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GECE AKADEMİ

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İÇİNDEKİLER

CHAPTER 1

CANCER METASTASIS IN THE ORAL CAVITY

Aydin KESKİNRUZGAR[1]

1. INTRODUCTION

Cancer is a complex disease in which cellular functions such as cell division, apoptosis, and cell migration are impaired. Cancer usually progressesthrough four different phases and is a malignant disease process where the prognosis worsens with the completion of each stage. The cancer progression begins with uncontrolled cell proliferation and, if not diagnosed and treated at an early stage, continues with the spread of cancer cells to distant tissues. The spread of cancer cells to other organs is called metastasis.The metastasis is a complex process where the regulation of cell functions, cell proliferation, and the immune system collapses. Uncontrolled metastases in cancer cases result in morbidity and ultimately mortality. (1)

2. CANCER METASTASIS

The pace of metastasis depends on the increase of the formation of blood vessels (angiogenesis) in the affected tissue. In cancer patients, an increase in the microvessel density accompanies the presence of an inflammatory response, and as a result, cancer cells spread by blood circulation more easily.(2) In general, increased vascularization in the tumor area and elevated release of proangiogenic factorsfrom cancer cells are associated with advanced tumor progression and poor prognosis of cancers. (3)

The rate at which malignant cells migrate into the bloodstream and metastasize increases with tumor size. Cancer cells can often remain inactive for months or even years without recurrence. (1) Following surgery or other treatments, passive cancer cells may cause *local recurrence* or distant metastases due to increased angiogenesis. (4)

Normally, metastasis of cancer cells is a rather difficult process, and only a small number of metastatic cells can successfully settle in remote tissues. (5) When cancer cells enter the bloodstream, they encounter several obstacles before they can localize in other organs. Metastasizingcancer cells in the blood circulation are often destroyed by mechanical stress or are killedby the immune cells. However, cancer cells that cannot be destroyed in the circulation can eventually metastasize if they settle on the capillaries and proliferate in distant tissues. (6)

Routes of metastasis include Batson's plexus and arterial, venous and lymphatic circulation. In addition, there are organ-specific routes that take place in

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only certain tissues. For example, metastasis routes of lung cancers include direct spreading by aspiration orindirect spreadingthrough a drain on the left side of the heart via pulmonary vein. Another example is the stomach cancer, where the movement of the stomach contents is also considered as spread routes. (7)

It is suggested that in various cancers, metastases to distant organs do not occur at random, but they are specific to the region. (8) This process was first described by Paget in the "seed and soil" hypothesis. The metastatic cells are compared to the seedswhile the organ microenvironment was compared to the soil. (9)Although some cancer types can metastasize into almost every organ, some of them usually metastasize to specific organs. For example, while prostate cancer usually metastasizes to the bones, stomach cancer usually metastasizes to the ovaries in women. Nevertheless, in most cases, the primary organs that are affected by metastases are bone, brain, liver, and the lung.

3. METASTASIS IN THE ORAL CAVITY

Metastasis is a complex biological process that begins with the departure of the cancer cells from the primary tumor site, continues with their spreading through the lymphovascular structures,and requires thesurvivalof these cancer cells after spreading to distant tissues. In this process, the cancer cells have to overcome a series of obstacles. Cancer cells should first leave the primary area, migrate inside the original tissueand spreadthrough the blood circulation to settle in remote tissues. These metastatic structures should then settle in the microvasculature and extravasateto the vessel walls. Finally, they proliferate in the target tissue. (10) When metastasis is diagnosed, it indicates eitherthe presence of a tumor of unknown origin at a distant site or a previously diagnosed tumorthat began to spread. (11) Thus, the detection of this condition is of great clinical importance.

The oral cavity is not considered as a very common area for the metastasis of cancer cells.Oral cavity metastases can affect the quality of life negatively by causing pain, dysphagia, difficulty in chewing, facial asymmetry and bleeding. (12)Although metastasis to the oral cavity is usually interpreted as a marker of a widely spread cancer, in some cases, cancer cells may initially metastasizeto the oral cavity.Cancer cases that metastasize to the oral cavity constitute only 1-3% of all neoplasms (13)It has been reported that oral metastases are generally seen in individuals aged 40-70 years and men are more affected than women. (1)

Lesions that metastasize to the oral cavity vary clinically depending on the specific region and the characteristics of these lesions can give clues about the disease. Maxillofacial bones and/or soft tissues are affected in the metastasis to the oral cavity; however, bones are affected more than the soft tissues.Metastases have been reported to affect the mandibular bone more than the maxillary bone. Especially the posterior region of the mandible has been shown to be more affected by metastasis. (1)

