortical synaptophysin levels were increased in old and environmentally enriched females compared to young and aged control groups. Moreover, it was observed that spatial memory performance of aged mice exposed to enriched environment was higher than aged controls (Frick & Fernandez, 2003). In conclusion, localization of synaptophysin in the hippocampus strengthens the possibility that it may play a prominent role in the regulation of higher cognitive functions such as learning and memory.

2.2. Synapsins

Synapsins are an important family of phosphoproteins that are abundant in the nerve terminal. Synapsins are neuronal proteins that coat synaptic vesicles and bind to the cytoskeleton. Membrane depolarization and neurotransmitter release into the synaptic cleft have been associated with the activity of synapsins. Structurally, there are 3 different types of synapsins in most vertebrate species. While synapsin I and II are specifically localized to presynaptic terminals, synapsin III is expressed at very low levels in cell

bodies and growth cones (Khvotchev & Sun, 2009). Moreover, due to the difference in the number of amino acids, synapsin I dissociates into two different isoforms, synapsin Ia and synapsin Ib (Sudhof, 1990). While synapsins have a widespread anatomical localization in the central and peripheral nervous system, they are expressed very little in non-neuronal cells. It is efficiently regulated by certain protein kinases and phosphatases, which modulate the association of synapsins with synaptic vesicles and their interaction with actin filaments and other synaptic proteins. The existence of different types of synapsins in mammals makes difficult to define the physiological role of the synapsin. However, it was observed that exocytosis and synaptic plasticity were not significantly impaired in synapsin knockout animal models. But, it has been reported that the absence of synapsin disrupts the reserve vesicle pool in presynaptic active sites (Abrams, 2009).

There are several approaches that show that synapsins are important for fine-tuning the molecular processes of higher cognitive functions such as learning and behavioral paradigms. Behavioral deficits have been reported in hippocampus-dependent contextual fear conditioning tests in synapsin I- and II-deficient mice (Silva et al., 1996). Alternatively, increased synapsin Ia and IIa levels have been reported in the hippocampus of mice treated with the cognitive enhancer SGS742 Indeed, altered synapsin I levels have been observed in some neurodegenerative diseases characterized by cognitive deficits. For example, synapsin I levels were found to be low in some parts of the brain in postmortem examinations of Alzheimer's patient brains (Perdahl et al., 1984). Similarly, decreased synapsin I levels have been reported particularly in CA1 and dentate gyrus regions of the hippocampus of Alzheimer's patients (Qin et al., 2004). Besides memory functions, recent evidence has reported that synapsins I and II can regulate neurogenesis in dentate gyrus of adult mice (Barbieri et al., 2018). These results revealed that synapsins play an extremely vital role in both the modulation of vesicles and maintenance of cognitive functions.

3. CONCLUSION

In this review, we partially summarize the current knowledge of the role of various members of the SNARE protein family in the molecular mechanism of synaptic transmission. Moreover, we focused on the potential role of SNARE proteins in the control and regulation of higher cognitive functions such as learning and memory. On the other hand, we present brief information on the relationship between genetic defects in these proteins and the pathophysiology of some neurodegenerative diseases in the central nervous system.

REFERENCES

Abrams, T. W. (2009). Cyclic AMP (cAMP) Role in Learning and Memory. *Encyclopedia of Neuroscience*, 265–277. https://doi.org/10.1016/B978-008045046-9.00819-6

Barbieri, R., Contestabile, A., Ciardo, M. G., Forte, N., Marte, A., Baldelli, P., Onofri, F. (2018). Synapsin I and Synapsin II regulate neurogenesis in the dentate gyrus of adult mice. *Oncotarget*, *9*(27), 18760–18774. https://doi.org/10.18632/oncotarget.24655

Boschert, U., O'Shaughnessy, C., Dickinson, R., Tessari, M., Bendotti, C., Catsicas, S., & Pich, E. M. (1996). Developmental and plasticity-related differential expression of two SNAP-25 isoforms in the rat brain. *Journal of Comparative Neurology*, *367*(2), 177–193. https://doi.org/10.1002/(SICI)1096-9861(19960401)367:2<177:AID-CNE2>3.0.CO;2-2

Calakos, N., & Scheller, R. H. (1994). Vesicle-associated membrane protein and synaptophysin are associated on the synaptic vesicle. *Journal of Biological Chemistry*, *269*(40), 24534–24537. https://doi.org/10.1016/s0021-9258(17)31422-9

Calhoun, M. E., Jucker, M., Martin, L. J., Thinakaran, G., Price, D. L., & Mouton, P. R. (1996). Comparative evaluation of synaptophysin-based methods for quantification of synapses. *Journal of Neurocytology*, *25*(1), 821–828. https://doi.org/10.1007/bf02284844

Dun, A. R., Rickman, C., & Duncan, R. R. (2010). The t-SNARE complex: A close up. *Cellular and Molecular Neurobiology*, *30*(8), 1321–1326. https://doi.org/10.1007/s10571-010-9599-4

Frick, K. M., & Fernandez, S. M. (2003). Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice, *24*, 615–626.

Gopaul, K. R., Irfan, M., Miry, O., Vose, L. R., Mogha Dam, A., Subah, G., Stanton, P. K. (2020). Developmental time course of SNAP-25 isoforms regulate hippocampal long-term synaptic plasticity and hippocampus-dependent learning. *International Journal of Molecular Sciences*, *21*(4), 1–18. https://doi.org/10.3390/ijms21041448

Gordon, S. L., Leube, R. E., & Cousin, M. A. (2011). Synaptophysin Is Required for Synaptobrevin Retrieval during Synaptic Vesicle Endocytosis. *Journal of Neuroscience*, *31*(39), 14032–14036. https://doi.org/10.1523/JNEUROSCI.3162-11.2011

Gosso, M. F., De Geus, E. J. C., Van Belzen, M. J., Polderman, T. J. C., Heutink, P., Boomsma, D. I., & Posthuma, D. (2006). The SNAP-25 gene is associated with cognitive ability: Evidence from a family-based study in two

independent Dutch cohorts. *Molecular Psychiatry*, *11*(9), 878–886. https://doi.org/10.1038/sj.mp.4001868

Grosse, G., Grosse, J., Tapp, R., Kuchinke, J., Gorsleben, M., Fetter, I., Bergmann, M. (1999). SNAP-25 requirement for dendritic growth of hippocampal neurons. *Journal of Neuroscience Research*, *56*(5), 539–546. https://doi.org/10.1002/(SICI)1097-4547(19990601)56:5<539::AID-JNR9>3.0.CO;2-Y

Gudi, V., Gai, L., Herder, V., Tejedor, L. S., Kipp, M., Amor, S., Skripuletz, T. (2017). Synaptophysin is a reliable marker for axonal damage. *Journal of Neuropathology and Experimental Neurology*, *76*(2), 109–125. https://doi.org/10.1093/jnen/nlw114

Han, J., Pluhackova, K., & Böckmann, R. A. (2017). The multifaceted role of SNARE proteins in membrane fusion. *Frontiers in Physiology*, *8*(JAN). https://doi.org/10.3389/fphys.2017.00005

Hou, Q., Gao, X., Zhang, X., Kong, L., Wang, X., Bian, W., Yu, L. (2004). SNAP-25 in hippocampal CA1 region is involved in memory consolidation. *European Journal of Neuroscience*, *20*(6), 1593–1603. https://doi.org/10.1111/j.1460-9568.2004.03600.x

Irfan, M., Gopaul, K. R., Miry, O., Hökfelt, T., Stanton, P. K., & Bark, C. (2019). SNAP-25 isoforms differentially regulate synaptic transmission and long-term synaptic plasticity at central synapses. *Scientific Reports*, *9*(1), 1–14. https://doi.org/10.1038/s41598-019-42833-3

Jahn, R., Südhof, T., Baumert, M., & Perin, M. (1989). A synaptic vesicle membrane protein is conserved from mammals to Drosophila. *Neuron*, *2*, 1475–1481.

Kádková, A., Radecke, J., & Sørensen, J. B. (2019). The SNAP-25 Protein Family. *Neuroscience*, *420*(September), 50–71. https://doi.org/10.1016/j.neuroscience.2018.09.020

Koo, S. J., Kochlamazashvili, G., Rost, B., Puchkov, D., Gimber, N., Lehmann, M., Maritzen, T. (2015). Vesicular Synaptobrevin/VAMP2 Levels Guarded by AP180 Control Efficient Neurotransmission. *Neuron*, *88*(2), 330–344. https://doi.org/10.1016/j.neuron.2015.08.034

Kumar, P., Guha, S., & Diederichsen, U. (2015). SNARE protein analog-mediated membrane fusion. *Journal of Peptide Science*, *21*(8), 621–629. https://doi.org/10.1002/psc.2773

Lang, T., & Jahn, R. (2008). Core proteins of the secretory machinery. *Handbook of Experimental Pharmacology*, *184*, 107–127. https://doi.org/10.1007/978-3-540-74805-2_5

Lee, A. (2009). Neuromodulation of Calcium Channels. *Encyclopedia of*

Neuroscience, 505–512. https://doi.org/10.1016/B978-008045046-9.01638-7

M V Khvotchev and J Sun. (2009). Synapsins. In *Neuron* (pp. 705–708).

Malsam, J., & Söllner, T. H. (2011). Organization of SNAREs within the Golgi stack. *Cold Spring Harbor Perspectives in Biology*, *3*(10), 1–17. https://doi.org/10.1101/cshperspect.a005249

Mcmahon, H. T., Bolshakov, V. Y., Janz, R., Hammer, R. E., Siegelbaum, S. A., & Südhof, T. C. (1996). Synaptophysin, a major synaptic vesicle protein, is not essential for neurotransmitter release. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(10), 4760–4764. https://doi.org/10.1073/pnas.93.10.4760

Mishima, T., Fujiwara, T., Sanada, M., Kofuji, T., Kanai-Azuma, M., & Akagawa, K. (2014). Syntaxin 1B, but not syntaxin 1A, is necessary for the regulation of synaptic vesicle exocytosis and of the readily releasable pool at central synapses. *PLoS ONE*, *9*(2). https://doi.org/10.1371/journal.pone.0090004

MK, B., N, C., & RH, S. (1992). Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. *Science*, *257*(5067), 255–259.

Najera, K., Fagan, B. M., & Thompson, P. M. (2019). SNAP-25 in Major Psychiatric Disorders: A Review. *Neuroscience*, *420*(March), 79–85. https://doi.org/10.1016/j.neuroscience.2019.02.008

Perdahl, E., Adolfsson, R., Alafuzoff, I., Albert, K. A., Nestler, E. J., Greengard, P., & Winblad, B. (1984). Synapsin I (protein I) in different brain regions in senile dementia of Alzheimer type and in multiinfarct dementia. *Journal of Neural Transmission*, *60*(2), 133–141. https://doi.org/10.1007/BF01245030

Prescott, G. R., & Chamberlain, L. H. (2011). Regional and developmental brain expression patterns of SNAP25 splice variants. *BMC Neuroscience*, *12*. https://doi.org/10.1186/1471-2202-12-35

Qin, S., Hu, X. Y., Xu, H., & Zhou, J. N. (2004). Regional alteration of synapsin I in the hippocampal formation of Alzheimer's disease patients. *Acta Neuropathologica*, *107*(3), 209–215. https://doi.org/10.1007/s00401-003-0800-4

Raptis, A., Torrejón-Escribano, B., Gómez De Aranda, I., & Blasi, J. (2005). Distribution of synaptobrevin/VAMP 1 and 2 in rat brain. *Journal of Chemical Neuroanatomy*, *30*(4), 201–211. https://doi.org/10.1016/j.jchemneu.2005.08.002

Redecker, P., & Grube, D. (1992). Synaptophysin in the nervous system and endocrine cells. *Acta Histochemica. Supplementband*, *42*, 33–38.

Rizo, J., & Rosenmund, C. (2008). Synaptic vesicle fusion. *Nature Structural and Molecular Biology*, *15*(7), 665–674. https://doi.org/10.1038/nsmb.1450

Rizo, J., & Südhof, T. C. (2012). The membrane fusion enigma: SNAREs, Sec1/Munc18 proteins, and their accomplices guilty as charged? *Annual Review of Cell and Developmental Biology*, *28*, 279–308. https://doi.org/10.1146/annurev-cellbio-101011-155818

Roberts, L. A., Morris, B. J., & O'Shaughnessy, C. T. (1998). Involvement of two isoforms of SNAP-25 in the expression of long-term potentiation in the rat hippocampus. *NeuroReport*, *9*(1), 33–36. https://doi.org/10.1097/00001756-199801050-00007

Sauvola, C. W., & Littleton, J. T. (2021). SNARE Regulatory Proteins in Synaptic Vesicle Fusion and Recycling. *Frontiers in Molecular Neuroscience*, *14*(August), 1–26. https://doi.org/10.3389/fnmol.2021.733138

Schoch, S., Deák, F., Königstorfer, A., Mozhayeva, M., Sara, Y., Südhof, T. C., & Kavalali, E. T. (2001). SNARE function analyzed in synaptobrevin/VAMP knockout mice. *Science*, *294*(5544), 1117–1122. https://doi.org/10.1126/science.1064335

Sheng, Z. H., & Leenders, A. G. M. (2009). Calcium Channels and SNARE Proteins. *Encyclopedia of Neuroscience*, *1*, 551–557. https://doi.org/10.1016/B978-008045046-9.01646-6

Silva, A. J., Rosahl, T. W., Chapman, P. F., Marowitz, Z., Friedman, E., Frankland, P. W., Bourtchuladze, R. (1996). Impaired learning in mice with abnormal short-lived plasticity. *Current Biology*, *6*(11), 1509–1518. https://doi.org/10.1016/S0960-9822(96)00756-7

Sørensen, J. B., Nagy, G., Varoqueaux, F., Nehring, R. B., Brose, N., Wilson, M. C., & Neher, E. (2003). Differential control of the releasable vesicle pools by SNAP-25 splice variants and SNAP-23. *Cell*, *114*(1), 75–86. https://doi.org/10.1016/S0092-8674(03)00477-X

Sudhof, T. C. (1990). The structure of the human synapsin I gene and protein. *Journal of Biological Chemistry*, *265*(14), 7849–7852. https://doi.org/10.1016/s0021-9258(19)39008-8

Takamori, S., Holt, M., Stenius, K., Lemke, E. A., Grønborg, M., Riedel, D., … Jahn, R. (2006). Molecular Anatomy of a Trafficking Organelle. *Cell*, *127*(4), 831–846. https://doi.org/10.1016/j.cell.2006.10.030

Vardar, G., Chang, S., Arancillo, M., Wu, Y. J., Trimbuch, T., & Rosenmund, C. (2016). Distinct functions of syntaxin-1 in neuronal maintenance, synaptic vesicle docking, and fusion in mouse neurons. *Journal of Neuroscience*, *36*(30), 7911–7924. https://doi.org/10.1523/JNEUROSCI.1314-16.2016

Washbourne, P., Thompson, P. M., Carta, M., Costa, E. T., Mathews, J. R., Lopez-Benditó, G., Wilson, M. C. (2002). Genetic ablation of the t-SNARE SNAP-

25 distinguishes mechanisms of neuroexocytosis. *Nature Neuroscience*, *5*(1), 19–26. https://doi.org/10.1038/nn783

Xu, L., Long, J., Su, Z., Xu, B., Lin, M., Chen, Y., & Long, D. (2019). Restored presynaptic synaptophysin and cholinergic inputs contribute to the protective effects of physical running on spatial memory in aged mice. *Neurobiology of Disease*, *132*(April), 104586. https://doi.org/10.1016/j.nbd.2019.104586

Yoon, T. Y., & Munson, M. (2018). SNARE complex assembly and disassembly. *Current Biology*, *28*(8), R397–R401. https://doi.org/10.1016/j.cub.2018.01.005

CHAPTER 5

AGGRESSIVE VARIANTS OF PAPILLARY THYROID CARCINOMA: HISTOPATHOLOGICAL AND MOLECULAR FEATURES

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INTRODUCTION

Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid with a generally good prognosis. It accounts for approximately 80% of all thyroid cancer cases (Abdullah et al., 2019). Although PTC can occur at any age, it is usually detected in the third and fifth decades of life, at a mean age of 40 years (LiVolsi, 2011). The most important factor known in its etiology is a history of radiation (Iglesias et al., 2017), and other risk factors include a benign thyroid nodule or a family history of PTC (Lee et al., 2016). The overall survival of patients with papillary carcinoma is excellent, with tumor-related deaths being reported at a rate of approximately 5% in most series (Rosai, 2014). Various variants of PTC have been previously described. Although the rate of local invasion, recurrence and metastasis (distant or regional) of PTCs is low, some histological variants have a small group of tumors that show more aggressive features with different clinical, pathological and molecular features. The aggressive variants of PTC include the tall cell variant (TCV), hobnail variant (HV), columnar cell variant (CV), and solid variant (SV) (Lloyd, 2017), which may have radioactive iodine resistance and higher recurrence and metastasis and lower survival rates than classical PTC. TCV tends to be more common in elderly patients. Extrathyroidal spread is more common, and it is also the most aggressive variant (Ito et al., 2008). SV has an aggressive course and is distinguished by the presence of mitosis, necrosis, and papillary nuclear features due to its growth pattern similar to poorly differentiated papillary thyroid carcinoma (Lloyd, 2017). CV has aggressive behavior and usually presents with distant metastases and a high risk of tumor-related death (Haugen et al., 2016). Aggressive behavior has been attributed to the histological subtype and/or clinicopathological features, but this remains a controversial issue. Rare variants of papillary carcinoma, the most common tumor of the thyroid, histologically differ, and due to their aggressive behavior, it is important to recognize these variants and differentiate them morphologically.

1. AGGRESSIVE VARIANTS OF PAPILLARY THYROID CARCINOMA

1.1. Tall Cell Variant of Papillary Thyroid Carcinoma

1.1.1. Epidemiology and classification

Although the definitions of PTC with tall cell morphology date back to 1948, it was first defined by Hawk and Hazard in 1976 (Nath & Erickson, 2018). The inclusion criteria for the diagnosis of PTC have been revised since its initial description. The 2004 classification of the World Health Organization (WHO) defined TCV as a variant of PTC consisting of cells whose height is at least three times their width with predominant tall cell histology. Patients with PTC with tall cell histology that did not meet this threshold were classified as PTC with long cell histological features (Lloyd, 2017). In studies investigating the threshold value of the tall cell histology for the diagnosis of TCV, the presence of a low percentage of cells and more than 50% cells showed similar clinical features, recurrence, and prognosis (Ganly et al., 2014; Ito et al., 2017). In a study using the 10% cut-off value, it was found that it showed more aggressive features, such as extrathyroidal spread, angiolymphatic invasion, positive surgical margins and lymph node metastasis. In addition, it was determined that aggressive properties increased with the increase in the tall cell component. It has also been associated with higher tumor stage, recurrence, and worse prognosis (Beninato et al., 2013; Dettmer et al., 2015; Oh et al., 2014).

Given these findings, the WHO Classification of Tumours of Endocrine Organs was updated in 2017 to redefine TCV as having more than 30% long cells. According to WHO, the required tall cell rate for the diagnosis of TCV is 30% or more, but the rate of the clinically important tall cell component is still a matter of debate (Lloyd, 2017). Beninato et al. and Oh et al. suggest that the tumor is an aggressive variant when it consists of more than 10% elongated cells (Beninato et al., 2013; Oh et al., 2014). Ganly et al. recommends a cut-off value of at least 30% (Ganly et al., 2014). Experienced pathologists, such as LiVolsi suggest that the presence of tall cells, regardless of percentage, should be indicated in the pathology report for the management of therapy, given the aggressiveness of the tumor (LiVolsi, 2010).

In a cohort study conducted between 2010 and 2016 with 35,812 patients with classical PTC and 5,447 with aggressive PTC, it was observed that the annual growth in the incidence of aggressive PTC variants, especially TCV significantly outstripped classical PTC (Ho et al., 2020). The incidence and prevalence of TCV are increasing, accounting for 1-19% of all PTC cases (Cartwright & Fingeret, 2020).

1.1.2. Clinicopathological Characteristics

Compared to classical PTC, TCV is more common in advanced ages (fifth to sixth decades of life) and women. Clinically, patients usually present with neck mass or other compression symptoms related to tumor size. Compared with classical PTC, TCV contains more aggressive clinicopathological features, such as larger tumor size, higher grade, extrathyroidal spread, capsule invasion, vascular invasion, multifocality, high mutation density including BRAFV600E mutation, and local and distant lymph node metastases. Tumor size is larger than conventional PTC and ranges from 1.9 to 4 cm (Cartwright & Fingeret, 2020; Holoubek et al., 2020). Numerous studies have shown multifocality in PTC or TCV with tall cell morphology (Cartwright & Fingeret, 2020). A study using the American College of Surgeons National Cancer database showed multifocality at a rate of 44% (Limberg et al., 2021). Radiographically, irregular shape, microlobulation, hypoechogenicity, heterogeneity, calcifications, and sonographic findings of extrathyroidal extension can be detected. Unlike classical PTC, classical spiculated and microlobular margins are observed in TCV (Baek et al., 2018).

1.1.3. Cytological Features

The recognition of TCV in preoperative fine-needle aspiration (FNA) biopsy is important for aggressive clinical management. However, it is difficult to distinguish TCV from classical PTC in FNA. Guan et al. showed that the FNA smears of TCV were composed of hypercellular and flat single cell layers (Guan et al., 2013). The presence of large follicular cells with abundant granular oncocytic cytoplasm and well-defined cell borders was the most common feature in all cases, while long columnar cells with cytoplasmic tails were detected in 56% of cases. In their cytological examination, Baum et

al. found elongated cells, with their length twice their width, in the periphery of follicular groups or as single cells (Baum et al., 2019). They identified the presence of cytoplasmic cuff and soap bubble pseudoinclusions along the periphery of cell clusters as specific features of TCV. Similarly, Tanaka et al. described five cytological findings indicative of TCV: 1) palisade pattern, 2) long columnar cells at least three times their width, 3) tombstone appearance, 4) spindle-like carcinoma cells, and 5) cytoplasmic elongation (Tanaka et al., 2019). Despite these unique cytological features that raise clinical suspicion, none is specific or sensitive enough for the diagnosis of TCV.

1.1.4. Histological Features

WHO defines TCV as a tumor composed of prominent papillary structures, two to three times the length of their width, dense eosinophilic (oncocytic-like) cytoplasm, and cells with the nuclear features of PTC (Lloyd, 2017) (Fig 1). The cut-off value for the acceptance of PTC as TCV was ≥30% when first defined, but since then, it has been reported to vary up to 10-75% in the literature. According to the WHO 2004 classification, TCV was defined as the presence of ≥50% predominant tall cell component (Lloyd, 2017). Tumors with a tall cell component of more than 10% have been associated with advanced tumor stage and lymph node metastases. These tumors have also been suggested to indicate adverse outcomes, and therefore this feature should be included in the pathology report (Dettmer et al., 2015). However, in line with the most recent (2017) classification of WHO, indicating that tall cells should constitute more than 30% of tumor cells for a TCV diagnosis, experienced researchers now emphasize that tall cell morphology should be specified regardless of percentage (LiVolsi, 2010; Lloyd, 2017).