Clinical manifestations of the oral cavity metastasis may include pain, swelling, paraesthesia, and limitation of mouth motion due to trismus.Neuropathic conditions such as numbness in the region indicate that the metastasis is affect-

ing the tissues surrounding the respective nerve.In this case, the mental nerve in the lower jaw or the inferior alveolar nerve are usually affected.(12)Trismus is considered to be an important discriminating factor in temporomandibular joint metastases. (14)

If cancer cells produce osteoblast-inducing factors (Bone Morphogenetic protein, endothelin-1, etc.), an excessive bone formation may occur; however, if they release osteoclast-inducing factors (IL-8, IL-11, etc.),osteolytic lesions occur.(15) Metastases that occur in the jawbone usually presentosteomyelitis-like multiple destruction sites or bone destructions.(13) In addition, osteolytic lesions are mostly seen in bone metastases of the lung, kidney and breast cancers, whereas osteoblastic lesions are often observed in bone metastasis of prostate cancers. Taking these conditions into consideration, the diagnosis of metastatic lesions in the oral cavity is very difficult and complicated.For example, osteolytic lesions in the jawbone can also occur in systemic conditions such as in bone infections, giant cell granulomas, primary malignant or benign tumors,multiple myeloma, histiocytosis x and fibro-osseous lesions. (13)

Several criteria have been proposed for the metastases to the jawbone, which are the radiological and histological diagnoses ofprimary cancer and the similarity of the histopathological features of the primary site and the cells in the metastatic region. (16)

One-thirds of oral metastases occur in soft tissues. (17) It is observed that the most commonly affected region of the metastasis in oral soft tissues is the adherent gingiva, followed by the submucosa areas on the tongue and in the mouth. (17) Clinically, gingival metastases present as vascularized areas that are polypoid or exophytic. For these reasons, the clinical presentation of gingival metastases resembles hyperplastic lesions such as pyogenic granuloma, giant cell granuloma, and peripheral fibroma. (18,19) Because oral metastatic lesions are both uncommon and resemble many lesions, diagnosis and detection of their primary origin are very difficult in terms of both clinical and pathological aspects.Thus, biopsies should be performed for all lesions in the oral area, and the patients with cancer history should especially be examined in more detail. (1)

The main primary cancers that cause metastasis to the oral region differ according to gender.Oral metastases frequently involve lung, kidney, liver and prostate cancers in males; while breast, genital organs, kidney, and colon-rectum cancers are usually responsible in females. (1)

It was observed that the specific regions metastasized in the oral area differ according to the location of the primary cancer area.In male patients, metastatic lesions were frequently seen in the jawbone (21%) and oral mucosa (31.3%) in the lung cancer, while the jawbone (11%) was mostly affected in the prostate cancer and the soft tissue was frequently affected in the kidney cancer (14%). In female patients,metastatic lesions were frequently seen in jaw bones (41%) and oral mucosa (24.3%) in breast cancer cases, whereas jaw boneswere usually affected in the cancers of the adrenal glands(7.7%), and soft tissues (14.8%)were frequently affected by cancers of the genital organs.In addition, the metastatic

targets of the adrenal, thyroid, and eye cancers have always been the jaw bones. (1)The primary cancer that metastasizes most frequently in soft tissues of the oral cavity is the lung cancer whereas breast cancer is the main cancer type that metastasizes to the jaw bones. (20) While lung is the most common primary organ in metastases, soft tissue metastases in the oral cavity are mostly located in the gingiva and less in the tongue and the tonsils. The majority of liver and breast cancers metastasize to the gingiva in the oral cavity (72.2% and 60.6%), whereas kidney cancers were reported to metastasize mostly to the tongue.(7) Most of the gingiva metastases were reported to be observed in the posterior region and mainly in toothed areas. The rate of metastases in toothless patients is very low. (7)

Histological examinationsrevealedthat most of the soft tissue metastases in the oral cavity areadenocarcinoma. These are followed by renal carcinoma, hepatocellular carcinoma, and less frequently malignant melanoma and squamous cell carcinoma. Although very rarely, the soft tissue metastases were also found to be associated with osteosarcoma, chondrosarcoma and Ewing sarcoma. (7)

Jaw-bone metastases are usually seen in the 5th decade of life, while oral soft-tissue metastases are observed in the 4th decade. (19) In younger patients,metastasis is more common in the jawbone than in soft tissues.Although gender distribution in jaw-bone metastases is almost 1:1, male to female ratio in oral soft tissue metastases was 2:1. (19)