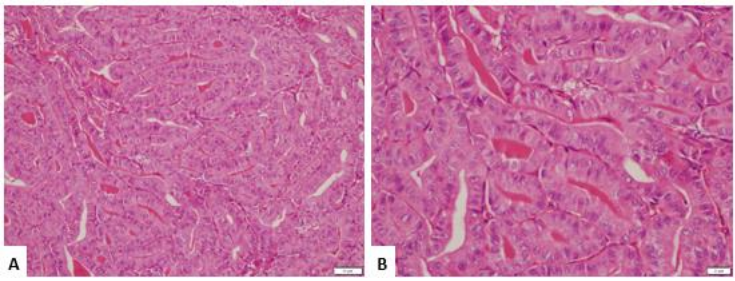

Figure 1: A.B. Tall Cell Variant (H&E, x200, x400)

1.1.5. Immunohistochemical Profile

Immunohistochemically, TCV shows positivity with thyroglobulin, paired box gene 8 (PAX8), pan-cytokeratin (CK), thyroid transcription factor (TTF)-1, Hector Battifora mesothelial cell (HBME)-1 and galectin-3 antibodies. It has also been reported to show a higher Ki-67 (MIB-1) proliferation index than conventional PTC (Rossi et al., 2019).

1.1.6. Mutational Analysis

BRAF is a protooncogene located in 7q24 that encodes a serine/threonine kinase from the RAF kinase family and plays a central role in the transduction of signals along the RAS/RAF/MEK/ERK pathway regulating cell growth, differentiation and apoptosis. BRAFV600E mutation is observed in 60-95% of TCV cases. Wong et al. found BRAF mutations at a higher frequency in subjects with more than 50% tall cell composition, and these cases often had a secondary mutation (in the TERT promoter gene) (Wong et al., 2019). Basolo et al. identified RET/PTC gene rearrangement in 35.8% of TCV cases (Basolo et al., 2002). The Cancer Genome Atlas reported that TCV cases clustered together at the mRNA level and were characterized by miR-21 expression ("Integrated genomic characterization of papillary thyroid carcinoma," 2014). These differences in the rate of driver mutations are considered to be due to differences in the definition of TCV.

1.2. Hobnail Variant of Papillary Thyroid Carcinoma

1.2.1. Epidemiology and classification

HV has been recently identified as a rare and aggressive variant of PTC. Histological features with hobnail morphology were first reported in 2004 by Kakudo et al., and then in 2009 by Motosugi et al. (Kakudo et al., 2004; Motosugi et al., 2009). In 2010, Asioli et al. described a new aggressive variant of moderately differentiated PTC with prominent hobnail features in a series of eight cases (Asioli et al., 2010). HV is more common in women and has been associated with increased rates of regional and distant metastases, radioactive iodine (RAI) refractoriness, higher mortality, and more aggressive behavior compared to classical PTC. A meta-analysis showed that the overall prevalence of HV in PTC cases was 1.08% (Donaldson et al., 2021).

1.2.2. Clinicopathological Characteristics

HV tumors can occur at any age but they are most common in the fifth and sixth decades of life (Nath & Erickson, 2018). Asioli et al. retrospectively evaluated 24 patients with HV, including eight previously described cases (Asioli et al., 2013). The mean age of the patients was 57.3 years, and HV was three times more common in women. The authors also found vascular invasion in 70.8% of the cases and extrathyroidal spread in 58.3%. In a 2017 article retrospectively analyzing 94 HV cases reported in the literature, the mean age at diagnosis was 53 years, and female predominance (F/M = 2:1) was observed. The clinical symptoms of the patients were mostly thyroid or cervical palpable masses. HV has also been associated with dyspnea due to tracheal compression, cervical lymphadenopathy, a history of multinodular goiter, and hemoptysis in one case (Ambrosi et al., 2017). Tumor size is larger in HV than conventional PTC and averages 3.1 cm (Donaldson et al., 2021). An HV tumor can be detected radiologically as a hypoechoic and highly vascularized thyroid nodule (Ambrosi et al., 2017).

1.2.3. Cytological Features

HV is observed cytologically in a cellular feature containing micropapillary or papilla-like structures in the hemorrhagic background containing a small amount of colloidal material. Cells range in size from small to medium with teardrop cytoplasm called comet-like cells. The nuclei are apically located, sometimes containing a groove, and form a surface protrusion with a high nuclear/cytoplasmic ratio resulting in a hobnail appearance. There may be nuclear pleomorphism and occasionally multiple soap bubble-like pseudoinclusions, and mitotic figures may also be seen. The presence of “comet-like” cells in FNA may be stimulatory for the HV of PTC (Ambrosi et al., 2017).

1.2.4. Histological Features

Histologically, HV is characterized by complex papillary structures, apically located nuclei forming a hobnail, and cells with a high nuclear/cytoplasmic ratio (Fig 2). Cells can vary in size and shape, from small lymphocytoid cells to larger cuboidal cells and even long/columnar cells (Coca-Pelaz et al., 2020). Although a 30% cut-off value is used for the

diagnosis HV according to the 2017 WHO calcification, even tumors with a <10% hobnail component can exhibit aggressive behavior (Lloyd, 2017). The hobnail pattern is associated with the loss of cellular polarity and cohesion and is important for epithelial-mesenchymal transition as a possible mechanism of metastasis (Ambrosi et al., 2017). In a recent study, it was reported that although there were cells with hobnail morphology in classical PTC cases, the tumor did not exhibit aggressive behavior. The authors suggested that in addition to hobnail morphology being sufficient for aggressive behavior, other aggressive histological features, such as a high mitotic rate and extrathyroidal spread were also important (Wong et al., 2020).

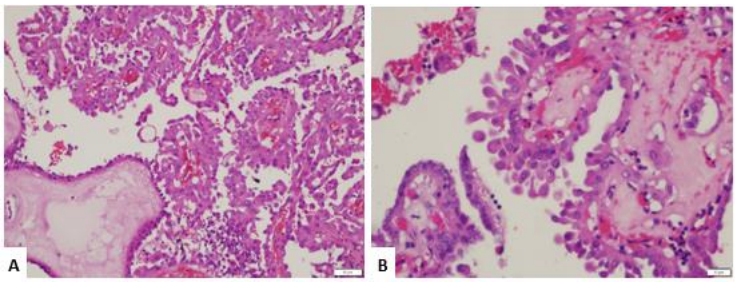

Figure 2: A.B. Hobnail Variant (H&E, x200, x400)

1.2.5. Immunohistochemical Profile

Immunohistochemically, thyroglobulin, TTF-1, CK7, CK19, HBME-1 and epithelial membrane antigen are positive in HV cases. Patchy lateral or basolateral membranes with β-catenin and E-cadherin show positivity. A high expression of p53 has been reported in more than 25% of hobnail cells in 77% of cases. The Ki-67 proliferation index is consistent with a high mitotic rate and is reported to be 10% on average (Coca-Pelaz et al., 2020).

1.2.6. Mutational Analysis

When HV was first defined, Asioli et al. detected BRAF mutations in 57.1% of cases, and later Lubitz et al. detected BRAFV600E mutation in 25.3% (Asioli et al., 2010; Lubitz et al., 2014). They also found that 80% of the tumors were BRAFV600E-positive and 20% contained RET/PTC1 fusions. The most common genetic abnormality detected in HV is the BRAFV600E mutation and RET/PTC rearrangement (Nassar et al., 2004;

Romei & Elisei, 2012). Rarely, gene amplifications and copy number gains involving tyrosine kinase receptor genes can also be observed (Abubaker et al., 2008; Lui et al., 2008; Santarpia et al., 2008). Morandi et al. confirmed that the most common primary genetic change in HV was the BRAFV600E mutation, and they also identified a rare mutation called A598V (Morandi et al., 2017). Other reported mutations include TP53 (55.6%), hTERT (44.4%), PIK3CA (27.8%), CTNNB1 (16.7%), EGFR (11.1%), AKT1 (5.5%), and NOTCH1 (5.5%). The mutation pattern remains unchanged in primary tumor and metastasis. This indicates a significantly increased risk of mortality in patients with BRAF mutations. The high prevalence of BRAF mutation and its association with other genes (TP53, PIK3AC, CTNNB1, and hTERT) reflect a complex pathogenetic mechanism for HV. The TP53 or PIK3CA-associated BRAF mutation results in greater mortality (Morandi et al., 2017).

1.3. Solid/Trabecular Variant of Papillary Thyroid Carcinoma

1.3.1. Epidemiology and classification

Although SV has been known since the 1970s, it was previously acknowledged as a separate entity. In 1985, Carcangiu et al. showed that the presence of solid component was associated with higher lung and lymph node metastases in patients with PTC (Carcangiu et al., 1985). Nikiforov et al. demonstrated a higher incidence of SV among children and young adult patients exposed to radiation after the Chernobyl nuclear accident in 1986 (Nikiforov et al., 1997). While this variant was first described as a tumor occurring in irradiated children and young adults, it has also been reported to occur sporadically in previously irradiated adult patients. This suggests that SV can affect patients of any age (Chiosea et al., 2009; Trovisco et al., 2005). SV is an extremely rare variant, accounting for only 1-3% of all PTC cases (Nikiforov et al., 2001).

1.3.2. Clinicopathological Characteristics

SV is more common in young adults and patients with a history of exposure to ionizing radiation. A study comparing this variant with classical papillary carcinoma reported that SV was associated with a larger tumor size, higher lymphovascular invasion, lymph node metastasis, extrathyroidal

spread, higher recurrence rate, and shorter disease-free survival (Ohashi, Kawahara, Namimatsu, Igarashi, et al., 2017). Adults who have not been exposed to radiation have a similar female-to-male ratio and mean age to classical PTC, although different values have been reported in the literature. Chang et al. evaluated 14 cases, 12 women and 2 men, with a mean age of 48.2 years, of whom none had been exposed to radiation (Chang et al., 2014). Guleria et al. reviewed nine cases of SV, five male and four female, with a mean age of 43.9 years (Guleria et al., 2018). Vural et al. reported a total of 28 cases, 24 female and four male, with a mean age of 45.2 years (Vural et al., 2021). The mortality rate of SV can reach 10% in adults. However, it has a better prognosis than poorly differentiated thyroid carcinoma despite their overlapping architecture (Nikiforov et al., 2001).

Although some authors stated that the clinical features of SV were the same as those of classical PTC, Vuong et al. found, in a meta-analysis of 11 studies including a total of 205 SV cases, that this variant had a high risk of recurrence and mortality and showed an aggressive course (Vuong et al., 2018). Similarly, in a study of 27 cases with SV, Ohashi et al. showed that this variant was associated with a larger tumor size, extracapsular invasion, higher recurrence rate, higher lymph node metastasis, lymphovascular invasion, and shorter disease-free survival compared to classical PTC (Ohashi, Kawahara, Namimatsu, Igarashi, et al., 2017).

1.3.3. Cytological Features

Regarding the FNA cytology of SV, the number of reports in the English literature is limited (Giorgadze et al., 2015; Higuchi et al., 2017; Troncone et al., 2008). While the classical cytological features of PTC, such as nuclear groove and pseudonuclear inclusions are preserved on a clean necrosis-free surface, the absence of a true papilla structure, cohesive syncytial fragments with a trabecular pattern, solid or trabecular sockets, pleomorphic overlapping, enlarged nuclei and nucleoli are important findings in the differentiation of SV from classical PTC (Guleria et al., 2018; Ohashi, 2019). The presence of cellular overlap, solid sockets, and trabecular patterns in FNA smears has been shown to be associated with a high recurrence rate (Ohashi, Murase, et al., 2017). Although poorly differentiated thyroid carcinoma and SV share structurally similar patterns (solid, trabecular, and

insular), tumor necrosis, high nuclear atypia, and increased mitosis are observed in the former. In addition, PTC nuclear features, such as pseudoinclusion and nuclear groove are not detected in the cytological examination of poorly differentiated thyroid carcinoma (Purkait et al., 2016).

1.3.4. Histological Features

SV is macroscopically defined as a well-circumscribed white-tan nodule in the thyroid gland without localized necrosis or hemorrhage in any lobe. Microscopically, it shows solid, trabecular and insular patterns in which the nuclear properties of PTC are preserved (Fig. 3). One of the problems in the diagnosis of SV is the inconsistency in the solid component ratio. Nikiforov and Gnepp used a 70% cut-off value, while Carcangiu et al. and Ohashi et al. suggested that there should be 50% solid component ratio (Carcangiu et al., 1985; Nikiforov & Gnepp, 1994; Ohashi, Murase, et al., 2017). The latest version of the WHO classification states that the term "solid variant" could be used when all or nearly all of the tumor has a solid, trabecular, or insular appearance (Lloyd, 2017). SV should be differentiated from follicular carcinoma with a solid growth pattern and poorly differentiated thyroid carcinoma.

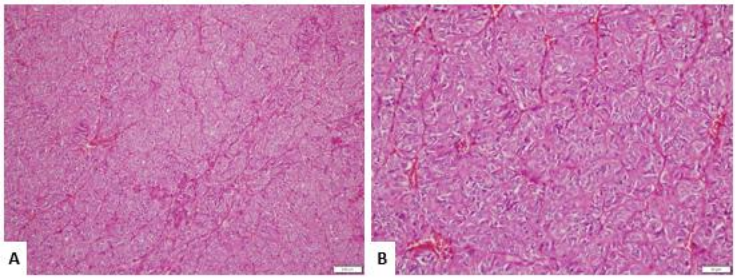

Figure 3: A.B. Solid Variant (H&E, x100, x200)

1.3.5. Immunohistochemical Profile

Frequently used markers used in the diagnosis of classical PTC are TTF-1, thyroglobulin, PAX8, CK19, HBME-1 and galectin-3. Kakudo et al. reported that a Ki67 proliferation index above 10% might be a predictor of aggressive thyroid tumors, including those that are poorly differentiated (Kakudo et al., 2015). In a recent study, it was shown that CK19 and HBME-1

expressions were low in patients with SV, and their tumor recurrence was high after surgery (Ohashi, Kawahara, Namimatsu, Okamura, et al., 2017).

1.3.6. Mutational Analysis

The mechanisms underlying the development of SV may differ between sporadic cases and those with a history of radiation. While RET/PTC1 rearrangements are more frequently observed in classical PTC, the prevalence of RET/PTC3 rearrangements has been found to be higher in PTC among pediatric patients exposed to radiation after the Chernobyl nuclear accident (Nikiforov et al., 1997; Thomas et al., 1999). Nikiforov et al. found similar types of RET/PTC rearrangements in both classical PTC and SV in patients without a history of radiation exposure (Nikiforov et al., 2001).

The presence of BRAF mutations in PTC is associated with aggressive behavior, such as extracapsular spread, tumor recurrence, metastasis, and higher mortality (Elisei et al., 2012; Xing et al., 2015). The BRAF mutation is found in most classical PTC (60%) and TCV (77%) cases, while the lowest prevalence of this mutation is observed in the follicular variant (Xing, 2005). In addition to V600E, the most common type of BRAF mutations, several rare types have been reported in thyroid cancer. Trovisco et al. described a triplet nucleotide deletion in coding nucleotides 1799-1801 in a patient with SV (Trovisco et al., 2005). Chiosea et al. found a complex BRAF mutation in a single case of SV (Chiosea et al., 2009).

1.4. Columnar Variant of Papillary Thyroid Carcinoma

1.4.1. Epidemiology and classification

CV was first reported in 1986 by Evans et al. (Evans, 1986). It is the most misdiagnosed and underrecognized variant among the aggressively behaving PTCs. It accounts for only 0.15-0.2% of all PTC cases. Since typical PTC nuclear features are not seen in CV, the Japanese classification of thyroid tumors proposed by the Clinical Practice Guideline for the Management of Thyroid Nodules in Japan in 2015 was omitted from the PTC lineage, and CV was classified within other tumors ("The General Rules for the Description of Thyroid Cancer", 2015). According to the 2017 WHO Classification of Tumours of Endocrine Organs, CV is among the PTC variants (Lloyd, 2017). This variant may be aggressive, metastasizing extensively and being

unresponsive to RAI therapy, although it has variable behavior. Compared with classical PTC, CV has been reported to be associated with higher extrathyroidal spread, metastasis (nodal and distant), and worse overall survival (Jiang et al., 2018; Wang et al., 2019).

1.4.2. Clinicopathological Characteristics

CV is a rare variant seen in a wide age range. Chen et al. reported nine cases, four female and five male, with a mean age of 57 years (Chen et al., 2011). While the clinical symptom of eight patients was a mass in the neck, it was detected incidentally in one case during autopsy. Cho et al. reported six cases, one male and five female, with a mean age of 41.7 years (Cho et al., 2018). Bongiovanni et al., examining 11 FNA cytology specimens obtained from three men and seven women with a mean age of 49.4 years, reported the mean tumor size as 31.6 mm (Bongiovanni et al., 2017). CV may present with an encapsulated or infiltrated mass, with the most common finding being a neck mass, while patients may also be asymptomatic.

In a study evaluating 48 cases of CV, it was found that 20 cases were associated with an indolent course and 28 cases with an aggressive course. While patients with indolent behavior either were asymptomatic or had a painless mass, a clinical mass and related compression symptoms (shortness of breath, hoarseness, stridor, hemoptysis, adenopathy, tracheal deviation, and dysphagia) were described in some patients with an aggressive course. Indolent behavior was observed mostly in women (18 women vs two men), younger patients (mean age, 45 years), and smaller tumors (mean size, 3.6 cm). Tumors with aggressive behavior were more predominant in men (10 women vs 13 men), older age (mean age, 56 years), and larger tumors (mean diameter, 6 cm). The authors demonstrated that when encapsulated and confined to the thyroid, CV had a similar prognosis to classical PTC (Coca-Pelaz et al., 2020). The American Joint Committee on Cancer classified the indolent group of CV as I and II, and the aggressive group as III and IV.

1.4.3. Cytological Features

Due to the rare nature of CV, it lacks cytologically distinctive cytomorphological features. Therefore, patients with CV are usually diagnosed with classical PTC based on FNA findings. After CV was first

described by Evans et al., cytological features were tried to be defined with several case reports (Evans, 1986; Verma & Paul, 2016). Bongiovanni et al. reported several cytomorphological features common to all FNAs from CV tumors, such as high cellularity, papillary fragments, medium or large cell size, single cells, and pseudostratified nuclei (Bongiovanni et al., 2017). They also detected colloid, tall cells, dark or densely packed chromatin, minimal or mild nuclear atypia, and inconspicuous nucleoli in more than half of the cases. They did not detect the presence of pseudoinclusion.

1.4.4. Histological Features

On histology, CV is defined based on the presence of papillae or gland-like structures lined with columnar cells with prominent nuclear stratification. The nuclear features of papillary carcinoma are not as well developed as in classical PTC or TCV. Pseudostratified nuclei and narrow cytoplasm distinguish CV from the long-cell variant of PTC. The morphological appearance of CV may mimic adenocarcinomas of the gastrointestinal tract and endometrium (Nath & Erickson, 2018). There is no consensus on the percentage of columnar cells required for this diagnosis. According to different authors, the required rate varies between 30% and 80%. For the diagnosis of CV, the WHO Classification of Tumours of Endocrine Organs indicate that tumors must comprise at least 30% of columnar cells with nuclear stratification and narrow cytoplasm (Lloyd, 2017). Tumors may be well circumscribed or encapsulated, or they may show a more aggressive infiltrative growth pattern and extrathyroidal spread. Mitotic activity is generally increased (Cho et al., 2018; Wenig et al., 1998).

1.4.5. Immunohistochemical Profile

CV tumors are positive for TTF-1 and thyroglobulin. They also show positivity for the expression of cyclin D1, B-cell lymphoma 2, β-catenin (membranous), estrogen, and progesterone. CDX2 positivity can also be seen (reported in 55% of cases) (Coca-Pelaz et al., 2020). Ki67 is usually 20%, but may be higher in aggressive tumors (Sujoy et al., 2013).

1.4.6. Mutational Analysis

Chen et al. showed that 33% of CV cases had the BRAFV600E mutation, supporting the idea that it is a variant of PTC (Chen et al., 2011).

Table 1: Clinicopathological and Molecular Features of Aggressive PTC Variants

Variant	Clinicopathologic Features	Histologic Features	Molecular
TCV	Incidence: 1-19% Age of occurrence: fifth and sixth decades of life Tumor size: large Extrathyroidal spread	PTC nuclear properties Cells with dense eosinophilic (oncocytic-like) cytoplasm, with lengths two-three times their width, consisting of prominent papillary structures WHO cut-off: 30%	BRAF mutation (60-95%) RET (30%) hTERT (5-30%)
HV	Incidence: 1.08% Age of occurrence: fifth and sixth decades of life Extrathyroidal spread High risk of lymph node metastasis	Micropapillary growth pattern Apically located nuclei Cells with a high nuclear/cytoplasmic ratio WHO cut-off: 30%	BRAF mutation (72%) TP53 mutation (55%) hTERT (44%)
SV	Incidence: 1-3% Affected populations: children, young adults exposed to radiation, and adults Tumor size: large Extrathyroidal spread High risk of metastasis	PTC nuclear properties Solid, trabecular and insular growth pattern WHO cut-off: "all or nearly all of the tumor"	RET/PTC3 * BRAF mutation
CV	Incidence: 0.15-0.2% Variable behavior Age of occurrence: third and fourth decades of life for the encapsulated form Indolent clinical course Age of occurrence: fifth and sixth decades of life for the infiltrative form Aggressive clinical course	Poorly developed PTC nuclear properties Hypercellular neoplasm with thin papillae and glandular structures Columnar cells with pseudostratified nuclei and narrow cytoplasm WHO cut-off: 30%	BRAF V600E mutation (33%)

*****in pediatric and radiation-related cases. **PTC:** Papillary thyroid carcinoma, **TCV:** Tall cell variant, **HV:** Hobnail variant, **SV:** Solid variant, **CV:** Columnar variant

REFERENCES

Abdullah, M. I., Junit, S. M., Ng, K. L., Jayapalan, J. J., Karikalan, B., & Hashim, O. H. (2019). Papillary Thyroid Cancer: Genetic Alterations and Molecular Biomarker Investigations. *Int J Med Sci*, *16*(3), 450-460.

Abubaker, J., Jehan, Z., Bavi, P., Sultana, M., Al-Harbi, S., Ibrahim, M., Al-Nuaim, A., Ahmed, M., Amin, T., Al-Fehaily, M., Al-Sanea, O., Al-Dayel, F., Uddin, S., & Al-Kuraya, K. S. (2008). Clinicopathological analysis of papillary thyroid cancer with PIK3CA alterations in a Middle Eastern population. *J Clin Endocrinol Metab*, *93*(2), 611-618.

Ambrosi, F., Righi, A., Ricci, C., Erickson, L. A., Lloyd, R. V., & Asioli, S. (2017). Hobnail Variant of Papillary Thyroid Carcinoma: a Literature Review. *Endocr Pathol*, *28*(4), 293-301.

Asioli, S., Erickson, L. A., Righi, A., & Lloyd, R. V. (2013). Papillary thyroid carcinoma with hobnail features: histopathologic criteria to predict aggressive behavior. *Hum Pathol*, *44*(3), 320-328.

Asioli, S., Erickson, L. A., Sebo, T. J., Zhang, J., Jin, L., Thompson, G. B., & Lloyd, R. V. (2010). Papillary thyroid carcinoma with prominent hobnail features: a new aggressive variant of moderately differentiated papillary carcinoma. A clinicopathologic, immunohistochemical, and molecular study of eight cases. *Am J Surg Pathol*, *34*(1), 44-52.

Baek, H. J., Kim, D. W., Shin, G. W., Heo, Y. J., Baek, J. W., Lee, Y. J., Cho, Y. J., Park, H. K., Ha, T. K., Kim, D. H., Jung, S. J., Park, J. S., & Ahn, K. J. (2018). Ultrasonographic Features of Papillary Thyroid Carcinomas According to Their Subtypes. *Front Endocrinol (Lausanne)*, *9*, 223.

Basolo, F., Giannini, R., Monaco, C., Melillo, R. M., Carlomagno, F., Pancrazi, M., Salvatore, G., Chiappetta, G., Pacini, F., Elisei, R., Miccoli, P., Pinchera, A., Fusco, A., & Santoro, M. (2002). Potent mitogenicity of the RET/PTC3 oncogene correlates with its prevalence in tall-cell variant of papillary thyroid carcinoma. *Am J Pathol*, *160*(1), 247-254.

Baum, J. E., Soong, L., Scognamiglio, T., Margolskee, E. M., Hoda, R. S., & Rao, R. (2019). Cytological diagnosis of papillary thyroid carcinoma with tall cells on ThinPrep liquid-based cytology. *Diagn Cytopathol*, *47*(6), 541-546.

Beninato, T., Scognamiglio, T., Kleiman, D. A., Uccelli, A., Vaca, D., Fahey, T. J., 3rd, & Zarnegar, R. (2013). Ten percent tall cells confer the aggressive features of the tall cell variant of papillary thyroid carcinoma. *Surgery*, *154*(6), 1331-1336; discussion 1336.

Bongiovanni, M., Mermod, M., Canberk, S., Saglietti, C., Sykiotis, G. P., Pusztaszeri, M., Ragazzi, M., Mazzucchelli, L., Giovanella, L., & Piana, S. (2017). Columnar cell variant of papillary thyroid carcinoma: Cytomorphological characteristics of 11 cases with histological correlation and literature review. *Cancer Cytopathol*, *125*(6), 389-397.

Carcangiu, M. L., Zampi, G., Pupi, A., Castagnoli, A., & Rosai, J. (1985). Papillary carcinoma of the thyroid. A clinicopathologic study of 241 cases treated at the University of Florence, Italy. *Cancer*, *55*(4), 805-828.

Cartwright, S., & Fingeret, A. (2020). Contemporary evaluation and management of tall cell variant of papillary thyroid carcinoma. *Curr Opin Endocrinol Diabetes Obes*, *27*(5), 351-357.

Chang, H., Kim, S. M., Chun, K. W., Kim, B. W., Lee, Y. S., Chang, H. S., Hong, S. W., & Park, C. S. (2014). Clinicopathologic features of solid variant papillary thyroid cancer. *ANZ J Surg*, *84*(5), 380-382.

Chen, J. H., Faquin, W. C., Lloyd, R. V., & Nosé, V. (2011). Clinicopathological and molecular characterization of nine cases of columnar cell variant of papillary thyroid carcinoma. *Mod Pathol*, *24*(5), 739-749.

Chiosea, S., Nikiforova, M., Zuo, H., Ogilvie, J., Gandhi, M., Seethala, R. R., Ohori, N. P., & Nikiforov, Y. (2009). A novel complex BRAF mutation detected in a solid variant of papillary thyroid carcinoma. *Endocr Pathol*, *20*(2), 122-126.

Cho, J., Shin, J. H., Hahn, S. Y., & Oh, Y. L. (2018). Columnar Cell Variant of Papillary Thyroid Carcinoma: Ultrasonographic and Clinical Differentiation between the Indolent and Aggressive Types. *Korean J Radiol*, *19*(5), 1000-1005.

Coca-Pelaz, A., Shah, J. P., Hernandez-Prera, J. C., Ghossein, R. A., Rodrigo, J. P., Hartl, D. M., Olsen, K. D., Shaha, A. R., Zafereo, M., Suarez, C., Nixon, I. J., Randolph, G. W., Mäkitie, A. A., Kowalski, L. P., Vander Poorten, V., Sanabria, A., Guntinas-Lichius, O., Simo, R., Zbären, P., . . . Ferlito, A. (2020). Papillary Thyroid Cancer-Aggressive Variants and Impact on Management: A Narrative Review. *Adv Ther*, *37*(7), 3112-3128.

Dettmer, M. S., Schmitt, A., Steinert, H., Capper, D., Moch, H., Komminoth, P., & Perren, A. (2015). Tall cell papillary thyroid carcinoma: new diagnostic criteria and mutations in BRAF and TERT. *Endocr Relat Cancer*, *22*(3), 419-429.

Donaldson, L. B., Yan, F., Morgan, P. F., Kaczmar, J. M., Fernandes, J. K., Nguyen, S. A., Jester, R. L., & Day, T. A. (2021). Hobnail variant of papillary thyroid carcinoma: a systematic review and meta-analysis. *Endocrine*, *72*(1), 27-39.

Elisei, R., Viola, D., Torregrossa, L., Giannini, R., Romei, C., Ugolini, C., Molinaro, E., Agate, L., Biagini, A., Lupi, C., Valerio, L., Materazzi, G., Miccoli, P., Piaggi, P., Pinchera, A., Vitti, P., & Basolo, F. (2012). The BRAF(V600E) mutation is an independent, poor prognostic factor for the outcome of patients with low-risk intrathyroid papillary thyroid carcinoma: single-institution results from a large cohort study. *J Clin Endocrinol Metab*, *97*(12), 4390-4398.

Evans, H. L. (1986). Columnar-cell carcinoma of the thyroid. A report of two cases of an aggressive variant of thyroid carcinoma. *Am J Clin Pathol*, *85*(1), 77-80.

Ganly, I., Ibrahimpasic, T., Rivera, M., Nixon, I., Palmer, F., Patel, S. G., Tuttle, R. M., Shah, J. P., & Ghossein, R. (2014). Prognostic implications of papillary thyroid carcinoma with tall-cell features. *Thyroid*, *24*(4), 662-670.

Giorgadze, T. A., Scognamiglio, T., & Yang, G. C. (2015). Fine-needle aspiration cytology of the solid variant of papillary thyroid carcinoma: a study of 13 cases with clinical, histologic, and ultrasound correlations. *Cancer Cytopathol*, *123*(2), 71-81.

Guan, H., Vandenbussche, C. J., Erozan, Y. S., Rosenthal, D. L., Tatsas, A. D., Olson, M. T., Zheng, R., Auger, M., & Ali, S. Z. (2013). Can the tall cell variant of papillary thyroid carcinoma be distinguished from the conventional type in fine needle aspirates? A cytomorphologic study with assessment of diagnostic accuracy. *Acta Cytol*, *57*(5), 534-542.

Guleria, P., Phulware, R., Agarwal, S., Jain, D., Mathur, S. R., Iyer, V. K., Ballal, S., & Bal, C. S. (2018). Cytopathology of Solid Variant of Papillary Thyroid Carcinoma: Differential Diagnoses with other Thyroid Tumors. *Acta Cytol*, *62*(5-6), 371-379.

Haugen, B. R., Alexander, E. K., Bible, K. C., Doherty, G. M., Mandel, S. J., Nikiforov, Y. E., Pacini, F., Randolph, G. W., Sawka, A. M., Schlumberger, M., Schuff, K. G., Sherman, S. I., Sosa, J. A., Steward, D. L., Tuttle, R. M., & Wartofsky, L. (2016). 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, *26*(1), 1-133.

Higuchi, M., Hirokawa, M., Suzuki, A., Takada, N., Yamao, N., Kuma, S., & Miyauchi, A. (2017). Cytological features of solid variants of papillary thyroid carcinoma: a fine needle aspiration cytology study of 18 cases. *Cytopathology*, *28*(4), 268-272.

Ho, A. S., Luu, M., Barrios, L., Chen, I., Melany, M., Ali, N., Patio, C., Chen, Y., Bose, S., Fan, X., Mallen-St Clair, J., Braunstein, G. D., Sacks, W. L., & Zumsteg, Z. S. (2020). Incidence and Mortality Risk Spectrum Across Aggressive Variants of Papillary Thyroid Carcinoma. *JAMA Oncol*, *6*(5), 706-713.

Holoubek, S. A., Yan, H., Khokar, A. H., Kuchta, K. M., Winchester, D. J., Prinz, R. A., & Moo-Young, T. A. (2020). Aggressive variants of papillary thyroid microcarcinoma are associated with high-risk features, but not decreased survival. *Surgery*, *167*(1), 19-27.

Iglesias, M. L., Schmidt, A., Ghuzlan, A. A., Lacroix, L., Vathaire, F., Chevillard, S., & Schlumberger, M. (2017). Radiation exposure and thyroid cancer: a review. *Arch Endocrinol Metab*, *61*(2), 180-187.

Integrated genomic characterization of papillary thyroid carcinoma. (2014). *Cell*, *159*(3), 676-690.

Ito, Y., Hirokawa, M., Fukushima, M., Inoue, H., Yabuta, T., Uruno, T., Kihara, M., Higashiyama, T., Takamura, Y., Miya, A., Kobayashi, K., Matsuzuka, F., & Miyauchi, A. (2008). Prevalence and prognostic significance of poor differentiation and tall cell variant in papillary carcinoma in Japan. *World J Surg*, *32*(7), 1535-1543; discussion 1544-1535.

Ito, Y., Hirokawa, M., Miyauchi, A., Higashiyama, T., Kihara, M., & Miya, A. (2017). Prognostic Significance of the Proportion of Tall Cell Components in Papillary Thyroid Carcinoma. *World J Surg*, *41*(3), 742-747.

Jiang, C., Cheng, T., Zheng, X., Hong, S., Liu, S., Liu, J., Wang, J., & Wang, S. (2018). Clinical behaviors of rare variants of papillary thyroid carcinoma are associated with survival: a population-level analysis. *Cancer Manag Res*, *10*, 465-472.

Kakudo, K., Tang, W., Ito, Y., Mori, I., Nakamura, Y., & Miyauchi, A. (2004). Papillary carcinoma of the thyroid in Japan: subclassification of common type and identification of low risk group. *J Clin Pathol*, *57*(10), 1041-1046.

Kakudo, K., Wakasa, T., Ohta, Y., Yane, K., Ito, Y., & Yamashita, H. (2015). Prognostic classification of thyroid follicular cell tumors using Ki-67 labeling index: risk stratification of thyroid follicular cell carcinomas. *Endocr J*, *62*(1), 1-12.

Lee, C., Abdullah, M., Junit, S., Ng, K. L., Wong, S. Y., Ramli, F., & Haji Hashim, O. (2016). Malignant transformation of benign thyroid nodule is caused by prolonged H2O2 insult that interfered with the STAT3 pathway? , *9*, 18601-18617.

Limberg, J., Ullmann, T. M., Stefanova, D., Buicko, J. L., Finnerty, B. M., Zarnegar, R., Fahey, T. J., 3rd, & Beninato, T. (2021). Does Aggressive Variant Histology Without Invasive Features Predict Overall Survival in Papillary Thyroid Cancer?: A National Cancer Database Analysis. *Ann Surg*, *274*(3), e276-e281.

LiVolsi, V. A. (2010). Papillary carcinoma tall cell variant (TCV): a review. *Endocr Pathol*, *21*(1), 12-15.

LiVolsi, V. A. (2011). Papillary thyroid carcinoma: an update. *Mod Pathol*, *24 Suppl 2*, S1-9.

Lloyd, R. V. O. R. Y. K. p. G. n. R. J. W. H. O. I. A. f. R. o. C. (2017). *WHO classification of tumours of endocrine organs*.

Lubitz, C. C., Economopoulos, K. P., Pawlak, A. C., Lynch, K., Dias-Santagata, D., Faquin, W. C., & Sadow, P. M. (2014). Hobnail variant of papillary thyroid carcinoma: an institutional case series and molecular profile. *Thyroid*, *24*(6), 958-965.

Lui, W. O., Zeng, L., Rehrmann, V., Deshpande, S., Tretiakova, M., Kaplan, E. L., Leibiger, I., Leibiger, B., Enberg, U., Höög, A., Larsson, C., & Kroll, T. G. (2008). CREB3L2-PPARgamma fusion mutation identifies a thyroid signaling pathway regulated by intramembrane proteolysis. *Cancer Res*, *68*(17), 7156-7164.

Morandi, L., Righi, A., Maletta, F., Rucci, P., Pagni, F., Gallo, M., Rossi, S., Caporali, L., Sapino, A., Lloyd, R. V., & Asioli, S. (2017). Somatic mutation profiling of hobnail variant of papillary thyroid carcinoma. *Endocr Relat Cancer*, *24*(2), 107-117.

Motosugi, U., Murata, S., Nagata, K., Yasuda, M., & Shimizu, M. (2009). Thyroid papillary carcinoma with micropapillary and hobnail growth pattern: a histological variant with intermediate malignancy? *Thyroid*, *19*(5), 535-537.

Nassar, H., Pansare, V., Zhang, H., Che, M., Sakr, W., Ali-Fehmi, R., Grignon, D., Sarkar, F., Cheng, J., & Adsay, V. (2004). Pathogenesis of invasive micropapillary carcinoma: role of MUC1 glycoprotein. *Mod Pathol*, *17*(9), 1045-1050.

Nath, M. C., & Erickson, L. A. (2018). Aggressive Variants of Papillary Thyroid Carcinoma: Hobnail, Tall Cell, Columnar, and Solid. *Adv Anat Pathol*, *25*(3), 172-179.

Nikiforov, Y., & Gnepp, D. R. (1994). Pediatric thyroid cancer after the Chernobyl disaster. Pathomorphologic study of 84 cases (1991-1992) from the Republic of Belarus. *Cancer*, *74*(2), 748-766.

Nikiforov, Y. E., Erickson, L. A., Nikiforova, M. N., Caudill, C. M., & Lloyd, R. V. (2001). Solid variant of papillary thyroid carcinoma: incidence, clinical-pathologic characteristics, molecular analysis, and biologic behavior. *Am J Surg Pathol*, *25*(12), 1478-1484.

Nikiforov, Y. E., Rowland, J. M., Bove, K. E., Monforte-Munoz, H., & Fagin, J. A. (1997). Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Res*, *57*(9), 1690-1694.

Oh, W. J., Lee, Y. S., Cho, U., Bae, J. S., Lee, S., Kim, M. H., Lim, D. J., Park, G. S., Lee, Y. S., & Jung, C. K. (2014). Classic papillary thyroid carcinoma with tall cell features and tall cell variant have similar clinicopathologic features. *Korean J Pathol*, *48*(3), 201-208.

Ohashi, R. (2019). Solid/Trabecular Variant of Papillary Thyroid Carcinoma. In K. Kakudo (Ed.), *Thyroid FNA Cytology: Differential Diagnoses and Pitfalls* (pp. 249-253). Springer Singapore.

Ohashi, R., Kawahara, K., Namimatsu, S., Igarashi, T., Sakatani, T., Sugitani, I., & Naito, Z. (2017). Clinicopathological significance of a solid component in papillary thyroid carcinoma. *Histopathology*, *70*(5), 775-781.

Ohashi, R., Kawahara, K., Namimatsu, S., Okamura, R., Igarashi, T., Sugitani, I., & Naito, Z. (2017). Expression of MRP1 and ABCG2 is associated with adverse clinical outcomes of papillary thyroid carcinoma with a solid component. *Hum Pathol*, *67*, 11-17.

Ohashi, R., Murase, Y., Matsubara, M., Watarai, Y., Igarashi, T., Sugitani, I., & Naito, Z. (2017). Fine needle aspiration cytology of the papillary thyroid carcinoma with a solid component: A cytological and clinical correlation. *Diagn Cytopathol*, *45*(5), 391-398.

Purkait, S., Agarwal, S., Mathur, S. R., Jain, D., & Iyer, V. K. (2016). Fine needle aspiration cytology features of poorly differentiated thyroid carcinoma. *Cytopathology*, *27*(3), 176-184.

Romei, C., & Elisei, R. (2012). RET/PTC Translocations and Clinico-Pathological Features in Human Papillary Thyroid Carcinoma. *Front Endocrinol (Lausanne)*, *3*, 54.

Rosai, J. D. R. A. C. M. L. F. W. J. T. G. R. J. D. R. A. A. R. o. P. (2014). *Tumors of the thyroid and parathyroid glands*.

Rossi, E. D., Faquin, W. C., & Pantanowitz, L. (2019). Cytologic features of aggressive variants of follicular-derived thyroid carcinoma. *Cancer Cytopathol*, *127*(7), 432-446.

Santarpia, L., El-Naggar, A. K., Cote, G. J., Myers, J. N., & Sherman, S. I. (2008). Phosphatidylinositol 3-kinase/akt and ras/raf-mitogen-activated protein kinase pathway mutations in anaplastic thyroid cancer. *J Clin Endocrinol Metab*, *93*(1), 278-284.

Sujoy, V., Pinto, A., & Nosé, V. (2013). Columnar cell variant of papillary thyroid carcinoma: a study of 10 cases with emphasis on CDX2 expression. *Thyroid*, *23*(6), 714-719.

Tanaka, A., Hirokawa, M., Higuchi, M., Suzuki, A., Yamao, N., Hayashi, T., Kuma, S., & Miyauchi, A. (2019). Diagnostic clues indicating tall cell variants of papillary thyroid carcinoma in fine needle aspiration. *Diagn Cytopathol*, *47*(5), 452-457.

The Japanese Society of Thyroid Surgery (JSTS). (2015). The General Rules for the Description of Thyroid Cancer. Kanehara-Printing Co, Tokyo, Japan.

Thomas, G. A., Bunnell, H., Cook, H. A., Williams, E. D., Nerovnya, A., Cherstvoy, E. D., Tronko, N. D., Bogdanova, T. I., Chiappetta, G., Viglietto, G., Pentimalli, F., Salvatore, G., Fusco, A., Santoro, M., & Vecchio, G. (1999). High prevalence of RET/PTC rearrangements in Ukrainian and Belarussian post-Chernobyl thyroid papillary carcinomas: a strong correlation between RET/PTC3 and the solid-follicular variant. *J Clin Endocrinol Metab*, *84*(11), 4232-4238.

Troncone, G., Russo, M., Malapelle, U., Accardo, M., Ferraro, A., Cozzolino, I., & Palombini, L. (2008). Cytological and molecular diagnosis of solid variant of papillary thyroid carcinoma: a case report. *Cytojournal*, *5*, 2.

Trovisco, V., Soares, P., Soares, R., Magalhães, J., Sá-Couto, P., & Sobrinho-Simões, M. (2005). A new BRAF gene mutation detected in a case of a solid variant of papillary thyroid carcinoma. *Hum Pathol*, *36*(6), 694-697.

Verma, R., & Paul, P. (2016). Columnar cell variant of papillary thyroid carcinoma: A diagnostic dilemma in fine-needle aspiration cytology. *Diagn Cytopathol*, *44*(10), 816-819.

Vuong, H. G., Odate, T., Duong, U. N. P., Mochizuki, K., Nakazawa, T., Katoh, R., & Kondo, T. (2018). Prognostic importance of solid variant papillary thyroid carcinoma: A systematic review and meta-analysis. *Head Neck*, *40*(7), 1588-1597.

Vural, Ç., Kiraz, U., Turan, G., Özkara, S. K., Sözen, M., & Çetinarslan, B. (2021). Solid variant of papillary thyroid carcinoma: An analysis of 28 cases with current literature. *Ann Diagn Pathol*, *52*, 151737.

Wang, S., Xiong, Y., Zhao, Q., Song, H., Yi, P., & Liu, C. (2019). Columnar cell papillary thyroid carcinoma prognosis: findings from the SEER database using propensity score matching analysis. *Am J Transl Res*, *11*(9), 6262-6270.

Wenig, B. M., Thompson, L. D., Adair, C. F., Shmookler, B., & Heffess, C. S. (1998). Thyroid papillary carcinoma of columnar cell type: a clinicopathologic study of 16 cases. *Cancer*, *82*(4), 740-753.

Wong, K. S., Chen, T. Y., Higgins, S. E., Howitt, B. E., Lorch, J. H., Alexander, E. K., Marqusee, E., Cho, N. L., Nehs, M. A., Doherty, G. M., & Barletta, J. A. (2020). A potential diagnostic pitfall for hobnail variant of papillary thyroid carcinoma. *Histopathology*, *76*(5), 707-713.

Wong, K. S., Higgins, S. E., Marqusee, E., Nehs, M. A., Angell, T., & Barletta, J. A. (2019). Tall Cell Variant of Papillary Thyroid Carcinoma: Impact of Change in WHO Definition and Molecular Analysis. *Endocr Pathol*, *30*(1), 43-48.

Xing, M. (2005). BRAF mutation in thyroid cancer. *Endocr Relat Cancer*, *12*(2), 245-262.

Xing, M., Alzahrani, A. S., Carson, K. A., Shong, Y. K., Kim, T. Y., Viola, D., Elisei, R., Bendlová, B., Yip, L., Mian, C., Vianello, F., Tuttle, R. M., Robenshtok, E., Fagin, J. A., Puxeddu, E., Fugazzola, L., Czarniecka, A., Jarzab, B., O'Neill, C. J., . . . Sýkorová, V. (2015). Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. *J Clin Oncol*, *33*(1), 42-50.

CHAPTER 6

USE OF HOMEOPATHY IN CHILD HEALTH

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INTRODUCTION

Homeopathy, "It has been one of the traditional and complementary methods that accepts that each person has a unique body structure and health condition, is applied by referring to the patient's verbal story, uses completely natural methods, is effective in physical, mental, spiritual and emotional healing, and is widely used in the treatment of various diseases" is defined as.

Various studies reveal the use of homeopathic methods, which are thought to initiate the natural healing process and cause less harm in children's health, which is the subject of our department. For this reason, it is recommended to conduct more studies with high level of evidence on homeopathy.

Human beings have been using traditional and complementary medicine methods from past to present. However, there has been an increase in the use of traditional and complementary medicine methods recently (Muslu & Öztürk, 2008). Diseases that are difficult to treat and care, which increase in direct proportion to the prolongation of the average life expectancy of people, high cost of treatments, difficulties in accessing treatment opportunities, and the inability of health care professionals to spare sufficient and productive time for their patients have been effective in this increase. In addition, doubts and concerns about current care and treatment methods and possible side effects have increased the interest in traditional and complementary medicine methods (Khorshid & Yapucu, 2005).

According to the World Health Organization (WHO); "Traditional and complementary medicine is the whole of knowledge, skills and practices based on theories, beliefs and experiences specific to different cultures, which can be explained or not, and which are used to protect, diagnose, cure or treat physical" (WHO, 2000). It has been reported that traditional and complementary medicine methods are highly used in cancer patients, chronic diseases, reproductive health problems, pregnancy, childbirth and postpartum period, among HIV/AIDS patients (Craig, Weinert, Walton, & Derwinski-Robinson, 2006; Duggan et al., 2001; Moschèn et al., 2001; Nayak et al., 2011; Ong, Petersen, Bodeker, & Stewart-Brown, 2002; Saha et al., 2016; White & Verhoef, 2006; Zarándi, 2016). It has also been used in pediatrics to improve the quality of life of the disabled and their families with disabled

children and elderly patients (Ai & Bolling, 2002; Boswell, et al., 2001; Haley, et al., 2001; Kelly et al., 2000; Mccurdy et al.,2003.

The most commonly used traditional and complementary medicine methods are massage, leech, reflexology, aromatherapy, apitherapy, mesotherapy, homeopathy, herbal therapies, nutritional therapies, hypnotherapy, acupuncture, cupping (Saha et.al., 2016). Among these methods, homeopathy is a method that is not widely known and used in Turkey. When the literature is examined, it is seen that homeopathy has found a wide area of use in some countries and is applied in various health problems. In this section, it is aimed to give information about homeopathy and to explain how it is used in the field of child health.

1. WHAT IS HOMEOPATHY?

The term homeopathy is derived from the Greek words homoios (similar) and pathos (sickness, pain). Homeopathy is a traditional and complementary treatment system that covers all diseases and allows the body to heal itself naturally by balancing the life force (Patient).

In the definition of medical science, three main subjects have been emphasized since Hippocrates, namely Natura Medicatrix (Law of Autonomous Biological Systems), Allopathy (Law of Contradictions) and Homeopathy (Law of Similarity)(Pekmezci & Gültiken, 2015). Hippocrates "disease occurs with like, and the patient returns to health with these likes." He became the first physician to talk about homeopathy by mentioning the law of similarity. Homeopathy, about 200 years ago, doctor, pharmacist, chemist Dr. It was developed and systematized by Samuel Hahnemann and spread all over the world, starting from his hometown of Germany (Ekins et al., 2005; Lockie, 2006; Walach et al., 2005).