The pathogenesis of the metastases in the jawbone is not understood, but it was suggested to be caused by the red bone marrow in the mandible. The diagnosis of metastatic cancers in the jawbone is difficult because lesions are formed in the center of the bone and there are very few clearindicators in the early period in addition to presenting no specific image on radiography. (2,13)

Although oral cavity metastases are more common in the jawbone than soft tissues, soft tissue metastases in the oral cavity can be more easily diagnosed than jawbone metastases.In the oral cavity, gingiva is the most common site for metastasis, and gingivitis or periodontitis may be critical factors that aid in the metastasis process. (21) In fact, it has been suggested that the observation of high metastatic frequency in the adherent gingiva might be due to these factors. (21)

In addition tothe gingiva, the tongue is also a vascularized organ, and the high frequency of metastatic cancersin the tongue is due to this enhanced vascularization. In general, inflammation and angiogenesis are known to play a role in the tissues where cancer metastases are present. (7)Tongue tissue has been reported to be a site where renal cell carcinoma metastasizes frequently. (22) It has also been shown that the second primary cancer type in tongue metastases is lung cancer. (7) When tongue metastasis occurs, there may be more extensive metastases of cancer to the other areas of the body through the lymph nodes adjacent to the tongue. (7)On the other hand, malignant melanomas and bronchogenic carcinomas have been identified as the most prevalent primary cancers that metastasizeto the tonsils. (23,24,25)

Metastasis pathways are mainly lymphatics and blood vessels, but they may also be caused by secretions or through surgical intervention. (26,27)General understanding is that the jaws do not have any lymphatic structures and therefore the only way to metastasize to the jaw bones is through the blood vessels. (28,29)However, the lymphatic structure may be responsible for the oral cavity metastases that are not in thejaw bones. The reason for the relatively low metastasis to the jaw bone in the elderly is the fact that the red bone marrow and blood vessels in the jawbone in elderly are significantly less compared to the young. (28,30) However, since the blood flow in the maxilla is greater than the mandible, the reason for the more frequent occurrence of mandibular metastases cannot be explained by this hypothesis. (31)

In some cases, metastasis occurs after a tooth extraction. It wasreported that metastatic cells move towards the post-extractioncavity and the wound regions around this cavity after tooth extraction. In addition,the growth factor release may also play a role in the formation of metastasis after tooth extraction. (17)The primary cancers of these patients may not have been diagnoseduntil that point. After the tooth extraction procedures, soft tissue lesions appear and / or aches start in the tooth extraction cavity. In the majority of these cases, metastatic cancer may cause pain, swelling and mobility of the teeth before the tooth extraction. Since the tooth is usually extractedfor these reasons,the metastatic lesions may reach the oral cavity afterwards. Because of these, tooth extraction can serve as a supporting factor in the metastatic process. (32,33)(Figure 1)