During his lifetime, when Hahnemann read the information about "quinine" used as a medicine in the treatment of malaria, he tried quinine on himself in order to understand its mechanism of action and took note of its effects. He observed that quinine, which reduces fever due to malaria in patients, raises body temperature in healthy individuals and creates situations similar to fever. Hahnemann, who experienced malaria attacks every time he used the remedy, concluded that "Medicines bring out the symptoms in healthy people that they improve in sick people." In addition, while

Hahnemann was alive, he tried nearly 100 remedies that would form the basis of homeopathy on himself (Pekmezci & Gültiken, 2015).

Dr. According to Hahnemann; The disease is the sum of the symptoms in the person, and these symptoms are an outward reflection of the deterioration of the balance of the life force. By balancing the life force in homeopathic treatment, the symptoms of the disease disappear and the disease is completely healed. Eliminated disease means health. In homeopathy, diseases are determined not by looking at a single symptom, but by examining all the symptoms as a whole (Samuel Hahnemann, 2002).

In homeopathy, there is acute or chronic treatment according to the course of the disease, and different frequency of use and dosage range are determined for both conditions. The main thing in homeopathy is to take a detailed anamnesis, to determine the disease and the causes of the disease, to determine the appropriate medicine for the patient, to start the remedy treatment in accordance with the principles of homeopathy, and to eliminate the disease in this way (Samuel Hahnemann, 2013).

The opening of homeopathy to the world has been through Hahnemann's students from different countries. Homeopathy, in the United States (USA); Dr. Constantine Hering and Dr. in James Tyler Kent, England; Dr. Introduced and developed through Frederick Hervey Foster Quin (Lockie, 2006). Turkey with homeopathy in 1815 homeopath Dr. Although he met with the arrival of John Martin Honigberger in Istanbul, the awareness of homeopathy in our country and the increase in interest in homeopathy found in the 2000s and studies on homeopathy began (Kutkan, 2017).

1.1 BASIC PRINCIPLES OF HOMEOPATHY

There are four basic principles that make up homeopathy. The first of these, the principle of "similia similibus curentur", explains Hahnemann as "A weaker dynamic influence is eliminated by a similar and stronger influence" (Samuel Hahnemann, 2013). The purpose of giving a similar remedy is to provide healing by stimulating the therapeutic power in the human body, that is, the life force (Owen, 2007).

"Materia medica", the second principle of homeopathy, is a work in which the names of homeopathic medicines are in alphabetical order in Latin, and there is information about the preparation, properties and use of

medicines (Lockie, 2006). It is a reference source for the science of homeopathy. From the emergence of homeopathy to the present day, remedy trials have been continued by homeopaths, thus enriching and updating the "Materia Medica" literature. (Bensky, Clavey, & Stoger, 2004).

In cases where more than one remedy is used in homeopathy, the third principle of homeopathy is the use of a single remedy in homeopathic treatment, since it is not known which remedy will be responsible for the changes that occur, and in some cases, there may be cases of suppressing the effects of each other (S Hahnemann, 2009). Homeopathy has found that the most effective treatment can be achieved when given in very small doses of a remedy that has similar characteristics with the symptoms of the disease identified, and has determined the fourth principle of homeopathy (von Timothy, 1995).

1.2 HOMEOPATHIC REMEDY

In homopathy, it is claimed that everything, animate or inanimate, has a hidden energy outside of its physical and chemical states. Based on this idea, it is defend that when the life forces of plants, minerals or animals are revealed, they can be used as powerful remedies (Lockie, 2006).

Homeopathic medicines, herbal substances (root, stem, leaf, flower), animal substances (such as organs of all animals, tissues, secretions, toxins), microorganisms (such as fungi, bacteria, viruses), human substances (such as tissues, secretions, hormones) It is produced from chemical elements and minerals. The quality of the main substances used in the production of homeopathic medicines is of great importance (Lockie, 2006).

These unprocessed main ingredients do not have homeopathic medicine properties. These substances gain the feature of homeopathic medicine only when they are determined in accordance with the principle of " similia similibus curentur " by taking correct and effective anamnesis, subjected to dilution and shaking processes and gain a certain concentration (Lockie, 2006). Homeopathic medicines are generally used in "globule" form. Apart from this form, it is available in different forms such as drops, fine powder, tablets and injection solution, ointment, cream (Cavaco, Arslan, & Sar, 2017).

When Hahnemann first introduced the rule of similarity, he was using remedies of natural origin in their main form without diluting. However, in cases where the main substance was treated purely, side effects of the remedies or signs of poisoning were observed. Based on Hahnemann's observations, he reduced the side effects by diluting the main ingredients of the remedies according to their properties, and reducing and mixing the non-dilutable solids in another inert solid. He observed that the effect of the remedies increased and the treatment period shortened when he rinsed the liquid remedies and crushed the solids to the patients he treated (Samuel Hahnemann, 2013).

1.3 USE OF HOMEOPATHİC REMEDİES

1.3.1 REMEDY SELECTİON

In homeopathy, the symptoms observed in patients are the symptoms caused by the external stimulus that negatively affects the life force or the reactions of the body due to internal imbalance. For this reason, in the detailed anamnesis taken for the selection of the remedy, all symptoms are taken into account and noted, whether they are related to the disease or not. In line with the anamnesis, the patient's information is recorded in electronic homeopathy programs, the most similar remedies are determined after the analysis of the program, and the most suitable remedies are selected by comparing the characteristics of the remedies with the patient's symptoms by applying to materia medica. In homeopathy, diseases are not classified according to organs or infectious agents. In addition, unlike classical medicine, in homeopathy, patient-specific symptoms are taken into account, not the disease (Samuel Hahnemann, 2013). In homeopathy, the patient should be evaluated as a whole and all the symptoms in the body should be learned. In addition, in the anamnesis taken for the diagnosis of the disease; The general habits and mental state of the person, the physical causes that may cause the disease, the changes in the mental state in case of illness, and the time of the day that the symptoms and signs related to the disease increase should be questioned.

Diagnosis in homeopathy is finding the right medicine. For this reason, the patient should be observed very well, carefully anamnesis should be taken and the most appropriate remedy should be selected (Jacobs et al., 2000).

1.3.2 ADMINISTRATION OF THE REMEDY

It is recommended to take homeopathic medicines on an empty stomach in the morning when the body is more fit or at night when the body is resting. Medicines are usually given in single doses, and the frequency and dose of the remedy are determined according to the patient's condition and the duration of action of the homeopathic medicine (Ernst, Pittler, & Stevinson, 2002). The most frequently used form of use, where the effects of remedies are observed more rapidly, is to put the remedies under the tongue. Thus, it is expected that the remedies will mix into the blood faster (Lockie, 2006).

2. HOMEOPATHY IN CHILD HEALTH

It is known that traditional and complementary medicine practices are used in children's health. One of these methods is homeopathy. Homeopathy is used in many areas such as diarrhea, hyperactivity, upper respiratory tract infections, and pediatric cancers (Brule et al., 2016; Langler et al., 2011; Liu, 2021; Schütze et al., 2016). When the studies and homeopathic remedies used are examined; It is known that success is achieved in the treatment of acute childhood diarrhea by using homeopathic medicines such as arsenicum album, chamomilla, mercurius vivus, podophylloum and sülfür (Jacobs, et al., 1994). As a result of studies evaluating the efficacy of homeopathy in the treatment of hyperactivity, the most commonly used remedies are Calcarea-carb., Sulfur, Chamomilla, Lycopodium, Silica, Hepar-sulph., Nux-vom., China, Ignatia, Mercurius, Capsicum, Causticum, Hyoscyamus, Phosphorus, Phosphoric- ac., Sepia, Staphysagria (Frei et al., 2005; Frei & Thurneysen, 2001).

In studies examining the use of homeopathy in the treatment of upper respiratory tract in children, it was stated that after determining the child-specific remedy and determining the appropriate dose, the cases were followed and improvement was observed (Ramchandani, 2010; Siqueira et al., 2016; Steinsbekk, et al., 2005). It is known that remedies such as tubercilinium are used in the treatment of rhinitis, hepar sulphur, medorrhinum in the treatment of tonsillitis, belladona, nux vomica, sulphur in the treatment of pharyngitis, and Sanguinaria canadensis in the treatment of sinusitis. When a study conducted with 169 children under the age of 10 with upper respiratory tract infections was examined, it was found that

homeopathic treatment was also successful compared to conventional treatment (Steinsbekk et al., 2005). In a study conducted with 208 children under the age of ten to evaluate the effectiveness of homeopathy in preventing upper respiratory tract infections, it was stated that the remedy calceraia carbonicum was used and its benefit was seen, but more randomized controlled studies are needed (Steinsbekk et al., 2007).

In the study in which the remedies used in the treatment of sinusitis were examined, throbbing in the sinuses, worsening in the middle of the night, feeling good when staying in the dark room, nausea and vomiting, arsenicum album, silicea, throbbing anterior headache with shaking, touching, bending forward, eye belladonna in children with a headache that increases with movement, redness of the face and eyes sensitive to light, Hepar sulphuricum in children who are extremely sensitive to cold, whose runny nose is intense and yellow, who is uncomfortable in breathing cold air, whose scalp is sensitive, and simple combing of the hair is painful, and it is difficult to move around, Thuja occidentalis remedy is used in children with green runny nose, nasal cavity dryness, chronic cold, nasal root pain, asthma diagnosis, worse at night. and it has been determined that the remedies are effective in the examinations made especially in the cases such as these examples (Das & Mondal). Bryonia, natrum muriaticum, Chamomilla, Mercurius solubilis remedies are used in the treatment of pediatric cancers and to cope with the side effects of oncological treatments (Brule et al., 2016; Gaertner et al., 2018). Studies have shown that homeopathy is one of the most commonly used traditional and complementary medicine methods in the treatment of children (Ağaoğlu, 2019; Ekins-Daukes et al., 2005; Huber et al., 2006).

3. CONCLUSION

Homeopathy, which has a history of approximately 200 years; It has been one of the traditional and complementary methods widely used in the treatment of various diseases. Various studies reveal that homeopathic methods, which are thought to initiate the natural healing process and cause less harm, are used in children's health problems. However, it is thought that more studies and evidence are needed on the safety of the use of homeopathic medicines in children's health problems. For this reason, it is recommended to apply homeopathy in a multidisciplinary approach within the scope of ethical

and legal procedures, in line with evidence-based results instead of traditional knowledge, and to conduct more studies with high levels of evidence.

REFERENCES

Ağaoğlu, A. (2019). The State of Homeopathy in the World and Europe. Journal of Biotechnology and Strategic Health Research, 3, 74-84.

Ai, A. L., & Bolling, S. F. (2002). The use of complementary and alternative therapies among middle-aged and older cardiac patients. *American Journal of Medical Quality*, *17*(1), 21-27.

Bensky, D., Clavey, S., & Stõger, E. J. C. H. M. (2004). Materia medica. 3-6.

Boswell, B. B., Knight, S., Hamer, M., & McChesney, J. (2001). Disability and spirituality: A reciprocal relationship with implications for the rehabilitation process. *Journal of Rehabilitation*, *67*(4), 20.

Brulé, D., Gillmeister, B., Lee, M., Alexander, S., Gassas, A., Hendershot, E.,& Sung, L. (2016). A feasibility pilot trial of individualized homeopathic treatment of fatigue in children receiving chemotherapy. *Integrative Cancer Therapies*, *15*(4), 495-501.

Cavaco, A. M., Arslan, M., & Sar, S. (2017). Informing the homeopathic practice for Turkish pharmacists: reviewing the example of Portuguese community pharmacies. *Homeopathy*, *106*(02), 93-102.

Craig, C., Weinert, C., Walton, J., & Derwinski-Robinson, B. (2006). Spirituality, chronic illness, and rural life. *Journal of Holistic Nursing*, *24*(1), 27-35.

Das, B. K., & Mondal, A. K. Sinusitis of children and its homoeopathic approach.

Duggan, J., Peterson, W. S., Schutz, M., Khuder, S., & Charkraborty, J. (2001). Use of complementary and alternative therapies in HIV-infected patients. *AIDS patient care and STDs*, *15*(3), 159-167.

Ekins-Daukes, S., Helms, P. J., Taylor, M. W., Simpson, C. R., & McLay, J. S. (2005). Paediatric homoeopathy in general practice: where, when and why?. *British journal of clinical pharmacology*, *59*(6), 743-749.

Ernst, E., Pittler, M. H., & Stevinson, C. J. (2002). Complementary/alternative medicine in dermatology. *3*(5), 341-348.

Frei, H., Everts, R., Von Ammon, K., Kaufmann, F., Walther, D., Hsu-Schmitz, S. F., ... & Thurneysen, A. (2005). Homeopathic treatment of children with attention deficit hyperactivity disorder: a randomised, double blind, placebo controlled crossover trial. *European journal of pediatrics*, *164*(12), 758-767.

Frei, H., & Thurneysen, A. (2001). Treatment for hyperactive children: homeopathy and methylphenidate compared in a family setting. *British Homeopathic Journal*, *90*(04), 183-188.

Gaertner, K., Lüer, S. C., Frei-Erb, M., & von Ammon, K. (2018). Complementary individual homeopathy in paediatric cancer care: A case series from a

University Hospital, Switzerland. *Complementary therapies in medicine*, *41*, 267-270.

Hahnemann, S. (2002). *Organon of medicine*: B. Jain publishers.

Hahnemann, S. (2009). The Chronic Diseases: Their Peculiar Nature and Their Homeopathic Cure. 12th impression. New Delhi, India: B. In: Jain Publishers, Ltd.

Hahnemann, S. (2013). *Organon der heilkunst*: Рипол Классик.

Haley, K. C., Koenig, H. G., & Bruchett, B. M. (2001). Relationship between private religious activity and physical functioning in older adults. *Journal of Religion and Health*, *40*(2), 305-312.

Huber, R., Koch, D., Beiser, I., Zschocke, I., & Luedtke, R. (2004). Experience and attitudes towards CAM--a survey of internal and psychosomatic patients in a German university hospital. *Alternative Therapies in Health & Medicine*, *10*(1).

Jacobs, J., Gloyd, S. S., Gale, J. L., Jiménez, L. M., & Crothers, D. (1994). Treatment of acute childhood diarrhea with homeopathic medicine: a randomized clinical trial in Nicaragua. *Pediatrics*, *93*(5), 719-725.

Jacobs, J., Jimenez, L. M., Malthouse, S., Chapman, E., Crothers, D., Masuk, M., & Jonas, W. B. (2000). Homeopathic treatment of acute childhood diarrhea: results from a clinical trial in Nepal. *The journal of alternative and complementary Medicine*, *6*(2), 131-139.

Kelly, K. M., Jacobson, J. S., Kennedy, D. D., Braudt, S. M., Mallick, M., & Weiner, M. A. (2000). Use of unconventional therapies by children with cancer at an urban medical center. *Journal of Pediatric Hematology/Oncology*, *22*(5), 412-416.

Khorshid, L., & Yapucu, Ü. (2005). The Role Of The Nurse In Complementary Treatments. Anatolian Journal Of Nursing And Health Sciences, 8(2), 124-130.

Kutkan, O. (2017). A Missionary Doctor In The Ottoman Empire And Their World: David H. Nutting (1854-1875).

Längler, A., Spix, C., Edelhäuser, F., Kameda, G., Kaatsch, P., & Seifert, G. (2011). Use of homeopathy in pediatric oncology in Germany. *Evidence-Based Complementary and Alternative Medicine*, *2011*.

Liu, A. (2021). Pediatric Complementary Medicine. *Pediatric Annals*, *50*(3), e91-e95.

Lockie, A. (2006). *Encyclopedia of Homeopathy: Encyclopedia of Homeopathy & Treatments for Common Ailments*. DK.

McCurdy, E. A., Spangler, J. G., Wofford, M. M., Chauvenet, A. R., & McLean, T. W. (2003). Religiosity is associated with the use of complementary medical

therapies by pediatric oncology patients. *Journal of Pediatric Hematology/Oncology*, *25*(2), 125-129.

Moschèn, R., Kemmler, G., Schweigkofler, H., Holzner, B., Dünser, M., Richter, R., ... & Sperner-Unterweger, B. (2001). Use of alternative/complementary therapy in breast cancer patients–a psychological perspective. *Supportive Care in Cancer*, *9*(4), 267-274.

Muslu, G. K., & Öztürk, C. (2008). Complementary and alternative therapies and their use in children. Journal of Child Health and Diseases, 51(1), 62-7.

Nayak, C., Singh, V., Singh, K., Singh, H., Gupta, J., Lamba, C. D., ... & Luxmi, K. S. V. B. (2011). Management of distress during climacteric years by homeopathic therapy. *The Journal of Alternative and Complementary Medicine*, *17*(11), 1037-1042.

Ong, C. K., Petersen, S., Bodeker, G. C., & Stewart-Brown, S. (2002). Health status of people using complementary and alternative medical practitioner services in 4 English counties. *American Journal of Public Health*, *92*(10), 1653-1656.

World Health Organization. (2000). *General guidelines for methodologies on research and evaluation of traditional medicine* (No. WHO/EDM/ TRM/2000.1). World Health Organization.

Owen, D. (Ed.). (2007). *Principles and practice of homeopathy: the therapeutic and healing process*. Elsevier Health Sciences.

Patient, H. Beyond Flat Earth Medicine.

Pekmezci, D., & Gültiken, N. (2015). Principles of Homeopathy and Its Use in Veterinary Medicine. Journal of Erciyes University Faculty of Veterinary Medicine, 12(1), 49-56.

Ramchandani, N. M. (2010). Homoeopathic treatment of upper respiratory tract infections in children: evaluation of thirty case series. *Complementary Therapies in Clinical Practice*, *16*(2), 101-108.

Saha, S., Koley, M., Saha, S., Singh, R., Hossain, M. M., & Pramanik, I. (2016). Obstetrics and gynecology outpatient scenario of an Indian homeopathic hospital: A prospective, research-targeted study. *Journal of Traditional and Complementary Medicine*, *6*(2), 168-171.

Schütze, T., Längler, A., Zuzak, T. J., Schmidt, P., & Zernikow, B. (2016). Use of complementary and alternative medicine by pediatric oncology patients during palliative care. *Supportive Care in Cancer*, *24*(7), 2869-2875.

Siqueira, C. M., Homsani, F., da Veiga, V. F., Lyrio, C., Mattos, H., Passos, S. R. L., ... & Quaresma, C. H. (2016). Homeopathic medicines for prevention of

influenza and acute respiratory tract infections in children: blind, randomized, placebo-controlled clinical trial. *Homeopathy*, *105*(01), 71-77.

Steinsbekk, A., Bentzen, N., & Brien, S. (2006). Why do parents take their children to homeopaths?–an exploratory qualitative study. *Complementary Medicine Research, 13*(2), 88-93.

Steinsbekk, A., Fønnebø, V., Lewith, G., & Bentzen, N. J. (2005). Homeopathic care for the prevention of upper respiratory tract infections in children: a pragmatic, randomised, controlled trial comparing individualised homeopathic care and waiting-list controls. *13*(4), 231-238.

Steinsbekk, A., Lewith, G., Fønnebø, V., & Bentzen, N. J. (2007). An exploratory study of the contextual effect of homeopathic care. A randomised controlled trial of homeopathic care vs. self-prescribed homeopathic medicine in the prevention of upper respiratory tract infections in children. *45*(4), 274-279.

von Timothy, R. D. (1995). Timothy R. Dooley Homeopathy: Beyond Flat Earth Medicine.

Walach, H., Jonas, W. B., Ives, J., Wijk, R. V., Weingärtner, O. J., & Medicine, C. (2005). Research on homeopathy: state of the art. *11*(5), 813-829.

White, M., & Verhoef, M. J. t. (2006). Cancer as part of the journey: the role of spirituality in the decision to decline conventional prostate cancer treatment and to use complementary and alternative medicine. *5*(2), 117-122.

Zarándi, I. (2016). Treating infertility with homeopathy. *European Journal of Integrative Medicine, 1*(8), 38.

CHAPTER 7

CONSTIPATION IN CHILDHOOD

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INTRODUCTION

The normal discharge of stool is considered to be a health indicator in children from all age groups, It is even more important in the first months of life. 3% of applications to general pediatric and pediatric surgery outpatient clinics and 25% of applications to pediatric gastroenterology outpatient clinics are due to defecation disorders (Molnar, D., Taitz, L. S., Urwin, O. M., & Wales, J. K. 1983, Abi-Hanna A and Lake AM 1998, Tobias N, Mason D, Lutkenhoff M, Stoops M, Ferguson D, 2008). Only a small percentage of constipation is due to organic causes. Among these are surgical pathologies like Hirschsprung's disease, Neuronal Intestinal Dysplasies and Spina Bifida which require prompt diagnosis and timely interventions.

Except for the neonatal period, most cases are considered as functional constipation and are called idiopathic constipation. The frequency of defecation in children at different ages has been determined and these are shown in Table 1. Newborns usually defecate four times a day during their first week of life. This frequency gradually decreases with age and may decrease to 1,7 stools per day at the age of two years and up to 1,2 stools per day at the age of four years (Nyhan WE 1952, Weaver LTand Steiner H. 1983). There are no significant changes in the number of defecations after the age of four. Functional constipation is defined as constipation without underlying pathological conditions. In this case, children often delay defecation in order not to feel pain, since they hold the stool because of the pain during defecation. There most common reasons leading to stool retention and consequent constipation are; toilet training, dietary changes, stressful events, intervening infections, lack of or unsuitable toilet, intense activities (playground, being with friends at school) (Molnar et al).

It has been reported that functional constipation is seen with a frequency of 7-30% in the world in 2009 and 10% of children in the USA suffer from chronic constipation (Abi-Hanna A et al). Clinicians often use Rome II or Rome III criteria to define constipation in children (http://www.romecriteria.org/criteria). According to the Rome II criteria, constipation is defined as the presence of two or more of the criteria listed (Table 2) for at least 12 weeks in the last 12 months.

There are periods when constipation is most likely to develop in childhood,

1. Conversion to supplementary food or cow's milk
2. Toilet training
3. Starting school (Di Lorenzo C.2001, Abrahamian FP, and Lloyd-Still JD. 1984).

The first encounter is the period when the baby is fed to foods other than breast milk. During this time, there is insufficient amount of fiber in the diet of the child, and this is often the main cause of constipation (Loening-Baucke V. 2000). Adequate fiber and fluid intake can help prevent or treat mild episodic constipation. In this period, the importance of informing the mothers can not be ignored.

Four main steps have been identified in the treatment of childhood constipation:

1. Education
2. Excretion
3. Prevention of re-accumulation of stool
4. Follow up (Tabbers MM, Boluyt N, Berger MY, Benninga MA 2011).

Education is informing the mother and if old enough the child, about the age-appropriate nutrition. In other words, the first thing to do in the treatment of constipation is to regulate the diet.

In one study it was observed that only 50% of the patients who received intensive treatment for constipation and have not been using laxatives for a year were successfully treated (Pijpers MA, Bongers ME, Benninga MA, Berger MY 2006). In another study, it was shown that 25% of patients with constipation before the age of five continued to have severe complaints of constipation after puberty (BongersME, vanWijkMP, Reitsma JB, Benninga MA 2010). In other words, although there is no underlying pathological condition, constipation continues for many years and can impair the quality of the lives of the child and the parents. One of the most important non-medication options that can be recommended to children or parents with constipation is to change the way and content of nutrition. Studies have shown that adolescents with constipation take less fluid and dietary fiber. Based on

similar information, the fiber that can be obtained from the diet and its importance will be mentioned briefly.

Dietary fiber

The interest in dietary fiber goes back to ancient times and this interest has increased especially in the last decades. The main reason for this is the hypotheses suggesting a relationship between some diseases that are common in developed countries and dietary fiber consumption. It has been shown that some diseases are less common in Africa than in western countries is due to the higher consumption of dietary fiber (Dönmez M, Cankurtaran M, İlseven S, Sancak N, İpekcioğlu P, Turan AR 2006, Saldamlı İ 1998). Studies have provided epidemiologically supportive results for the relationship between dietary fiber deficiency and some diseases such as constipation, hemorrhoids, colon diseases, and obesity, which are named as civilization diseases (Kahlon TS, Chow FI, Hudson CA, Lindgren FT, Betschart AA 1989, Gül H. 2007).

The indigestible components that make up the plant cell wall were first named as "dietary fiber" in 1953 (Devries JW, Prosky L, Li B, Cho S. 1999, Köksel H, Ozboy O.1993). It is one of the basic elements of edible plant parts that are resistant to digestion and absorption in the small intestine of humans and undergo full or partial fermentation in the large intestine (Harris, P. J., & Ferguson, L. R. 1999).

Lignin which is found in the plant cell Wall, lignin derivatives such as kutin, wax, suberin, structural polysaccharides such as cellulose, hemicellulose, pectin, and oligosaccharides such as inulin and oligofructose are defined as dietary fiber. In addition, gum substances such as "gum arabic" and "guar gum", which are not structural compounds, and seaweed polysaccharides such as carrageenan, agar, alginate are also reported to be dietary fibers. Dietary fiber is also expressed as non-starch polysaccharide derivatives (Ekici L, Ercoşkun H. 2007).

Although dietary fibers have been divided into many subgroups, in recent years they have been evaluated in two main groups as soluble and insoluble dietary fibers according to their solubility in water by the FAO (United Nations Food and Agriculture Organization) and the World Health Organization (WHO) (Ramulu, P., & Udayasekhara Rao, P. 2003).

Soluble fibers:

Soluble fibers include pectin, gum, mucilages, and water-soluble pentosans (Ralapati, S., & LaCourse, W. 2002, Jalili, T., Wildman, R., & Medeiros, D. 2006). Soluble fibers bind water, forming a gel and tight structure. Soluble dietary fiber has been found to be effective in lowering blood cholesterol and reducing the absorption of glucose in the gut.

Insoluble fibers:

Fibers containing cellulose, hemicellulose, lignin and water-insoluble pentosans. Insoluble fibers absorb 20 times their weight of water, but do not form a viscous structure.

Insoluble dietary fiber increases stool volume, shortens transit time and helps preventing constipation (Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M., Bemiller JN, Whistler RL. 1996). Insoluble dietary fibers directly increase in stool mass while soluble dietary fibers undergo fermentation to form gas with short-chain fatty acids, and these compounds change the pH of the intestinal contents, causing an increase in the bacterial mass in the intestine. However, it has been reported that soluble dietary fiber may also cause an increase in stool volume, considering its role in water holding capacity and gas formation (Roberfroid, M. 1993).

Dietary fiber is present in different proportions in our foods. The amount of fiber in a vegetable depends on the breed, variety, growing conditions, cultural practices. Total fiber content of fresh fruit may vary depending on the harvest time (Erbilir Ozel F. 2006, Grigelmo-Miguel, N., Gorinstein, S., & Martín-Belloso Olga 1999). Fruits and vegetables other than legumes contain less fiber compared to cereals due to their high water content. There are more fibers in the outer tissues of the cereal grain and the outer layers of fruits and vegetables. (Köksel, H. & Özboy, Ö. 1993).

In general, foods rich in dietary fiber are classified as follows;

a) Grain products; wholemeal breads (90% of the bran is fiber), crackers, breakfast cereals such as cereal, bulgur, rye, oats, barley, brown (shell) rice
b) Fruit; apples, pears, berries, citrus fruits, figs, apricots, plums, dried fruits

c) Vegetable; broccoli, cabbage, carrot, corn, peas, potatoes, zucchini, eggplant, okra
d) D. cookie; hazelnuts, peanuts, almonds, chickpeas, kernels, popcorn (Dönmez M et al 2010).

In foods containing dietary fiber, soluble and insoluble fibers are also present in varying proportions. From the soluble fiber group, pectin in foods such as apple and quince, gums in resin, β-glucan in foods such as oats, mucilages in plants and resistant starches are found in legumes.

From the insoluble dietary fiber group, cellulose is abundant in bran, hemicellulose in cereals and lignin in wheat (Erbilir Ozel F 2006). Dietary fiber types and sources are given in Table 3 (Dönmez M et al 2010, Tamer CE, Aydoğan N, Çopur U. 2004). The most beneficial intake in terms of healthy nutrition is consuming foods containing both fiber groups. It has been stated that consuming both fiber types together is more effective against diseases than they are alone (Dönmez M et al 2010, Tamer CE et al 2004).

Water-insoluble fibers have positive effects on bowel movements and intestinal transit time. It has been shown that faecal volume increases and intestinal transit time is shortened with increased dietary fiber intake. The increase in stool volume is mainly due to the water-binding properties of dietary fibers. Dietary fibers are also protective against colorectal cancers. They achieve this by changing the colon bacterial microbiota, preventing the production of toxic metabolites, and shortening the contact time of these metabolites with intestinal cells by accelerating fecal excretion (Dönmez M et al 2010).

There is an inverse relationship between the consumption of water-insoluble fiber and colon cancer. For this reason, it is recommended that foods high in water-soluble fiber, such as wheat and corn bran, should be consumed more in daily diets (Sullivan K. 1998). Foods with high dietary fiber content contain more mineral substances than refined foods. 100 grams of wheat bran daily meets almost all of the daily potassium, phosphorus, copper, zinc, sulfur and magnesium needs of the human body (Özer MS 1998).

Studies on constipation and dietary fiber

More research has been published in the past decade on fiber and constipation in children. A population-based review of the prevalence of

constipation in children aged 3-5 years in Hong Kong stated that almost 30% of children had constipation according to the Rome II criteria. Mean fiber consumption was found to be low (4,1 g/day) in these children which is less than half of the dietary fiber recommended by the American Academy of Pediatrics (APA) (Lee, W. T. K., Ip, K. S., Chan, J. S. H., Lui, N. W. M., & Young, B. W. Y. 2008). Similarly, one-third of children (mean age 10 years) in the United Kingdom have been found to have constipation (Glackin LM, Fraser M, O Neill MB. 2008). In both studies, higher fiber consumption was found in children without constipation (Jennings, A., Davies, G. J., Costarelli, V., & Dettmar, P. W. 2009). In a study conducted in Ireland in children aged 5-8 years, the incidence of constipation was found to be twice as high (13.6% and 6%) in children with insufficient fiber consumption compared to children with adequate fiber consumption (Glackin LM et al 2008).

These findings support a relationship between constipation and low fiber intake in children (de Morais MB, Vítolo MR, Aguirre AN, Medeiros EH, Antoneli EM, Fagundes-Neto U 1996). In a randomized controlled study using different methods comparing fiber intake and placebo, dietary fiber was found to be more effective than placebo (Loening-Baucke, V., Miele, E., & Staiano, A 2004). In a randomized controlled study comparing dietary fiber and lactulose, it was reported that no difference was found between the groups in terms of stool frequency and fecal incontinence (Kokke, F. T. M., Scholtens, P. A. M. J., Alles, M. S., Decates, T. S., Fiselier, T. J. W., Tolboom, J. J. M., Kimpen, J. L. L., Benninga, M. A. 2008). There are studies showing that dietary fiber intake has a positive effect in the treatment of constipation in children (Lee, W. T. K et al 2007, Chao, H.-C., Lai, M.-W., Kong, M.-S., Chen, S.-Y., Chen, C.-C., & Chiu, C.-H. 2008, Maffei, H. V., & Vicentini, A. P. 2011, Burnett C, Wilkins G. Managing children with constipation: a community perspective 2002, Walia, R., Mahajan, L., & Steffen, R. 2009), and increasing fiber consumption is the first treatment option for chronic constipation in healthy children (Walia, R. Et al 2009, Johnson, D. A. 2006). If dietary and lifestyle changes are not successful, laxatives may be prescribed (McClung HJ, Boyne L, Heitlinger L. 1995). Laxatives are highly effective drugs. However, it is worth noting that the big problem is that even after nutrition education and interventions, many

children have difficulty meeting fiber recommendations (Guimarães, E. V., Goulart, E. M. A., & Penna, F. J. 2001).

However, not all studies show a positive correlation between low fiber consumption and constipation; constipated children may have lower, the same, or higher dietary fiber consumption than non-constipated children (Morais, M. B., Vítolo, M. R., Aguirre, A. N., & Fagundes-Neto, U 1999, Roma, E., Adamidis, D., Nikolara, R., Constantopoulos, A., & Messaritakis, J. 1999, Speridião, P. G. L., Tahan, S., Fagundes-Neto, U., & Morais, M. B. 2003, Kranz, S., Mitchell, D. C., Siega-Riz, A. M., & Smiciklas-Wright, H. 2005). To date, the majority of findings support fiber consumption as an important factor in the development of constipation in children. The APA states that current research is insufficient to support firm recommendations on fiber consumption for the treatment of constipation. However, he recommends high fiber sourced whole grains, fruits and vegetables as part of a balanced diet to reduce constipation in children. However, the actual consumption level of these food groups in the society shows that this approach is not effective enough. Parents, caregivers and schools need to regulate fiber-dense foods so that children can get the recommended daily amount of fiber. It is also worth noting the importance of the difference between the fiber content in the total diet consumed by a child versus the total energy consumed (Castillejo, G., Bulló Mònica, Anguera, A., Escribano, J., & Salas-Salvadó Jordi 2006). Although there is no definite consensus on the recommended amount of fiber and the type of fiber that may be most beneficial for children, the available findings show that fiber contributes to a healthy gastrointestinal function and to the prevention and treatment of childhood constipation. According to most guidelines, the amount of fiber consumed should be gradually increased to treat childhood constipation.

Although dietary fiber certainly has important contributions to the digestive system of children, assessing the effect of fiber on constipation is easiest by evaluating each fiber in isolation, because different fibers have variable effects on the body. In a study in which constipated children were given wheat bran (average consumption 20 g/day). They have reported children whose constipation improved had higher wheat bran (Maffei, H. V., & Vicentini, A. P. 2011). In children aged 3-10 years with chronic idiopathic constipation, consumption of cocoa shells resulted in a reduction in the

formation of hard stools compared to children taking placebo (41,7% vs. 75%) (Tse, P. W., Leung, S. S., Chan, T., Sien, A., & Chan, A. K 2000).

The effects of fiber on constipation have also been studied in children with developmental disabilities. It was determined that the basic fiber consumption of these children was 2 g per day and they were using laxatives to keep their constipation under control. In the two-stage dietary fiber intervention, fiber consumption was increased from 2 g/day to 17 g/day in phase 1 and was increased to 21 g/day in the second phase. As a result, while the use of laxatives decreased in the 1st phase it decreased even more in the 2nd phase (Tse, P. W., et al 2000).

The effect of a specific fiber, glucomannan, has been evaluated in children with chronic functional constipation. Improvement in stool consistency was reported by parents and children in both the glucomannan group (62%) and the placebo group (23%). In addition, complaints of irregular bowel movements and abdominal pain were detected in fewer children in the glucomannan group. Loening et al. reported that constipation resolved at higher rates after treatment with fiber (Loening-Baucke et al 2004). The most common benefit associated with dietary fiber is overall gastrointestinal health, particularly in defecation. However, other benefits of fiber are not well defined in children. Coccorullo et al. obtained conflicting results regarding the role of dietary fiber, as the findings suggest that fiber consumption is lower, equivalent, or higher in constipated children (Coccorullo, P., Quitadamo, P., Martinelli, M., & Staiano, A 2009).

The lack of consensus in current research is due to the difficulty in assessing the effects of dietary fiber. More research is needed to understand the appropriate amount of fiber needed for all children in general, not only in constipated ones. However, until the benefits are well proved, it is important that these children continue to be encouraged for increased dietary fiber consumption with current Institute of Medicine (IOM) fiber consumption recommendations.

Who should recommend dietary fibers?

Thomais Karagiozoglou-Lampoudi et al. in their study on 86 children aged 1-11 years with idiopathic constipation, 44 of the patients were given a personalized diet accompanied by a dietitian, and treatment and diet was

recommended to the remaining 42 by the physician (Karagiozoglou-Lampoudi, T., Daskalou, E., Agakidis, C., Savvidou, A., Apostolou, A., & Vlahavas, G. 2012). When all the patients were re-evaluated one month later, it was observed that the amount of water, fiber and energy intake increased significantly in the children who were fed with the recommendation of a dietitian compared with the physician group.

When compared according to the first visit, dietary fiber intake increased by 32% and water intake by 38% in those on a personalized diet with the help of a dietitian.

The relationship of dietary fibers with intestinal functions

While foods are generally broken down with the help of digestive enzymes, dietary fibers are not affected and are broken down only by beneficial bacteria in the intestine. This phenomenon is called fermentation in the colon. According to the percentage of fermentation in the colon, dietary fiber is reported to better protect intestinal health. About half of the total dietary fiber undergoes fermentation in the gut. Soluble fiber is more fermented. E.g; While legumes are 100% fermented, bran and wheat are fermented between 20-80%. For this reason, it has been reported that the regular consumption of legumes is quite positive in terms of intestinal health.

Suggestions

The recommended daily fiber intake is 14 grams/1000 kcal (Center for Nutrition Policy and Promotion (CNPP 2002). For young children and teenagers, this value is about 20 grams/day.

Although the minimum daily fluid intake varies according to the child's weight, it is recommended as 960 ml for a 10 kg child, 1260 ml for a 15 kg child, and 1500 ml for a 20 kg child. While dietary changes may be helpful in preventing and treating mild constipation, there is little evidence that adding liquids or fiber-rich foods to the diet is effective in treating severe chronic constipation.

Current recommendations for dietary fiber intake vary widely among the various authorities. The AAP makes two different recommendations for fiber intake.

a) Child's age in years + 5 g

b) 0.5 g fiber/kg, with a maximum of 35 g/day.

According to IOM, the consumption amount varies between 14 g fiber/1000kcal or 19-38 g/day depending on age. According to the FDA Labels guide, 12 g/1000 kcal should be consumed.

Water Consumption

In general, low fluid intake is thought to play an important role in the development of constipation. In clinical practice, it is often recommended to increase fluid intake for constipated patients. However, in a controlled case study, although children increased their water consumption, it was reported that no change was observed in stool frequency and consistency.

They suggest that if the fluid intake required for the age and activity level of the child is not lower than the required amount, increased fluid intake in children is not helpful in the treatment of constipation and should not be recommended (Jennings, A., Davies, G. J., Costarelli, V., & Dettmar, P. W 2009). In a study that evaluated three-year data of 10914 adults over the age of 20, published in 2013 in the USA, low fluid intake was found to be associated with constipation for men and women, but the same result was not found for fiber. Based on the results of this study, researchers emphasized that increasing fluid intake for constipation is important in the treatment (Markland, A. D., Palsson, O., Goode, P. S., Burgio, K. L., Busby-Whitehead, J., & Whitehead, W. E. 2013).

CONCLUSİON

More than 90% of constipation is functional in childhood. While children with functional constipation are being evaluated for treatment, information on nutrition and fluid intake should be obtained in the presence of a dietitian who has experience with the pediatric patients. The amount of fiber suitable for the patient should be gradually increased and the treatment of constipation should be provided. Laxative treatment should be started in patients who do not improve even though the amount of fiber exceeds 10 g/day.

REFERENCES

Molnar, D., Taitz, L. S., Urwin, O. M., & Wales, J. K. (1983). Anorectal manometry results in defecation disorders. *Archives of Disease in Childhood*, *58*(4), 257–261. https://doi.org/10.1136/adc.58.4.257

Evaluation and treatment of constipation in infants and children. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Abi-Hanna A, Lake AM. Constipation and encopresis in childhood. Pediatr Rev 1998;19:23-30. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Tobias N, Mason D, Lutkenhoff M, Stoops M, Ferguson D. Management principles of organic causes of childhood constipation. J Pediatr Health Care 2008;22:12-3. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Nyhan WE. Stool frequency of normal infants in the first weeks of life. Pediatrics 1952;10:414-25. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Weaver LT, Steiner H. The bowel habits of young children. Arch Dis Child 1983;59:649-52. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

http://www.romecriteria.org/criteria/. (2006). *Journal of Pediatric Gastroenterology &Nutrition,43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Di Lorenzo C. Pediatric anorectal disorders. Gastroenterol Clin North Am 2001;30:269. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Abrahamian FP, Lloyd-Still JD. Chronic constipation in childhood: a longitudinal study of 186 patients. J Pediatr Gastroenterol Nutr 1984;3:460-7. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Loening-Baucke V. Clinical approach to fecal soiling in children. Clin Pediatr (Phila) 2000;39:603-7. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3).

https://doi.org/10.1097/01.mpg.0000233159.97667.c3

11. Tabbers MM, Boluyt N, Berger MY, Benninga MA. Clinical practice Diagnosis and treatment of functional constipation Eur J Pediatr 2011;170:955-63. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Pijpers MA, Bongers ME, Benninga MA, Berger MY. Functional constipation in children: a systematic review on prognosis and predictive factors. J Pediatr Gastroenterol Nutr 2010;50:256-68. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3).

https://doi.org/10.1097/01.mpg.0000233159.97667.c3

BongersME, vanWijkMP, Reitsma JB, Benninga MA. Longterm prognosis for childhood constipation: clinical outcomes in adulthood. Pediatrics 2010;126:156-62. (2006). *Journal of Pediatric Gastroenterology & Nutrition*, *43*(3). https://doi.org/10.1097/01.mpg.0000233159.97667.c3

Dönmez M, Cankurtaran M, İlseven S, Sancak N, İpekcioğlu P, Turan AR. Diyet fifleri ve insan sağlığı üzerindeki etkileri MYO-OS 2010-Ulusal Meslek Yuksekokulları Öğrenci Sempozyumu.21-22 Ekim 2010, Düzce. (2006).

Saldamlı İ. "Gıda Kimyası." Hacettepe Üniversitesi Yayınları. Ankara. 1998., *43*(3).

Kahlon TS, Chow FI, Hudson CA, Lindgren FT, Betschart AA. Influence of wheat bran particle size on vitamins A and E and cholesterol in rats. Cereal Chemistry 1989; 66:103-6.

Gül H. Mısır ve Buğday Kepeğinin Hamur ve Ekmek Nitelikleri Üzerindeki Etkilerinin İncelenmesi. Çukurova Universitesi Fen Bilimleri Enstitüsüu Gıda Mühendisliği Anabilim Dalı Doktora Tezi, Adana, 2007.

Devries JW, Prosky L, Li B, Cho S. A historical perspective of defining dietary fiber. Cereal Foods World 1999;44:367-9.

Köksel H, Ozboy O. Besinsel liflerin insan sağlığındaki rolü. Gıda 1993;18:309-14.

Harris, P. J., & Ferguson, L. R. (1999). Dietary fibres may protect or enhance carcinogenesis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *443*(1-2), 95–110. https://doi.org/10.1016/s1383-5742(99)00013-7 .

Ekici L, Ercoşkun H. Et Ürünlerinde diyet lif kullanımı. Gıda Teknolojileri Elektronik Dergisi 2007;1:83-90.

Ramulu, P., & Udayasekhara Rao, P. (2003). Total, insoluble and soluble dietary fiber contents of Indian fruits. *Journal of Food Composition and Analysis*, *16*(6), 677–685. https://doi.org/10.1016/s0889-1575(03)00095-4

Ralapati, S., & LaCourse, W. (2002). Carbohydrates and other electrochemically active compounds. *Methods of Analysis for Functional Foods and Nutraceuticals*. https://doi.org/10.1201/9781420014679.ch8

Jalili, T., Wildman, R., & Medeiros, D. (2006). Dietary fiber and coronary heart disease. *Handbook of Nutraceuticals and Functional Foods, Second Edition*, 131–144. https://doi.org/10.1201/9781420006186.ch6

Thebaudin, J. Y., Lefebvre, A. C., Harrington, M., & Bourgeois, C. M. (1997). Dietary fibres: Nutritional and technological interest. *Trends in Food Science & Technology*, *8*(2), 41–48. https://doi.org/10.1016/s0924-2244(97)01007-8

Bemiller JN, Whistler RL. Dietary fiber and carbonhydrate digestibility. Food Chemistry, Marcel Dekker, 1996:157-224.

Roberfroid, M. (1993). Dietary fiber, inulin, and oligofructose: A review comparing their physiological effects. *Critical Reviews in Food Science and Nutrition*, *33*(2), 103–148. https://doi.org/10.1080/10408399309527616

Erbilir Ozel F. Değişik meyveler ve bu meyvelerden yapılan recellerde NDF, ADF ve hemiselüloz içeriğinin belirlenmesi. Kahramanmaras Sütçü İmam Universitesi Fen Bilimleri Ana Bilim Dalı Yuksek Lisans Tezi, 2006.

Grigelmo-Miguel, N., Gorinstein, S., & Martín-Belloso Olga. (1999). Characterisation of peach dietary fibre concentrate as a food ingredient. *Food Chemistry*, *65*(2), 175–181. https://doi.org/10.1016/s0308-8146(98)00190-3

Köksel, H. & Özboy, Ö. (1993). Besinsel Liflerin İnsan Sağlığındaki Rolü . Gıda , 18 (5)

Retrieved from https://dergipark.org.tr/tr/pub/gida/issue/6976/92974

Tamer CE, Aydoğan N, Çopur U. Besinsel liflerin sağlık üzerine etkileri. Turkiye 8. Gıda Kongresi, 26-28 Mayıs, Bursa 2004.

Kahlon, T. S., Chow, F. I., Hoefer, J. L., & Betschart, A. A. (2001). Effect of wheat bran fiber and bran particle size on fat and fiber digestibility and gastrointestinal tract measurements in the rat. *Cereal Chemistry Journal*, *78*(4), 481–484. https://doi.org/10.1094/cchem.2001.78.4.481

Sullivan K. Fiber and its role in health and disease. Int J Food Sciences Nutr 1998; 49:9-12.

Özer MS. Kepekli ekmeklerin bazı niteliklerinin incelenmesi ve kalitelerinin iyilestirilmesi olanakları. Çukurova Üniversitesi Fen Bilimleri Enstitusu Gıda MuhendisliğiAnabilim Dalı Doktora Tezi, Adana. 1998.