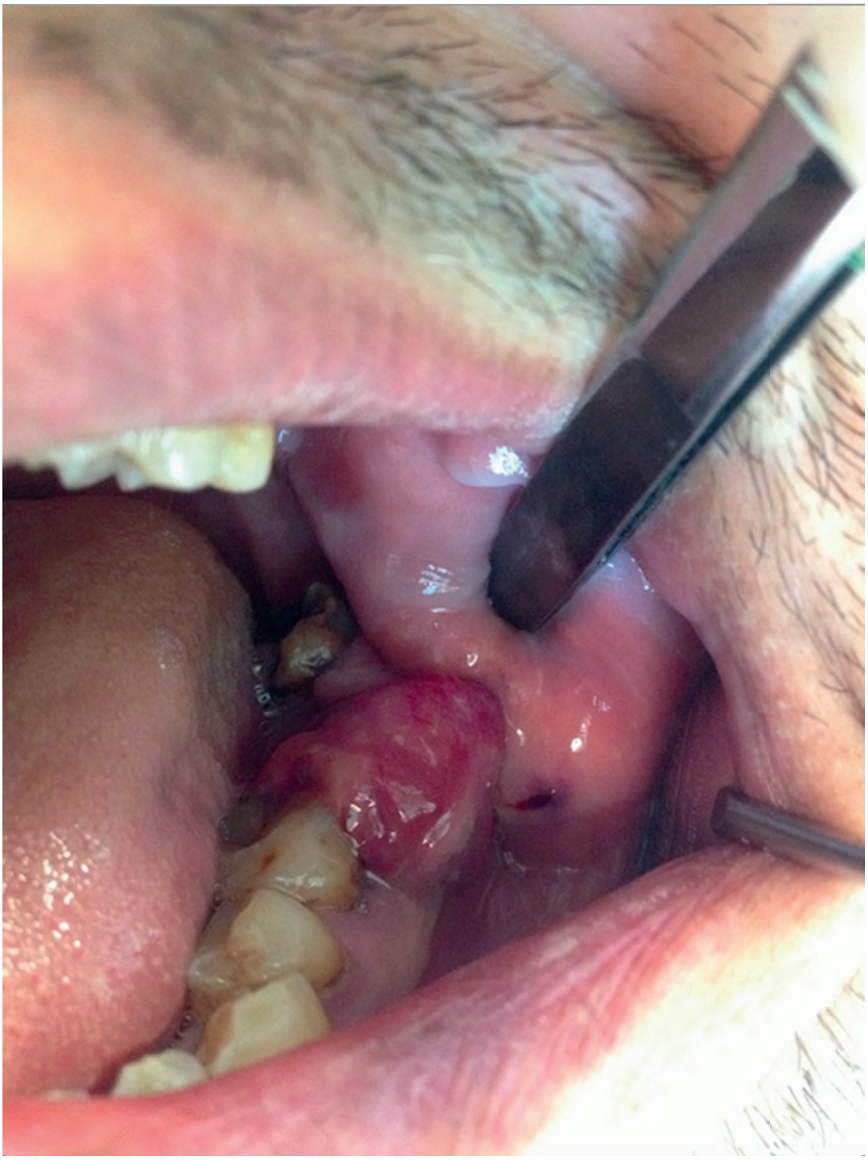

Figure 1.An oral lesion with erythema and swelling of the left mandible. This lesion developed after tooth extraction. According to the biopsy result, metastasis of renal collecting duct cancer was diagnosed. (34)

In some cases, metastatic lesions can be misdiagnosed because they appear like pulpal, periapical-induced dental pain. (35) It has been reported that some breast cancer metastases mimic mandibular periodontal abscess. (36) The incidence of metastases to the jaw bones is considered to be more frequent than that reported so far since they are difficult to detect, and some jaw bone metastases have only been identified in autopsies. (37)

If the organ with primary cancer is treatable and the medical condition of the patient is appropriate, the metastatic lesions should be treated radically. Basic cancer treatment protocols such assurgical resection, chemotherapy and/or radiotherapy can be applied. If the primary cancer cannot be controlled, then metastatic oral lesions should be treated conservatively. The reason for this is to try to improve the quality of life of the patient and to maintain basic oral functions. Therefore, radiotherapy, chemotherapy and local surgical excisions can be applied in conservative treatment options. (38)

Care should be taken to distinguish the primary intraoral cancer from oral cavity metastases. Salivary gland tumors show histological features that are similar to the metastatic lesions in the oral cavity.Thus, in the oral cavity,primary ductal carcinoma of the salivary gland may be confused with metastatic breast carcinoma. Furthermore, intraoral clear cell carcinoma of the salivary gland may be confused with metastatic renal cell carcinoma. (39) For these reasons, in case of cancer-like lesions that occur in the oral cavity,the lesion should be analyzed in detail in order to understand whether the lesion is a metastatic lesion or not, otherwise the prognosis of primary cancer in the remote side would be negatively affected when metastases are diagnosed as oral cancer.

It has been reported that the interval between the diagnosis of primary cancer and the detection of oral metastases may range between 40 months and 10 years. In most cases, the average survival time was 7 months after diagnosis of oral metastasis. Most of the patients lost their lives within 1 year and the survival rate was as low as 10% within 4 years following metastasis. Therefore, the main objective of the treatment is to increase the quality of life of the patient, and even in advanced cases, treatments such as surgical resection, radiotherapy and chemotherapy can be applied for this purpose.(40)

4. CONCLUSION

Oral metastases are rare, so in order to determine whether these lesions are primary or metastatic lesions, the patient should be evaluated through a multidisciplinary approach. Oral cavity lesions should be approached with caution, each lesion should be evaluated individually, and biopsy results should be examined in detail.

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CHAPTER 2

A RELIABLE MINIMALLY INVASIVE SURGICAL TECHNIQUE FOR THE TREATMENT OF LUMBAR SPINAL STENOSIS "UNILATERAL HEMILAMINECTOMY AND BILATERAL FLAVECTOMY."