Lee, W. T. K., Ip, K. S., Chan, J. S. H., Lui, N. W. M., & Young, B. W. Y. (2008). Increased prevalence of constipation in pre-school children is attributable to under-consumption of Plant Foods: A community-based study. *Journal of Paediatrics and Child Health*, *44*(4), 170–175. https://doi.org/10.1111/j.1440-1754.2007.01212.x

Glackin LM, Fraser M, O Neill MB. The adequacy of dietary fibre intake in 5-8 year old children. Ir Med J. 2008 Apr;101(4):118-20. PMID: 18557515

Jennings, A., Davies, G. J., Costarelli, V., & Dettmar, P. W. (2009). Dietary fibre, fluids and physical activity in relation to constipation symptoms in pre-

adolescent children. *Journal of Child Health Care*, *13*(2), 116–127. https://doi.org/10.1177/1367493509102469

de Morais MB, Vítolo MR, Aguirre AN, Medeiros EH, Antoneli EM, Fagundes-Neto U. Teor de fibra alimentar e de outros nutrientes na dieta de crianças com e sem constipação intestinal crônica funcional [Intake of dietary fiber and other nutrients by children with and without functional chronic constipation]. Arq Gastroenterol. 1996 Apr-Jun;33(2):93-101. Portuguese. PMID: 9109975

Loening-Baucke, V., Miele, E., & Staiano, A. (2004). Fiber (glucomannan) is beneficial in the treatment of childhood constipation. *Pediatrics*, *113*(3). https://doi.org/10.1542/peds.113.3.e259

Kokke, F. T. M., Scholtens, P. A. M. J., Alles, M. S., Decates, T. S., Fiselier, T. J. W., Tolboom, J. J. M., Kimpen, J. L. L., & Benninga, M. A. (2008). A dietary fiber mixture versus lactulose in the treatment of childhood constipation: A double-blind randomized controlled trial. *Journal of Pediatric Gastroenterology & Nutrition*, *47*(5), 592–597. https://doi.org/10.1097/mpg.0b013e318162c43c

Chao, H.-C., Lai, M.-W., Kong, M.-S., Chen, S.-Y., Chen, C.-C., & Chiu, C.-H. (2008). Cutoff volume of dietary fiber to ameliorate constipation in children. *The Journal of Pediatrics*, *153*(1). https://doi.org/10.1016/j.jpeds.2007.12.044

Maffei, H. V., & Vicentini, A. P. (2011). Prospective evaluation of dietary treatment in childhood constipation: High dietary fiber and wheat bran intake are associated with constipation amelioration. *Journal of Pediatric Gastroenterology & Nutrition*, *52*(1), 55–59. https://doi.org/10.1097/mpg.0b013e3181e2c6e2

Burnett C, Wilkins G. Managing children with constipation: a community perspective. J Fam Health Care. 2002;12(5):127-32. PMID: 12449063.

Walia, R., Mahajan, L., & Steffen, R. (2009). Recent advances in chronic constipation. *Current Opinion in Pediatrics*, *21*(5), 661–666. https://doi.org/10.1097/mop.0b013e32832ff241

Johnson, D. A. (2006). Treating chronic constipation. *Clinical Drug Investigation*, *26*(10), 547–557. https://doi.org/10.2165/00044011-200626100-00001

McClung HJ, Boyne L, Heitlinger L. Constipation and dietary fiber intake in children. Pediatrics. 1995 Nov;96(5 Pt 2):999-1000. PMID: 7494681.

Guimarães, E. V., Goulart, E. M. A., & Penna, F. J. (2001). Dietary fiber intake, stool frequency and colonic transit time in chronic functional constipation in children. *Brazilian Journal of Medical and Biological Research*, *34*(9), 1147–1153. https://doi.org/10.1590/s0100-879x2001000900007

Morais, M. B., Vítolo, M. R., Aguirre, A. N., & Fagundes-Neto, U. (1999). Measurement of low dietary fiber intake as a risk factor for chronic constipation in children. *Journal of Pediatric Gastroenterology and Nutrition*, *29*(2), 132–135. https://doi.org/10.1097/00005176-199908000-00007

Roma, E., Adamidis, D., Nikolara, R., Constantopoulos, A., & Messaritakis, J. (1999). Diet and chronic constipation in children: The role of Fiber. *Journal of Pediatric Gastroenterology & Nutrition*, *28*(2), 169–174. https://doi.org/10.1097/00005176-199902000-00015

Speridião, P. G. L., Tahan, S., Fagundes-Neto, U., & Morais, M. B. (2003). Dietary fiber, energy intake and nutritional status during the treatment of children with chronic constipation. *Brazilian Journal of Medical and Biological Research*, *36*(6), 753–759. https://doi.org/10.1590/s0100-879x2003000600011

Kranz, S., Mitchell, D. C., Siega-Riz, A. M., & Smiciklas-Wright, H. (2005). Dietary fiber intake by American preschoolers is associated with more nutrient-dense diets. *Journal of the American Dietetic Association*, *105*(2), 221–225. https://doi.org/10.1016/j.jada.2004.11.005

Castillejo, G., Bulló Mònica, Anguera, A., Escribano, J., & Salas-Salvadó Jordi. (2006). A controlled, randomized, double-blind trial to evaluate the effect of a supplement of cocoa husk that is rich in dietary fiber on colonic transit in constipated pediatric patients. *Pediatrics*, *118*(3). https://doi.org/10.1542/peds.2006-0090.

Tse, P. W., Leung, S. S., Chan, T., Sien, A., & Chan, A. K. (2000). Dietary fibre intake and constipation in children with severe developmental disabilities. *Journal of Paediatrics and Child Health*, *36*(3), 236–239. https://doi.org/10.1046/j.1440-1754.2000.00498.x

Coccorullo, P., Quitadamo, P., Martinelli, M., & Staiano, A. (2009). Novel and alternative therapies for childhood constipation. *Journal of Pediatric Gastroenterology & Nutrition*, *48*(Suppl 2). https://doi.org/10.1097/mpg.0b013e3181a15fd7

Karagiozoglou-Lampoudi, T., Daskalou, E., Agakidis, C., Savvidou, A., Apostolou, A., & Vlahavas, G. (2012). Personalized diet management can optimize compliance to a high-fiber, high-water diet in children with refractory functional constipation. *Journal of the Academy of Nutrition and Dietetics*, *112*(5), 725–729. https://doi.org/10.1016/j.jand.2012.01.021

Center for Nutrition Policy and Promotion (CNPP). Center for Nutrition Policy and Promotion (CNPP) | Food and Nutrition Service. (n.d.). Retrieved February 28, 2022, from https://www.fns.usda.gov/cnpp

Farvid, Eliassen, E, C., X, L., WY, C., & Willett. (2019, October 28). *Fiber*. The Nutrition Source. Retrieved February 28, 2022, from https://www.hsph.harvard.edu/nutritionsource/carbohydrates/fiber/

Jennings, A., Davies, G. J., Costarelli, V., & Dettmar, P. W. (2009). Dietary fibre, fluids and physical activity in relation to constipation symptoms in pre-adolescent children. *Journal of Child Health Care*, *13*(2), 116–127. https://doi.org/10.1177/1367493509102469

Markland, A. D., Palsson, O., Goode, P. S., Burgio, K. L., Busby-Whitehead, J., & Whitehead, W. E. (2013). Association of low dietary intake of fiber and liquids with constipation: Evidence from the National Health and Nutrition Examination Survey. *American Journal of Gastroenterology*, *108*(5), 796–803. https://doi.org/10.1038/ajg.2013.73

Table 1. Normal Defecation Frequency in Childhood (2)

Age	**Weekly**	**Daily**
0-3 months -Mother's Milk -Formula Feeding	 5-40 5-28	2,9 2,0
6-12 months	5-28	1,8
1-3 years	4-21	1,4
Over 3 years	3-14	1,0

Table 2. Functional constipation according to Rome III criteria (At least two of the following criteria for 12 weeks in the last 12 months)

• Straining (more than half of the defecations)
• Lumpy or hard stools – (more than half of the defecations)
• The feeling of incomplete emptying –(more than half of the defecations)
• The feeling of anorectal obstruction or blockage- (more than half of the defecations)
• Manual maneuvers to facilitate the defecation (finger evacuation, supporting of the pelvic floor) - (more than half of the defecations)
• And/Or Less than 3 defecations per week

Table 3. Dietary fiber types and source

Diet Fiber	**Properties**	**Resources**
Soluble		
Pectin	High content of galacturonic acid, rhamnose, arabinose, galactose, found in the middle lamina and primary wall	Whole grains, apples, legumes, cabbage, root vegetables
Gum	It consists mainly of hexose and pentose monomers	Oatmeal, dried beans, legumes
Mucilages	Components that contain glycoprotein which is synthesized in plants	Food additives
Insoluble		

Cellulose	Major component of the cell wall consisting of glucose monomers	Whole grain, bran, peas, root vegetables, beans, apples
Hemi Cellulose	Primary and secondary cell walls	Bran, whole grains
Lignin	Composed of aromatic alcohols and other cell wall components	Vegetables, flour

CHAPTER 8

SEXUAL DEVELOPMENT THEORIES

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INTRODUCTION

The World Association for Sexual Health (WAS 2014) defines sexuality as an integral part of the human personality. Therefore, human sexuality includes the concepts of gender, sexual role and identity, sexual intimacy and orientation, sexual eroticism, sexual pleasure and reproduction. In addition, sexuality includes sexual organ functions, the level of perception of sexuality and the way of expressing one's sexuality (WAS 2014).

The World Health Organization (WHO) defines the concept of sexual health as "a state of complete sexual, sociocultural, physical and psychosocial well-being and the continuity of this state". At the same time, sexual health is a positive and important dimension of the individual health of people of all age groups (WHO, 2010).

Sexual development is generally seen as a biological and physiological concept. But these perceptions have started to change in recent years. Sexual development began to get out of the biological concept and gain a broader perspective. Sexual development includes individual and communication skills such as taking responsibility and making decisions, and informing about sexual health and sexuality (San-Bayhan et al.2011). Therefore, in addition to the physiological development of the person in sexual development, skills such as individuals' ability to express themselves, get to know themselves, talk about their bodies and make decisions are also mentioned. Cognitive, language, emotional, motor and social development areas of individuals have an important place in their childhood development, as well as their sexual development. Sexuality continues from birth to death and forms an important part of personality. The child, who has developed in every aspect since infancy, also develops sexually (Deniz, & Gözütok, 2016).

Adolescents' interest in sexuality also increases during early childhood, the period when human development is the fastest. Sexual development during adolescence has an important place in terms of showing its effect in the future, as in other adolescent development areas. As a result, it is important and necessary to know the sexual development characteristics of individuals and to take appropriate approaches in raising healthy individuals in the society (Deniz & Yıldız, 2018).

1. SEXUAL DEVELOPMENT THEORIES

It is a fact that guidance regarding the attitudes, roles and behaviors that a child will adopt as a girl or a boy is important in understanding the concepts related to sexual development more easily. In order for this orientation to be healthy, it is important to consider sexual development theories.

There are five theories on the development of sexuality;

1. Cognitive - developmental theory
2. Social learning theory
3. Psychoanalytic theory
4. Information-processing theory (gender schemas)
5. Learning theory (operant conditioning theory)

1.1. Cognitive-Developmental Theory

Lawrence Kohlberg has proposed an alternative theory of sexual development based on Piaget's theory. This theory draws the cognitive side of children's understanding of gender, emphasizing that three stages must pass before children can fully understand sexuality. According to the cognitive-developmental theory (McLeod, S. A. 2016). children first learn the male-female distinction and realize that they are a girl or a boy, with the formation of sexual schemas as boys and girls physically. Later, the child realizes that his/her gender will remain fixed and will grow with his/her gender (MEB 2013). Finally, the child understands that even if people make some superficial changes such as hair style, clothing, activities, their gender will remain the same. This last stage is defined as around the age of six or seven, when sexual protection is acquired (Deniz & Yıldız, 2018).

1.2. Social Learning Theory

In this theory (Bandura and Walters 1963), the child learns new behaviors, information about sexual roles, by observing and imitating people. Mothers and fathers often act as identification models, both in terms of communication density and because of their characteristics of being the strongest and warmest according to the child's perception. Boys learn their sexual behaviors by imitating and observing the masculine behaviors of their

fathers, and girls by imitating the feminine behaviors of their mothers (Bandura, 1989).

According to the social learning theory put forward in the 1960s, the learning process occurs by observing other people cumulatively (holistic), and in this way identity formation is provided. The learning process through cumulative (holistic) observation has a critical importance as it occurs in the early development period with the perceptions, attitudes and impressions of individuals. Researchers advocating social learning suggest that children learn by observing gender roles (Grusec, & Brinker, 1972).

Social learning theory, especially emphasizing role modeling, argued that children learn many behaviors through observation. When children learn about gender roles, they prefer the same sex rather than the opposite sex (Parlaz ve ark. 2012) because they are similar to themselves. In their experimental study, Grusec and Brinker (1972) watched 5-7 year old children watch a video about male and female models, and observed that boys remembered more the behavior of the male model, and that the girls remembered the behavior of the female model more, which supports the aforementioned thesis of the social learning approach. This helps children to identify by comparing their own characteristics with those of others (Grusec, & Brinker, 1972).

Social learning theory deals with sexuality and the learning process of information about sexuality in five stages (Grusec, & Brinker, 1972, Bandura, 1989):

Phase 1: Personalization; It includes the individual's perception of basic content and information and learning processes in situations related to their own lives.

Stage 2: Sensitivity; It includes the process of assessing the risks of many behaviors of the person, including sexual activity. It is important to inform children about this issue and to support them to analyze their risk situations.

Stage 3: Self-efficacy; Many people do not believe that they can do anything to reduce the risks associated with sexuality, even if they think they are susceptible. For a successful sexuality education, it is necessary for children to gain the necessary skills to manage risks in sexual matters.

Stage 4: Social norms; It includes the process of emphasizing and reinforcing positive behaviors.

Stage 5: Skills; It is important in making knowledge functional, but not sufficient in influencing behavior. Skill development covers the process of applying the main contents in daily life (Grusec, & Brinker, 1972, Bandura, 1989).

1.3. Information-Processing Theory (Gender Schemas)

According to the information-processing approach, the main determinant of sexual typing is schema. Schemas must exist to organize all our daily behavior. Gender-based schemas enable children to categorize information and gain knowledge of the distinction between their roles as boys and girls. Without a clear formation of sexual identity, children first form schemas of both male and female types. Later, when they encounter examples suitable for their gender, they are interested in this and reinforce this interest with various questions, so that children create additional schemes suitable for their gender (Deniz, & Yıldız, 2018, MEB, 2013).

Learning theory, this theory is referred to as operant conditioning theory. According to the basic principle of this theory, sexual role acquisition is based on reward and punishment. Children are rewarded if they behave in accordance with their gender, whereas they are punished if they do not behave in accordance with their gender (MEB, 2013).

1.4.Psychoanalytic Theory

Freud, as the representative of psychoanalytic (psychosexual) theory, argues that the child shapes his personality by going through some developmental stages and sexual development is the basis of this process. Freud states that there is an internal energy defined as libido (sexual drive) in humans and that this energy is located in different places in the body according to periods. Freud states that the relationship between mother-boy and father-daughter in the phallic period is resolved with the oedipal and electra complex, and that personality development is shaped and this process is a process of sexual development (McLeod, 2016).

The Oedipus complex is determined by the formation of sexual feelings towards the different-sex parent.

Sexual Development Periods (Psychosexual Period) According to Freud's Sexual Development Theory

Although Sigmund Freud (1856-1939) worked on personality and abnormal behaviors in adults, he has an important place because he was the first theorist to indicate the importance of infancy and childhood years in the structure of personality. Freud emphasized the importance of the first six years of life in the formation of personality and drew attention to the importance of parental attitudes in raising children (MEB, 2013).

According to Freud, personality consists of three parts: id, ego and superego. The id is the cornerstone of personality. It is innate and is the source of spiritual energy. At the same time, the id is the source of instincts (libido and aggression). Spiritual energy emerges in the form of instincts and wants to be fed as soon as possible. When spiritual energy emerges in the form of instinct with the desire to be satisfied, the ego steps in. The ego is the executive organ of the personality. It deals with the matching of the outer world (superego) with the wishes of the id. The ego tries to delay the wishes of the id when necessary, to choose pleasant lives, and to stay away from unpleasant ones. The ego is rational, logical, in a sense, it is the decision organ of the personality. Superego, on the other hand, includes social moral rules. In this sense, the superego means conscience. The person's value judgments and moral rules are found in the superego. People who always act as they please and disregard social rules are id dominant, those who always take into account moral rules and what others will say and adhere strictly to the rules are superego dominant; The ego is dominant in the person who constantly tries to act rationally. (psycho-sexual) developmental periods and the characteristics of these periods are given (CETAD, Gençlik ve Cinsellik).

- Oral period (0-1 years)
- Anal period (1-3 years)
- Phallic period (4-6 years)
- Latent period (7-11 years old)
- Genital (puberty) period (12-18 years)

1.4.1. Oral Period (0-1 years)

In this period, the pleasure zone is the mouth. Sucking or taking in can be shown as the main behavior. During this period, the baby tries to pick up the stimuli around him. It tries to do this both in the form of sucking and with other sense organs. For example, he tries to absorb what he sees around with his eyes and what he hears with his ears. In the second part of this period, biting behavior begins to appear with teething. If this period is not spent properly, some behaviors related to mouth and ingestion can be seen frequently. Smoking is cited as an example of these behaviors (Çakır, 2007).

1.4.2. Anal Period (1-3 years)

Anal period means the period when pleasure and interest are concentrated in the defecation area. During this period, the child intensively uses stool retention and release behaviors. This is the period when toilet training predominates. The child learns to hold and release his stool and pee by controlling his muscles. In the toilet training phase, the mother asks the child to hold the stool and do it at the appropriate time and place. It uses rewards and punishments for this. Stool is important to the child. It can play with its feces and rub it into the environment. In this case, he encounters the reaction of his mother. Sometimes the mother is happy and sometimes angry about the same defecation process, which creates surprise in the child. As a result of his mother's pressure, the child suppresses his unwanted impulses. During this period, the mother must be very patient and loving (FOSE, 2012).

Toilet training applied to the child can be effective on the acquisition of personality traits (CETAD, Gençlik ve Cinsellik).

1.4.3. Phallic Period (4-6 years)

During this period, the person's attention, interest and sense of pleasure are directed to the genitals. Freud built his theory on the oedipus and electra complexes, which he thought were experienced during this period. Oedipus complex means that the boy feels a desire (sexual) towards his mother and perceives his father as a rival. In this period, the child who discovers his/her gender, admires his father on the one hand, and fears his father if he understands his feelings for his mother, on the other hand. The

Electra complex is the situation in which girls take an interest in their fathers and see their mothers as rivals (Özyürek, 2015).

Freud was more interested in the oedipus complex. The sexual preferences of both girls and boys, which first start with their parents, change direction in the process with the efforts of the girl to resemble her mother and the boy to resemble her father. Children try to resolve this conflict by trying to be like them by identifying with their rival mother and father models. The girl tries to win the admiration of her father by being like her mother, and the boy tries to win the admiration of his mother by being like his father. This struggle forms the basis of Freud's theory (Çakır, 200, & MEB,2013).

Every child identifies with the parent of his own sex. According to Freud's theory, a girl prefers her father as an ideal spouse, and a boy prefers his mother (CETAD, Gençlik ve Cinsellik).

1.4.4. Latent Period (7-11 years)

The seven-eleven-year-old period, which covers the primary school period, is called the latent period according to Freud. During this period, the child suddenly forgets about his previous sexual interests. The turmoil and conflicts experienced in the previous years in the spiritual and sexual fields are calmed. With the onset of school, a decrease in sexual activities and socialization are observed. Social rules are adopted. During this period, teachers and peers were added to parents and family members. The child now identifies with other people besides his parents. During this period, sexual roles are strengthened and consolidated. The quality of the games of boys and girls differ (Özyürek, 2015).

In the latent period, the child benefits from the wide learning opportunities of the society (CETAD, Gençlik ve Cinsellik).

1.4.5. Genital (Puberty) Period (12-18)

Freud calls the adolescence period as the genital period. The sexuality of the child is not intended for reproduction, but rather for pleasure. With puberty, one's sexuality becomes aimed at reproduction. This is a period between childhood and adulthood, with plenty of mental problems. Parallel to this, the transition from the dependency period of childhood to the independent period of the adult begins. The aim of this period is for the young

person to learn to establish mature relationships with the opposite sex outside the family by breaking away from his dependence on his parents (Özyürek, 2015).

CONCLUSION

As a result, besides the cognitive, social, physical and biological development of the child, sexual development also gains importance. The healthy development of individuals is necessary for the family and society. For this reason, it is important for mothers and fathers to know the sexual development processes from birth. Health professionals should have information about sexual development and parents should be informed about sexual development and should provide counseling and support to parents when any problems are encountered.

REFERENCES

Bandura, A. (1989). Human Agency İn SocialCognitiveTheory. American Psychologist, 44(9), 1175.

CETAD. Gençlik ve Cinsellik. https://www.cetad.org.tr/CetadData/Books/43/pdf-dosyasini-indirmek-icin-tiklayiniz.pdf Erişim Tarihi: 06.02.2022.

Çakır, M.A. (2007). Sosyal Bilişsel Öğrenme Kuramı. Alim Kaya (Ed.), Eğitim Psikolojisi

Çalışandemir, F., Bencik, S., & Artan, i. (*2010*).Çocukların Cinsel Eğitimi: Geçmişten Günümüze Bir Bakış 2010 http://egitimvebilim.ted.org.tr /index.php/EB/article/view/627/97 Erişim Tarihi: 02.02.2022.

Çankırı , A. (2018). Okul Öncesi Dönem Çocuklarının Cinsel Eğitimi İle İlgili Anne Baba Görüşlerinin İncelenmesi.Eğitim Bilimleri Enstitüsü, Temel Eğitim Anabilim Dalı, Okul Öncesi Eğitim Bilim Dalı. Yüksek Lisans Tezi,.Çanakkale: On Sekiz Mart Üniversitesi.

Deniz, Ü., & Gözütok, A. (2016). Okul öncesi dönem çocuk kitaplarının cinsel gelişim ve cinsel eğitim açısından incelenmesi. Journal of International Social Research, 10(49),424-431.

Deniz, Ü., & Yıldız, R . (2018). Milli Eğitim Bakanlığı Okul Öncesi Eğitim Programında Gelişim ve Cinsel Eğitim. Gazi Üniversitesi Gazi Eğitim Fakültesi Dergisi, 38 (2) , 431-447.

Future Of Sex EducationInitiative (FOSE, 2012). National Sexuality Education Standards: Core Content AndSkills, K-12 [A Special Publication Of TheJournal Of School Health]. Http://Www.Futureofsexeducation.Org/ Documents/Josh-Fose Standards-Web.Pdf/ Erişim Tarihi: 02.02.2022.

Grusec, J. E., & Brinker, D. B. (1972). Reinforcement For Imitation As A Social Learning Determinant WithİmplicationsFor Sex-Role Development. Journal Of Personality And Social Psychology, 21(2), 149.

McLeod, S. A. (2016, Febuary 05). Bandura - social learning theory. Simply Psychology. www.simplypsychology.org/bandura.html Erişim Tarihi: 06.02.2022.

Milli Eğitim Bakanlığı Temel Eğitim Genel Müdürlüğü (MEB, 2013). Okul Öncesi Eğitim Programı, Ankara.

Özyürek, A. (2015). Erken Çocukluk Döneminde Gelişim. Ankara. Vize Yayıncılık.

Parlaz, E. A., Tekgül, N., Karademirci, E., & Öngel, K.(2012). Ergenlik Dönemi: Fiziksel Büyüme, Psikolojik ve Sosyal Gelişim Süreci, Turkish Family Physician, 3(2), 10-16.

San-Bayhan, P., & İsmihan Artan, i. (2011). Cinsel Gelişim ve Cinsel Eğitim, Çocuk Gelişimi ve Eğitimi, İstanbul; Morpa Yayınları.

World Association For Sexual Health, (WAS,2014).Cinsel Haklar Bildirgesi. Http://Www.Worldsexology.Org/ Erişim Tarihi: 01.02.2022.

World Health Organization. Developing Sexual Health Programmes. Https://Www.Who.İnt/Reproductivehealth/Publications/Sexual_Health/Rhr_Hrp_10_22/En/ Erişim Tarihi: 06.02.2022

CHAPTER 9

PRECONCEPTIONEL CARE

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INTRODUCTION

There are two basic and complementary approaches to improving maternal and child health, namely antenatal care and pre-pregnancy care. Today, it has been understood that prenatal care alone is not effective enough to protect and improve maternal and child health, even when applied widely and with high quality, and better results are obtained when pre-pregnancy care is given (Dunlop et al., 2007; Kirkham et al., 2005). . Continuity of care is required to reduce maternal, neonatal and child mortality. This continuity includes pre-pregnancy, pregnancy, birth and postpartum periods (Coşkun, 2012).

1. PRECONCEPTIONEL CARE

Preconceptional care; It is a life-long service that should be provided to every couple of reproductive age, providing the couples with the opportunity to prepare physically and psychologically for a healthy pregnancy in the pre-conception period, aiming to eliminate or reduce maternal-fetal risks. It also covers some pre-pregnancy interventions and primary prevention to achieve a healthy pregnancy and baby (Baysoy & Ozkan, 2012).

1.1. The Basic Logic/Key to Preconceptional Care

The basic logic of pre-pregnancy care is to apply primary protection. In other words, it is to prevent/reduce the problems related to pregnancy by eliminating or minimizing the negativities that may affect the mother-child health, thus achieving a healthier mother and baby. The only solution to prevent unwanted pregnancy is preconception care. Starting a desired pregnancy in the healthiest way is possible with pre-pregnancy care.

The key to preconception care is when healthcare professionals at all levels ask women of childbearing age (and if possible men) who come to them for any reason, "Do you plan to have a child in the next year?" (Moos et al., 2008). If this question is not asked, there is a great possibility of late detection of pregnancies and missing real opportunities

for early intervention. If pregnancy is not planned, the couple should use an appropriate contraceptive method (Guler Baysoy & Ozkan, 2012).

1.2. Importance of preconceptional care

The health behaviors of the couples before pregnancy can affect the duration of pregnancy and the postnatal health of the baby and mother (Coskun, 2011). The preconceptional period begins when couples decide to have a baby (Stephenson et al., 2018). Preconceptional care focuses on the pre-pregnancy period to have a healthy baby and basically aims to improve the health of the spouses and indirectly the baby (Coskun & Karakaya, 2016).

It is possible to prevent unwanted pregnancy and start the desired pregnancy in a healthy way with preconceptional care. Until the woman realizes that she is pregnant and goes to a health institution (2 months in urban areas, 3 months in rural areas), the organogenesis phase is completed, and it is late to prevent adverse events (Baysoy & Ozkan, 2012). 17-56 after fertilization for the embryo. The risk is high between days. The first check-up for pregnancy starts after a missed menstrual cycle in any month. However, the delayed health check as a result of the denial of pregnancy affects the pregnancy negatively. Therefore, preconceptional care is of great importance (Kızılkaya Beji, 2016).

Benefits of preconceptional care:

- Develops fertility awareness and pregnancy planning culture.
- It provides the preliminary preparation of parents for a healthy pregnancy.
- It gives a chance for early diagnosis and treatment.
- It affects maternal and fetal health, thus public health.
- It gives women a lifelong positive health behavior.
- It allows all members of the family to develop positive health behaviors (Coskun, 2011).

2. RISK ASSESSMENT PARAMETERS IN THE PRECONCEPTIONAL PERIOD

Different guidelines suggest questioning the following parameters for pre-pregnancy risk assessment:

1) Reproductive history
2) Environmental hazards and toxins
3) Drugs known as teratogens

4) Nutrition, folic acid intake and weight management

5) Genetic conditions and family history

6) Substance use, including tobacco and alcohol

7) Chronic diseases (diabetes, hypertension, etc.)

8) Infectious diseases and vaccines

9) Family planning

10) Social and mental health problems (depression, social support, domestic violence, etc.) (American College of Obstetricians and Gynecologists [ACOG], 2019; Centers for Disease Control and Prevention [CDC], 2006).

2.1. Individual story

2.1.1 Age Factor

Being under the age of 18 or over the age of 35 is a risk factor for pregnancy. Adolescent girls and adult women are less likely to have a healthy pregnancy when they become mothers without the knowledge, skills and support needed (Dean et al., 2014).

2.1.2. Nutrition and Exercise

Maternal health affects the health of the offspring. Environmental and genetic interactions can affect not only the health of children and adults, but also the fetus and, in some cases, their future progeny. Excessive weight or obesity resulting from malnutrition adversely affects perinatal and maternal outcomes. Obesity; It can cause difficulty in conceiving, preeclampsia, gestational diabetes, maternal mortality, macrosomic baby history, congenital anomaly, unsuccessful breastfeeding and stillbirth. For this reason, overweight women should be given nutrition education in the preconceptional period and help them lose weight (Stephenson et al., 2018).

2.1.2.1. Folic Acid Supplement:

Republic of Turkey Ministry of Health (2014), starting folic acid supplementation in the pre-pregnancy period reduces the possibility of neural tube defects in pregnancy. Smokers, alcohol users, and women who regularly use oral contraceptives or take diuretics are at higher risk for folic acid deficiency and should use folic acid supplements (World Health Organization [WHO], 2017). This supportive treatment (400 microgram/day, 4 mg/day for

risky groups) should be started 1-3 months before the planned pregnancy and should be continued until the 10-12th week of pregnancy (Turkish Republic Ministry of Health, 2014).

2.1.2.2. Iron Supplement

In the guideline prepared by the Ministry of Health, it is recommended that all pregnant women (hemoglobin >11 g/dl, if there is no pallor) use 40-60 mg/day iron for a total of 9 months starting from the second trimester and for three months after delivery. 100-120 mg/day is recommended for pregnant women with a hemoglobin level of 7-11 g/dl, pallor in the palms or conjunctiva and moderately anemic pregnant women (Republic of Turkey, Ministry of Health, 2014).

2.1.2.3. Zinc, Calcium and Iodine Supplement

It has been reported that zinc deficiency negatively affects fetal and placental growth and prevents neural tube closure (Stephenson et al., 2018). It has been reported that maternal and newborn outcomes are improved by administering zinc and calcium to women during pregnancy (Dean et al., 2014).

2.1.3. Consanguineous Marriage Story:

Consanguineous marriage is one of the negative risk factors affecting maternal and child health. It causes recurrent fetal anomalies in the family and increases the risk of genetic disease (Coşkun, 2011).

2.1.4. Exposure to teratogens:

Although teratogenicity is a complex process, it depends on the duration, dose and type of exposure (Dunlop et al., 2008). It is important to take a history about the drugs used by the woman in the preconception period. Drug use during pregnancy may cause teratogenic effects on the fetus. The use of drugs and whether the drugs used are suitable for pregnancy should be questioned (Öztürk, 2014).

In addition, toxoplasma, syphilis, varicella, rubella, cytomegalovirus, herpes simplex and HIV etc. passed before or during pregnancy infections have a teratogenic effect. Therefore, it is important for couples to raise awareness on this issue and to stay away from teratogens (Lassi et al., 2014a).

2.1.5. Smoking:

Nicotine in cigarettes; Due to its high transplacental transmission rate, it is considered to be one of the most harmful substances to the fetus. It is thought that smoking of the father-to-be affects sperm morphology negatively. For this reason, it is an issue that should be emphasized that the mother and father-to-be should quit smoking in the preconception period in order to have a healthy pregnancy and healthy newborn (Lassi et al., 2014a).

2.1.6. Alcohol Use

It has been determined that the alcohol consumed by men and women before conception has a negative effect on germ cells and increases the risk of congenital heart defect three times (Coşkun, 2011; Lassi et al., 2014a). It has been determined that alcohol taken during pregnancy has a negative effect on fetal development. For this reason, couples with a history of alcoholism should receive support before pregnancy (Coşkun, 2011).

2.1.7. Exposure to Domestic Violence:

A history of domestic violence adversely affects the health of both the mother and the baby (Coşkun, 2011). violence experienced during pregnancy; The risk of preterm birth is associated with poor health outcomes such as antepartum hemorrhage, low birth weight infant, fetal loss, sexually transmitted infection (STI), and postpartum depression (D'Angelo et al., 2007).

2.1.9. Psychosocial Evaluation

Mental health is as important as physical health (Lassi et al., 2014b). Therefore, precautions should be taken before pregnancy. A woman with mental health deterioration should be treated before pregnancy (Coşkun, 2011).

2.2. Disease history

2.2.1 Chronic Diseases

Chronic and metabolic diseases of women should be controlled before pregnancy (Coşkun, 2011). Women with chronic diseases such as diabetes, thyroid and asthma that require medication should become pregnant after determining that there is no risk in terms of pregnancy. (Republic of Turkey

Ministry of Health, 2014). In this context, the importance of preconceptional care emerges.

2.2.1.1. Diabetes

In diabetes, pre-pregnancy regulation of a woman's blood sugar reduces the risk of congenital anomalies and macrosomic babies (Coşkun, 2011). It has been reported that diabetes is associated with miscarriage, stillbirth, macrosomia, and intrauterine growth retardation during pregnancy. Diet and blood glucose monitoring in the preconceptional period affect pregnancy and pregnancy outcome positively. Pregnant women should be educated about their diseases and possible risks, and they should be informed about diet and exercise (Lassi et al., 2014b).

2.2.1.2 Hypertension

Hypertension should be controlled before pregnancy (Coşkun, 2011).

2.2.1.3. Epilepsy

Anti-epileptic drugs used by women with epilepsy during childbearing and their teratogenic effects should be considered. The drugs of women who are considering pregnancy should be replaced with drugs that are least harmful to the fetus (Lassi et al., 2014b).

2.2.1.4 Heart Disease

One of the most important causes of mortality and morbidity in pregnancy is heart disease. It is necessary to become pregnant after the condition of the heart disease is determined and treated before pregnancy (Çim, 2014).

2.2.1.5. Asthma

It has been reported that the complaints of women with severe asthma before pregnancy increase during pregnancy. Oral corticosteroid use in the first trimester has been associated with the risk of preeclampsia (Lassi et al., 2014b).

2.2.1.6. Thyroid Disease

Thyroid disease is one of the important chronic diseases of women of reproductive age. In the first trimester, thyroid hormone imbalances in

women; It causes complications such as hypertension, preeclampsia, anemia, postpartum hemorrhage, preterm birth, mental retardation in children and fetal death (Lassi et al., 2014b).

2.3. Screenings and vaccinations

2.3.1 Genetic Disease Screening

Genetic disease screening; The prevalence of paternal and maternal genetic disorders affecting pregnancy varies according to many factors such as the age of the parents and medical history. Genetic tests performed before pregnancy are of great importance in reducing morbidity and mortality rates and in the birth of healthy individuals (Douglas Wilson et al., 2011). In preconceptional care to prevent genetic diseases; It is emphasized that a large-scale family history should be taken, genetic counseling should be given, screening tests should be performed, and appropriate treatment should be given in the presence of genetic disorders. In addition, health professionals should remind individuals living in places where the disease is common, that consanguineous marriage is an important factor that increases the risk, and should provide counseling on this issue (T.R. Ministry of Health, Mother Child Health, 2014).

2.3.2 Vaccines

During the preconceptional period, the up-to-dateness of women vaccinations should be evaluated. During this period, it is recommended that all women of childbearing age should be immune to hepatitis-B, influenza, measles, rubella, mumps, tetanus, diphtheria and poliomyelitis (Coşkun, 2011). After evaluating the vaccination status, it was stated that measles, rubella, tetanus, diphtheria, poliomyelitis, hepatitis B and flu vaccinations should be administered before pregnancy occurs. It has been reported that these infections adversely affect the health of the baby and cause an increase in preterm birth, fetal morbidity and mortality when passed during pregnancy (Basgol & Oskay, 2012; Coşkun, 2011).

REFERENCES

American College of Obstetricians and Gynecologists. (2019). Committee on Gynecologic Practice. Prepregnancy counseling. Retrieved January 4, 2022 from https:// pubmed. ncbi. nlm. nh. gov/ 306 1 1411/

Basgol, S., Oskay, U. (2012). Evidence-based approaches in preconception and pregnancy International Journal of Human Sciences, 9(2), 1524-1534.

Centers for Disease Control and Prevention. (2006). Recommendations to improve preconception health and health care. Retrieved January 2, 2022 from https:// www.cdc.gov/ mmwr/ pdf/rr /rr 55 06.pdf

Coşkun, A. (2011) Preconceptional care and counseling: its place and importance in women's life. Journal of Education and Research in Nursing, 8(3), 8-15.

Coşkun, A., (2012). Genetic counseling and preconceptional care.
In Coşkun, A., Güngör, İ., (Ed), Women's Health and Diseases Nursing Handbook (p.122-128). Koç University Publications

Çim, N. (2014), Heart Disease and Pregnancy, Turkey Clinics J Fam-Med Special Topics, 5(5), 95-99.

D'Angelo, D., Williams. L., Morrow, B., Cox, S., Harris, N., Harrison, L., Posner, S. F., Hood, J. R., Zapata, L. (2007). Preconception and interconception health status of women who recently gave birth to a live-born infant. MMWR Surveill Summ., 56(10), 1-35

Dean, S. V., Lassi, Z. S., Imam, A. M., Bhutta, Z. A. (2014). Preconception care: promoting reproductive planning. Reproductive Health, 11(3), 1-17. https://doi.org/10.1186/1742-4755-11-S3-S2

Douglas Wilson, R., Audibert, F., Brock, J. A., Cartier, L., Désilets, V. A. (2011). Genetic Considerations for a Woman's Pre-conception Evaluation. Journal of Obstetrics and Gynaecology Canada, 33(1), 57–64. https://doi.org/10.1016/S17012163(16)34774-0

Dunlop, A. L., Jack, B., Frey, K. (2007). National recommendations for preconception care: the essential role of the family physician. J Am Board Fam Med., 20(1), 81-84. https://doi.org/10.3122/jabfm.2007.01.060143

Guler Baysoy, N., Ozkan, S. (2012). Preconceptional care: A public health perspective. Gazi Med J., 23, 77-90. https://doi.org/10.5152/gmj.2012.25

Kızılkaya Beji, N. and Toothless, M. (2016). Pregnancy and nursing approach. In Kızılkaya Beji, N. (Ed), Women's health and diseases (pp. 308-357). Nobel Medical Bookstores

Kirkham, C., Harris, S., Grzybowski, S. (2005). Evidence-based prenatal care: Part I. General prenatal care and counseling issues. Am Fam Physician, 71(7), 1307-1316.

Lassi, Z. S., Imam, A. M., Dean, S. V., Bhutta, Z. A. (2014a). Preconception care: caffeine, smoking, alcohol, drugs and other environmental chemical/radiation exposure. Reproductive Health, 11(3), 1-12. https://doi.org/ doi:10.1186/1742-4755-11-S3-S6

Lassi, Z. S., Imam, A. M., Dean, S. V., Bhutta, Z. A. (2014b). Preconception care: screening and management of chronic disease and promoting psychological health. Reproductive Health, 11(3), 1-20. https://doi.org/10.1186/1742-4755-11-S3S5

Moos, M. K., Dunlop, A. L., Jack, B. W., Nelson, L., Coonrod, D. V., Long, R., Boggess, K., Gardiner, P. M. (2008). Healthier women, healthy reproductive outcomes: recommendations for the routine care of all women of reproductive age. Am J Obstet Gynecol, 199(6), 280-289. https://doi.org/10.1016/j.ajog.2008.08.060

Ozturk, Z. (2014). Approach to pregnant women using drugs: Teratogenicity risk and counseling service. Sted, 23(5), 201-205.

Stephenson, J., Heslehurst, N., Hall, J., Schoenaker, DAJM, Hutchinson, J., Cade, JE, Poston, L., Barrett, G., Crozier, SR, Barker, M., Kumaran, K. ., Yajnik, CS, Baird, J., Mishra, GD (2018). Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. Lancet, 391(5), 1830-1841. https://doi.org/10.1016/S0140-6736(18)30311-8.

T.R. Ministry of Health, General Directorate of Maternal Child Health and Family Planning. (2014). genetic diseases. https: //sbu. health. Retrieved from gov.tr /Ekutuphane /kitaplar/genetik.pdf on January 24, 2022.

World Health Organization. (2017). Proper Maternal Nutrition during Pregnancy Planning and Pregnancy: a Healthy Start in Life Recommendations for health care professionals – the experience from Latvia, Retrieved January 2, 2022 from https://www.euro.who.int/en/health-topics/disease-prevention / nutrition/publications/2017/proper-maternal-nutrition-during-pregnancy-planning-and-pregnancy-a-healthy-start-in-life-2017

CHAPTER 10

FORENSIC AND LEGAL RESPONSIBILITIES

OF DENTISTS

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INTRODUCTION

The number of penal actions and actions for damage related to medical malpractice has been increasing remarkably in Turkey recently (Göktürk, 2014). This increase can be clearly observed both in the archives of the courts and in statistical studies of the institutions and agencies researching this subject. There are various reasons causing this increase in medical malpractice complaints. The increases in the number of individuals getting medical service, medical interventions, doctors and the population have relatively increased the number of suits for responsibility. The most important reasons leading to this increase in the number of suits include the rising expectations of patients, both of the health care system in general and of individual health care workers. The rising expectations are due mainly to new legal regulations and practices related to health care policy, the sensitivity of society due to the popularity of the topic in media, the proliferation of internet usage, and the fact that some lawyers make these kinds of suits their area of expertise (Göktürk, 2014). In a circular dated 18.01.2005 issued by the Ministry of Health, it was requested that since the number and amount of indemnities due to service faults are increasing, measures against faulty practices shall be taken.

1. LEGAL RESPONSIBILITIES OF DENTISTS IN TURKEY

The legal responsibility of dentists working in the public sector is a matter of public law. On the other hand, the patients harmed as a result of private sector dentistry practice can open a case against their dentist according to private law provisions. In this situation, the case is opened against the dentist practicing the medical intervention (Özdemir, 2011).

1.1. Regulations in the New Turkish Penal Code (TPC) and The Responsibility of the Dentist

According to Turkish laws, a person may open a case in penal court or civil court to claim malpractice (Erol, 2017). Penal cases may result in penalties of imprisonment. And in civil courts, an indemnity penalty may be given. These law articles are the same both for doctors and dentists.

Some of the articles and descriptions in the New Turkish Penal Code (TPC) about deliberate or negligent acts related to the occupational practices of dentists are listed below (Turkish Penal Code, Law no: 5237):

Negligent homicide

Article 85. - (1) *An individual committing negligent homicide is punished with imprisonment for a term of between two years and six years.*

If the patient dies by faulty action of the dentist in the faulty action highlighted here, this characterizes an action objectively determined to be a faulty action in dentistry due to lack of knowledge or skill. With this article of the law, the dentist is kept responsible for the lack of his/her knowledge and skill during treatment.

Deliberate injury

Article 86. - (1) *A person causing any pain to somebody's body or deteriorating the health or perception ability of somebody deliberately is punished with imprisonment for a term between one years and three years.*

(2) *Provided that the deliberate injury has a mild effect on the victim and can be remedied by a simple medical intervention, the perpetrator, following the complaint of the victim, is punished with imprisonment for a term of four months or with a judicial fine.*

Harming a patient deliberately/willfully is seldom the case in the professional practice of dentists and the crime category shown in the second paragraph of this article is generally applied to this case. An example is the case of extracting the dentures from the mouth of a patient following a conflict about a fee.

If the patient has any persistent problem in speaking due to an action of the dentist which is thought to be a criminal act, the fine is increased one fold according to the Article 87 of the Law. If any bone of the patient is broken as a result of the act (in the dentistry practice, it may be the jaw bone), the fine issued to the dentist will be between one year and six years of imprisonment according to paragraph 3 of Article 88 of the Law.

Although the dentists know that it is necessary to make an intervention on the patient, if they do not make an intervention deliberately and cause an injury due to this refusal, the resulting situation is deliberate

injury caused by neglectful act. In this case, the aforementioned fines are lowered by two-third according to the Article 88 of the Law.

Negligent injury

Article 89. - (1) *The person causing a pain in somebody's body or deteriorating the health or perception ability of somebody negligently is punished with imprisonment for a term between one years and three years or judicial fine.*

Generally, it is the Article 89 of the Law that the dentists will face with when their occupational practice harms the patients (Özdemir, 2011). According to this article, even though there is no deliberate act, harming the patient through neglectfulness, lack of knowledge and skill in the profession and carelessness are punished. The assumed punishment in the Law varies between six or one years of imprisonment, or judicial fine depending on the severity of the crime. If severe consequences of the acts of the dentist arise, the punishment will increase one fold or decrease by half depending on the severity of the consequences. In accordance with this article, in order to punish the dentist, except for cases when the act is committed with conscious negligence, the person being harmed from the crime should issue a complaint.

If the dentist predicts that the related action will harm the patient but still does this action, this action is called conscious negligence. In this case, the punishment will rise by between one-third or half and complaint will not be need to start a prosecution.

1.2. Legal status of the contract between the dentist and the patient

The contract between the dentist and patient is the same with one between other doctors and patients and is subject to the provisions of contract of mandate (Hakeri, 2007; Erol, 2017). The dentist takes over the treatment of the teeth of the patients and eliminates their complaints about their teeth in accordance with the contract. And the patient accepts to pay a certain amount for this service of the dentist. In the relationship emerging between the dentist and the patient, the dentist realizes the requirements of his/her profession against the uncalculated reactions and complex structure of the human body. Thus, provisions of the contract of mandate are applied to the contract between the dentist and the patient (Özdemir, 2011). In accordance with the contract of mandate, dentist can terminate the contract or refuses the patient

any time. Similarly, the patient can terminate it any time. However, neither of the parties can terminate the contract in an inconvenient time.

Within the scope of the freedom of making a contract with the patient, the dentists may also decide to undertake a certain consequence. Agreement is classified as contract of work and there is a guarantee of a result. For example, there may be a commitment that promises the porcelain bridge denture will have certain features, colors or shapes. The agreements undertaking a certain result by the dentist are regarded as contract of work by the Turkish Court of Appeals (Özdemir, 2011). The liabilities of the dentist are much more in the contract of work. In this case the dentist shall fix any faulty manufacture and do his/her work as required. The insurance put aesthetic interventions into the contract of work and since they are not regarded as necessary for treatment, they are not covered by them. Recently, insurance companies began selling insurance policies covering these kinds of interventions as well, yet policy fees are quite high.

The dentists can undertake both treating the teeth of the patient and making dentures in the agreement with the patients. In this case, the agreement is an contract of mandate in view of the treatment and an contract of work in view of the dentures. These kinds of agreements are regarded as mixed agreements (Mosenheuer, 2008). The law to be applied to these agreements depends on the elements arising. In Germany, court decisions have been made according to the problem creating act in relationships bearing elements of both agreements. For example, if there is a fault in the manufacture of the dentures in an agreement in which the dentist undertakes to make a denture, the dentist is regarded as responsible according to the contract of work (Şenocak, 1998).

Increasing numbers of symposia on health care law have been organized recently in Turkey. However, the responsibility of the dentists has always been described in a compressed manner among the responsibilities of other physicians with much depth. The number of decisions made by the Turkish Court of Appeals is very few. In this small number of decisions, it has automatically regarded all interventions of dentists within the scope of "contract of work." For the solution of problems arising from the medical interventions of the dentists, the provisions of Turkish Code of Obligations are applied. While making a decision about the legal responsibilities of the

dentists, the Court of Appeal decides on the application of provisions that are exceptional to the agreement between the parties without discrimination. However, considering the complexity of the human body and the restorations made by the dentist, each concrete case shall be assessed differently and legal evaluation shall be made accordingly.

1.3. The liability of the dentist to inform the patient

The dentist is liable for keeping the confidentiality of the patient, explaining the case to him/her, being careful, doing the operation in-person and being loyal; however, the most important duties of the dentist are informing the patient and obtaining his or her consent. Limits of the manner and extent of informing of the patient about the treatment are much debated. The preparation of a printed consent form is beneficial as constituting proof. When there is any legal action, the witness of a relative of the patient or secretaries is important as well. Expert discretion is mostly left to the court in the topic of whether the consent of the person has been taken after informing him/her (Özdemir, 2011).

The occupational mistakes made in Turkey when obtaining the consent of the patient are as follows: forcing the patient to give consent; making an intervention to an incapable patient without the consent of his/her guardian, curator or the court; making the patient sign an empty paper or a paper that says “I accept any intervention” (Öztürkler, 2003).

For the full approval of an intervention, the patient should be informed about the meaning and consequences of the intervention, its dangers and expected benefits. Additionally, the patient should give consent while knowing everything and through his/her will without any outside force. “Any approval of the patient without being informed about the consequences is regarded as a fundamentally flawed approval which does not eliminate the responsibility of the doctor” (Özaslan and Kolusayın, 2011). Article 17 of Turkish Constitution, Article 24 of Turkish Civil Law, Article 26 of TPC, Article 70 of Medicine Law no. 1219 and Section 5 of Patient Rights Bylaw state that the consent of the patient shall be obtained after informing the patient about the intervention.

1.4. Malpractice in dentistry

Medical malpractice and complications may result from medical interventions. “Medical intervention” can be defined as “intervening in the normal processes of human nature within the framework of medicine science” and can range between a simple tooth filling and the most complicated surgical operation (Savaş, 2012).

Within the scope of dentistry, medical malpractice includes failure to reach medical standards in practice due to a fault, neglect, lack of knowledge or skill and harm to the patient. There are many different approaches for determining standard medical practice (Yavuz, 2007). Regionalism rule, average physician rule etc… However, considering practice, since these kinds of cases require an expert knowledge of dentistry, expert opinion is always obtained in the process of judgment. And the court gives the decision informed by the report of the expert. Henceforth, the dentists have a right to say something about these standards. Since 2005, the parties have right to take opinion from an expert and resort to expert opinion in their defense in criminal procedure. Through a new regulation enacted in 2005, in accordance with the Article 67 and 68 of Turkish Code Of Criminal Procedure (TCCP), the parties can take their own expert opinion and put them into the file (Turkish Code Of Criminal Procedure, Law no:5271; Çetin, 2006). Hence, the defense rights of the person being tried have been extended and empowered in accordance with the equality-of-arms principle by means of expert examination in the file or specialist consideration regarding the report of the expert (Demircan, 2006).

There are factors such as the human factor (lack of knowledge, reasoning errors, and carelessness), environmental factors and medical-device errors which may increase fault risk. These factors may cause medical practice faults individually. However, since medical care is team work, the relevant factors causing fault generally occur together. The number of penal or legal suits opened with the claim of medical practice fault and the indemnity amount paid by the physicians is increasing day by day. The number of complaints increased significantly especially in 1998 and the reason for this is thought to be the enforcement of the Patient Rights Bylaw (Şenocak, 2007).

According to the judicial file archive review report of the Dentistry Faculty of Istanbul University, of the 101 malpractice cases between 1984-2008, 44% of them were faulty dentures, 29% of them were faulty surgical operations, 9% of them were faulty orthodontic treatments, 8% of them were implant treatments, 5% of them were faulty periodontal treatments and 5% of them were faulty teeth treatment. 57% of these malpractice cases were brought to court and the remaining ones were enacted at the level of the police station or prosecution office (Kocaeli and Karaman, 2009). Karaarslan et al. report that of the 14 cases to which a report was issued by the High Council of Health between 2002-2006, six received surgical treatment, four received prosthetic treatments and one received endodontic treatment while three cases were only examined (Karaarslan et al., 2008). As a result of the assessment, medical practice fault was found in nine cases and was not be found in four cases, and in a single case assessment could not be made due to documentary insufficiency in the hospital. According to the study of Göktürk, it is reported that of the 145 cases that came to the Council of Forensic Medicine in order to get an opinion, 40% of them were complaints related to denture treatment and 30% of them were complaints related to tooth, jaw-bone and soft tissue damages (Göktürk, 2014). Following the examination, it is stated that medical practice fault was found in 39% of the cases and not found in 52% of them, while no opinion was given to the 9%. Of the cases in which medical practice fault was found, 36.6% of them had insufficient treatment, 13.2 of them had failure to manage complications and 5.9% of them had false treatment. In 135 cases for which expert opinion was requested about medical practice fault, practitioner dentists were sued in 120 cases (89%), academicians in six cases (4%), specialist dentists in two cases (1%), and persons who are not dentists in seven cases (6%). When we exclude the persons who are not dentists from total of 145 cases and look at the remaining 138 cases which complain about dentistry practice, these complaints were found: complaints about denture treatment in 40 cases (29%), complaints about problems related to tooth, alveolar process, jaw bone, soft tissue damage in 30 cases (22%), complaints about implant treatment in ten cases (7%), complaints about infection in nine cases (6%), complaints about neurological problems in nine cases (6%), complaints about healthy tooth loss claim in nine cases (6%), accusation of causing death in seven cases (5%), complaints about root canal treatment in

five cases (4%), complaints about problems related to local anesthesia administration in five cases (4%), complaints about filling treatment in four cases (3%), complaints about diagnosis and treatment faults other than tooth treatment in four cases (3%), complaints of forgetting a foreign body in three cases (2%), complaints about complaint treatment in two cases (2%), complaint about the development of malign hyperthermia related to general anesthesia (Göktürk, 2014).

1.5. Legal procedures in the suits for damage against dentists due to malpractice

Mainly there are two systems of compensation for damage in malpractice cases. The first of these systems is "fault based (tortious act)." In fault-based systems, the responsibility of the individual or organization is decided after a judgment process and the damage is compensated by means of an insurance system or directly by the individual or organization. Although fault-based compensation systems used commonly across the world and are supported by powerful insurance companies, they are criticized because of the increase in application, constraints in judgment process and the burden they create on the health care budget.The system executed in Turkey for malpractice cases is the one based on fault (Özdemir, 2011).

1.5.1. Period of limitation for opening a case

If a case is to be opened according to the provisions of a tortious act, the period of limitation is one year. If the case will be opened for a violation of agreement, the period of limitation is five years for contracts of work and of mandate. These periods of limitation rise to ten years when the dentist deliberately harms the patient severely. According to TPC, this period begins when the damage arises and a right to open a case is acquired from that time on.

1.5.2. Opening a case against private health care workers

For the compensation of damage by private health care workers, suits for damage can be opened in jurisdiction within the scope of the general principles of Code of Obligations. Other than suit for damages, suits for penalty can also be opened and administrative investigation can begin (Çetin and Yorulmaz, 2006).

1.5.3. Process of a lawsuit for the public services

If the dentist committing malpractice works in the public sector, according to the fifth paragraph of Article 129 of the Constitution, the patient can open a case and lawsuits claiming compensation can only be opened against the administration. Other than contracts of mandate and work, the relationship between the patient and the dentist may result from public service. Here there is a difference. If the service is provided in a public hospital, a case cannot be opened against the dentist directly since he/she is public sector personnel (Demircan, 2006). The law about judging public sector personnel in the Constitution states that "you should open this case against the administration." For example, if a dentist working in a public hospital commits a faulty act, his/her patient can open a moral and material indemnity case but cannot write the name of the dentist. Since public hospitals are bound to the Ministry of Health, the patient should open the case against Ministry of Health. Therefore, the person shall open the case in Administrative Court not in the Civil Court of First Instance. In these kinds of cases, the respective health institution shall definitely inform its employee and ask him/her "will you participate?" These situations mean: "There is a case against myself, come and defend yourself. If I lose the case and pay indemnity, I will retract to you." Since the number of cases has increased recently and the amount of indemnity paid has increased, the administrations retract the indemnity to the public personnel (Demircan, 2006).

1.5.4. Indictments about public sector dentist

There is a regulation protecting public personnel against penal applications. According to this regulation, the state says that "law cannot judge public servants immediately." The prosecutor first applies to the respective administrative board (provincial or county administrative boards) and says "there is an indictment about a certain public servant and I would like to begin an investigation about these personnel." Since the Provincial Administrative Board also consists of public personnel, it makes an investigation about the related public servant; if there is enough evidence to judge these personnel, the prosecutor starts the criminal prosecution of the personnel.

1.5.5. The notice of the case petition and collection of the documents

The dentist is informed about the indemnity case against him through a legal notice made to him. The legal notice is the point that starts the legal process of the case. All legal periods begin as of the notice date. In this phase, the dentist shall check all the information and documents related to the intervention and if there are missing documents and records, he/she should find them immediately and create a file. For the solidity of the defense, other than the data and information that the dentist has about the patient, the data and information in the hospital and in the other physicians shall also be filed.

1.5.6. Notification of the insurance company

When a lawsuit petition requesting indemnity is issued to the dentist, the insurance company shall be notified about the issue within five days. Aside from the indemnity claim against the dentist in the civil court, in case of indictment to the prosecution office or administrative tracking and discipline prosecution, the insurance company shall be notified immediately. At the same time, it is important that a lawyer shall be determined and the preparation of the defense shall begin. The insurance company does not have the liability to send a lawyer for the dentist. The dentist determines his/her own lawyer and follows the case.

1.5.7. Presentation of defense of action and evidence

This issue shall be determined carefully since the procedure is limited in terms of time in civil cases. The dentist shall submit his/her defense and all the evidence within two weeks. If this period is not enough for obtaining the documents, the dentist can request additional time from the court within these two weeks. When both parties submit their defenses and evidence to each other and the court requests the evidence to be collected, from the related institutions, the file becomes ready for the assessment of the expert.

1.5.8. Expert assessment in the cases against public sector dentists

The phase after the collection of the evidence is the phase of expert assessment and it is the most important part of the case (Özdemir, 2011).

Since this topic requires technical knowledge, the consideration of the judge depends on the report of the expert. The parties can object to the expert report by presenting justification. The judge assesses these objections and may send the file back to the expert. The parties can also object to the new report presented to the file. Aside from any objections of the parties, if the judge sees a conflict between the reports, he/she may decide to send the report for the third time to the expert in order to correct the conflict.

The expert institutions resorted to for medical practice faults in Turkey are High Council of Health, Council of Forensic Medicine and Universities (Koç, 2004). It was compulsory for only the penal courts to apply to High Council of Health but the cancellation decision dated 22.10.2010 of Constitution Court eliminated this requirement.

The Council of Forensic Medicine (CFM) provides scientific and technical opinion about topics related to forensic science sent by courts, judgeships and prosecution offices. It provides training on forensic science expertise and sub-branch expertise within the framework of specialty in medicine bylaw, organizes seminars, symposia, conferences and similar events about forensic medicine and forensic sciences. It also applies training programs about these issues, assists related institutions and councils in the preparation and execution of training programs regarding forensic medicine and provides the compulsory health services that are needed during the execution of forensic medicine services (Koc, 2004). CFM is an official institution that is bound to Ministry of Justice and provides official expertise on topics related to forensic medicine at the demand of the courts, judgeships and public prosecution offices. With its decisions, CFM affects Turkey's justice and judicial system extensively. Although it is stated in TCCP that judicial offices can resort to universities, other institutions and individuals as experts other than CFM, in practice, the main institution of expertise is CFM. Since the Courts and the Court of Appeal regards CFM as the supreme expert institution, the reports of CFM become more influential and that makes the CFM General Board the ultimate expertness report body.

1.5.9. Conclusion of the case and the decision

After the collection of all the evidence and receipt of the expert reports, the judge will give the ultimate decision about the case. After the

decision hearing, the judge writes the justification of the decision and sends notice to the parties. The party finding the decision wrong has the right to appeal for correction. Appeal assessment is made by the Court of Appeal. If an indemnity decision against the dentist is given and the claim can be covered by the insurance policy, the payment is made by the insurance company.

1.5.10. Consensus and arbitration about malpractice

The judgment process in malpractice cases is very long and problematic because the crime should be proven, namely, that the normal undesired consequences of treatment shall be distinguished from wrongful neglect (Ünver, 2009). In order to find a solution for this problem, the idea of founding consensus boards and arbitration councils that may reconcile the patient who claims that he/she has been harmed and the related persons or institutions was brought into the agenda and this application entered into force in some countries. Since this application shortens the judgment period and provides savings, it can be claimed that it facilitates access to justice (Çokar, 2009). The patient rights' boards in public hospitals that were brought into the agenda with the application of Patient Rights Bylaw in Turkey have similar functions. However, private health care centers do not have these boards and they generally function as a consultation board not as a consensus board. In other words, their ability to decide without appealing to the courts is very limited.

The most radical regulation about the arbitration board was brought by the 2nd law draft. The draft proposed the foundations of "Supreme Board of Medical Malpractice Tracking and Arbitration" at the level of the Ministry of Health and "Boards of Medical Malpractice Tracking and Arbitration" at the provincial level. These boards consist of administrative personnel and representatives of professional chambers, and the membership of an expert member from the area of law will only be possible in the supreme board.

1.5.11. Discipline investigations about the dentists

Claims of malpractice by the dentist may also be the subject of disciplinary investigation. Depending on the result of the investigation by the chamber of dentists, if the dentist is found to have made a faulty practice,

he/she may get one of the punishments of warning, reprimand, fine or temporary dismissal from the profession.

The Turkish Dental Association issued a directive for eliminating conflicts between dentists and patients and patients' relatives due to occupational practice (Discipline regulation of Turkish Dental Association and Dentists Unions). According to this directive, the complaint application shall be made in a written form to the chamber that the dentist is a member. The appointed expert shall have been practicing dentistry for at least ten years. The expert is to be chosen and appointed from individuals who can provide information for understanding the reason and consequences of a conflict arising from an occupational practice. If the dentist who is subject to the complaint is a member of the management or boards of the chamber of dentistry, the arbitrator is appointed by the Turkish Dental Association Central Execution Board. The parties can object to the appointed expert by justifying their objection. The objection is evaluated by the appointing body and if found valid, a new expert is appointed. When necessary, the expert submits three copies of the report that he/she prepares within three weeks after listening to and/or examining the related parties.

1.6. Record keeping liability in dentistry practices

Between Articles 135-139 in the TPC, a new type of crime has been defined for protecting personal data. It is stated that persons who illegally record the personal data of individuals or publish them will be punished by imprisonment for term between six months and three years (Turkish Penal Code, Law no: 5237). And if the aforementioned crimes are committed by the facilities of occupation or art of perpetrator, the punishment shall be increased by half. Although dentists can record the data of patients legally, if they share this data with third parties without the permission of the owner of the data, they are considered to have committed this crime.

One of the basic components of the transformation program in the health system has been created by the Ministry of Health is National Health Information System (NHIS). NHIS is an electronic record system that contains all the citizens. Every citizen can access their information, and it has a database that covers all the health records of each individuals starting from his/her birth. Therefore, this database creates a central communication

platform for sharing this data and includes technologies of tele-medicine practices. This system is designed to include the records of all institutions and organizations that provide health care such as human resources, assets, estates, administrative and financial data (Ülgü, 2013). However, since it is hard to prevent third parties from stealing the data in the process of saving and sharing information, some ethical problems occur. The hospital always expects confidentiality or it should be assumed that the patient requests that.

Compulsory interventions and the usage of the patients in training introduce very serious private life and private life violation topics. All of the health information and personal information such as addresses and identity information are collected in a pool in the system of the Ministry of Health. This information has critical importance in cases moved into its jurisdiction, in tracking the treatment process and when the patient goes to a different health board. However, the insurance companies especially can see these files and check whether the interventions are made and follow the related payments. In fact, in some private hospitals, the dentists of the insurance company work for the benefit of the insurance company. And this may bring about some ethically unfavorable situations.

Professional secrecy shall be protected according to both international documents and Turkish law. There are many legal regulations about these protections. Occupational organizations, patient rights associations and other non-governmental organizations have made various studies about debated issues.

1.7. The failure of the health professionals to inform about a crime

According to the Article 280 of TPC, when the dentist witnesses some symptoms implying that a crime is committed, he/she is liable for informing authorized bodies without any delay and if he/she fails to do that, he/she may be imprisoned as much as one year (Turkish Penal Code, Law no:5237). Since this regulation creates an issue of trust, it is one of the most complained-about topics among dentists.

2. DETERMINING TRAUMA DAMAGES RELATED TO DENTISTRY (FORENSIC TASK)

Following the change of the TPC in 2005, the forensic medicinal assessment of the damages of persons by the changing punishment concepts, the basic assumptions and many evaluations have been changed. In June 2005, the Ministry of Justice Council of Forensic Medicine, Association of Forensic Medicine Experts and Association of Forensic Medicine prepared a guideline for forensic reports to be prepared within the framework of the new TPC (Balcı et al., 2004). Then on 22.09.2005, Ministry of Health issued a circular letter called "The Principles to be used for the execution of forensic medicine services" within the scope of this guideline (Notice regarding the rules which are to be followed during the application of forensic medicine services 2005). In this guideline, the teeth are classified in three groups: incisors, canine and molars. Considering chewing and speaking functions, different scoring is made for different teeth types. If the total scoring of tooth loss is between 15 and 30, it is considered to be *constant weakness of function* and if it is over 30, it is accepted to be *loss of function.* In scoring, the canine gets 4.5; incisor 4; premolar 3; molar 3 and the third molar 0.5. Let us consider this as an example:

Let us assume that a person has lost their canine and two premolars in the left bottom jaw bone and two incisors and the first premolar in the left top jaw bone due to teeth trauma:

- Canine loss degree: 4.5
- 1. Premolar loss degree: 3
- 2. Premolar loss degree: 3
- Lateral incisor loss degree: 4
- Central incisor loss degree: 4
- 1. Premolar loss degree: 3

$$4.5+3+3+4+4+3=21.5$$

In this case, since the total scoring of teeth loss is between 15-30; it is considered to be *constant weakness of function*.

REFERENCES

Balcı, Y.G., Çetin G, Güzel S (eds) (2004). *Yeni Türk Ceza Kanunu'nda tanımlanan yaralama suçlarının adli tıp açısından değerlendirilmesi ve adli rapor tanzimi için rehber [Guide for the evaluation of injuring offenses according to the new Turkish Penal Code and for the report setting]*. http://atud.org.tr/kutuphane/Rehber.pdf (Retrieved: 14.02.2021).

Çetin, G. and Yorulmaz, C. (2006). Yeni yasalar çerçevesinde hekimlerin hukuki ve cezai sorumluluğu [Legal and penal responsibilities of doctors in the light of new laws]. *Medical malpractice and the arrangement of forensic reports symposium series*, 48,31-42.

Çokar, M. (2009) Türkiye'de malpraktis (yanlış tedavi) konusunda yasal düzenleme girişimleri: zorunlu mali sorumluluk sigortası bir çözüm müdür? [Regulation attempts on malpractice in Turkey: Is the obligatory financial responsibility insurance a solution?]. http://www.turkhukuksitesi.com/makale 1123.htm (Retrieved: 12.03.2021)

Demircan, T.Y. (2006). *Diş hekimlerinin tıbbi müdahalelerden doğan hukukî sorumlulukları [Dentists' legal responsibilities born out of medical treatments]*. Dissertation, İstanbul.

Erol, G.H. (2017). *Diş hekimlerinin hukuki sorumluluğu ve hasta hakları*. 1. Edition, Seçkin Publishing, Ankara. pp 328.

Göktürk, C. (2014). *Adli Tıp Kurumunda diş hekimliği alanında tıbbi malpraktis iddiası bulunan olguların değerlendirilmesi [Evaluation of malpractice claims in the field of dentistry in the Council of Forensic Medicine]*. Dissertation, İstanbul.

Hakeri, H. (2007). *Tıp hukuku. [Medical jurisprudance].* Seçkin Publishing, Ankara. pp.192, 294.

Karaarslan, B., Karaarslan, E.Ş., Çelik, S. et al. (2008). 2001-2007 yılları arasında Yüksek Sağlık Şurasında görüşülen diş hekimliğinde malpraktis olgularının değerlendirilmesi [Evaluation of malpractice cases in dentistry that were discussed in the High Council of Health between the years of 2001-2007]. *13rd meeting of dental illnesses and treatment*, Sivas.

Kocaeli, H., Karaman, F. (2009). İstanbul Üniversitesi Diş Hekimliği Fakültesi adli dosya inceleme raporu [Forensic file analysis report of Istanbul University Faculty of Dentistry] *İ.Ü Diş Hekimliği Fakültesi Dergisi* 43(3-4),75-80.

Koç, S. (2004). Tıbbi bilirkişilik ve Adli Tıp Kurumu [Medical Expertise and the Council of Forensic Medicine] *Türkiye Barolar Birliği Dergisi* 50,99-104.

Mosenheuer, A. (2008). Hekimlerin cezalandırılabilirliğinin sınırı olarak varsayımsal rıza [Conjectural consent as the limit of punishability of the medical personals]. *5th Turkish-German Medicine Law Symposium "Current Problems of Medical Penal Code".* Turkish Bar Association Publishing, Ankara, pp 76.

Özaslan, A. and Kolusayın, Ö. (2011). Hekimin yasal sorumlulukları [Legal responsibilities of doctors] *Forensic medicine course book*. Cerrahpaşa Faculty of Medicine Publishing, İstanbul, pp 13-40.

Özdemir, H. (2011). Diş hekimlerinin hukuki sorumluluğu [Legal responsibility of dentists]. *EÜHFD* 1-2,177-229.

Öztürkler, C. (2003). *Hukuk uygulamasında tıbbi sorumluluk, teşhis tedavi ve tıbbi müdahaleden doğan tazminat davaları [Medical responsibility in legal action, diagnostic, treatment, and action for damage upon medical treatments]* Seçkin Publishing, 1 st. edition, Ankara, pp 276, 277.

Savaş, H. (2012). *Tıbbi malpraktis ve komplikasyondan doğan sorumluluklar "sağlık hukuku makaleleri" [Responsibilities born out of medical malpractices and complications "Medical law essays"].* Istanbul Bar Association Publishing, İstanbul, pp 259-294

Şenocak, Z. (1998). *Özel hukukta hekimin sorumluluğu [Responsibility of doctors in private law]* Ankara University Faculty of Law Publishing, Ankara, pp 37.

Şenocak, Z. (2007). Hekimin hukuki sorumluluğunun özel sorunları:Tıbbi Standartlar ve İspat [Specific problems of doctors' legal responsibilities: Medical standards and proof]. *Medical Law Congress Manual*, Ankara, pp 241-254.

Turkish Code of Criminal Procedure, Law no.: 5271. Accepted date: 04.12. 2004. Official Gazette: 17.12.2004; (25673) (in Turkish).

Turkish Penal Code. Law no.: 5237. Accepted date: 12.10. 2004. Official Gazette: 1.6.2005; (25611) (in Turkish).

Ülgü, M. (2013). Sağlık Bakanlığında kişisel verilerin toplanması, saklanması,işlenmesi ve paylaşılması neden ve nasıl yapılıyor [The procedure of gathering, keeping, analysing and sharing of private datum in the Ministry of Health] In:Türk Diş Hekimleri Birliği, sağlıkta sır saklama ve veri paylaşımı [Turkish Dental Association, keeping secrets and data sharing in medical field]. *18th education series seminer*, Ankara, pp 35-90.

Ünver, Y. (2009). Doktorların malpraktis nedeniyle ceza hukuku sorumluluğu ve malpraktis-komplikasyon ayrımı [Penal code responsibility of doctors due to malpractice, and the difference between malpractice and complication]. *Legal Responsibility born out of medical treatment symposium book*, Mersin Bar Association Publishing, Mersin, pp 167.

Yavuz, F. (2007). Malpraktis komplikasyon ayrımı [Malpractice and Complication Difference] *In: A'dan Z'ye Sağlık Hukuku Sempozyum Notları, [Notes from A to Z Medical Law Symposium]* Istanbul Bar Association Publishing, İstanbul.

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