Mustafa KARADEMİR[1]

Lumbar spinal stenosis (LSS) is a familiar source of leg and back pain, and it is typically caused by degenerative facet-joint arthrosis, ligamentum flavum hypertrophy, and broad-based disc bulging, leading to compression of the spinal canal and lateral recess, resulting in walking disability. It refers to a narrowing in the vertebra, in the areas of the central canal, lateral recess or the neural foramen. When lateral recess and neural foramen are stenosed, symptoms of lumbar radiculopathy may also be demonstrated. These changes lead to pain in the legs and back, as well as impaired ambulation and other disabilities. Patients may present with neurogenic claudication, which can be exacerbated by standing and relieved by flexion at the waist, such as when in the seated position. Patients may also experience tingling, numbness, and weakness of the lower extremities (Phan K. et al., 2017, Moisi M. et al., 2016).

Degenerative spondylosis is a significant etiology of lumbar spinal stenosis. With aging, wear-and-tear changes and traumas, amongst other factors, the intervertebral discs can degenerate and protrude posteriorly, causing increased loading of the posterior elements of the vertebrae. This can lead to posterior vertebral osteophyte formation, facet hypertrophy, synovial facet cysts, and ligamentum flavum hypertrophy, which in turn will cause spinal stenosis. Degenerative spondylolisthesis is another cause of lumbar spinal stenosis. When degenerative changes of the spine occur, the pars interarticularis can be fractured, and the resulting instability can lead to forwarding translation of the vertebra. Sufficient anterior slippage of one vertebra on top of the next vertebral segment can narrow the spinal canal, leading to stenosis. Other acquired conditions, although rarer than the conditions as mentioned above, should also be considered by the clinician. These include space-occupying lesions, post-surgical fibrosis, and rheumatologic conditions as well as other skeletal diseases such as ankylosing spondylitis or diffuse idiopathic skeletal hyperostosis. Even more rarely, lumbar spinal stenosis may be secondary to congenital causes such as achondroplasia, which can lead to short pedicles with medially placed facets(Wu L.& Cruz R., 2018).

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LSS is a progressive degenerative condition most common in patients over the age of 65 years and leads to progressive disability. However, there is no singleobjective standard for identifying LSS and diagnosis relieson complex judgments that integrate symptoms, signs,imaging findings, and comorbid conditions (Lurie J & Lane CT, 2016).

There is no objective standard for the clinical diagnosis ofLSS. In the absence of valid objective criteria, it has been suggested that expert opinion is considered the "goldstandard" in diagnosis. The clinical syndrome of LSS is generally diagnosed using a combination of clinical signs from the history, physical examination, and imaging.The most useful findings from the history are age, radiating leg pain that is exacerbated by standing up or walking, the absence of pain when seated, the improvement of symptoms when bending forward, and a wide-based gait. Balance impairment, neuromuscular deficits in the lower extremities including decreased strength, sensory deficits, and absent or decreased Achilles tendon and patellar reflexes are also highly associated with LSS. Although neurogenic claudicationis the cardinal symptom of LSS, it is usually seen importantly (Lurie J & Lane CT, 2016).

As mentioned previously, there is no consensus on the definition of lumbar spinal stenosis diagnostic criteria. However, in evaluating low back pain, neuroimaging is indicated when there are new-onset symptoms and suspicion of lumbosacral radiculopathy or spinal stenosis. In patients without suspicion of infection, metastasis or postoperative spine symptoms, non-contrast Magnetic resonance imaging (MRI) of the lumbosacral spine is the modality of choice for lumbar spinal stenosis, and computerized tomography (CT) myelography can be utilized when MRI is contraindicated. Many authors use an intraspinal canal area of less than $76mm^2$ and $100mm^2$ for severe and moderate stenosis, respectively. Anteroposterior spinal canal diameters of less than 10mm are also frequently used for the diagnosis of lumbar spinal stenosis MRI or CT myelography with axial loading may be a useful adjunct to routine imaging. Electromyography and nerve conduction studies (EMG) are also used to aid the diagnosis of diagnosis, as this distinguishes polyneuropathy, radiculopathy or other peripheral nerve disorders from the lumbar spinal stenosis. EMG exams are often standard in patients with lumbar spinal stenosis (Wu Lite & Cruz Ricardo, 2018).

LSS can significantly impact the quality of life and daily activities. Therefore, it is considerable for clinicians to recognize and treat lumbar spinal stenosis effectively. For the natural history of spinal stenosis, most patients do not improve with conservative treatment alone. Non-surgical management is the first-line therapy for most patients with LSS and may include physiotherapy, hydrotherapy, painmedication, and epidural injections of corticosteroids oranesthetics. Surgery is indicated in the acute setting for patients with rapidly progressive neurological impairment or sphincteric dysfunction. Surgical decompression is also a viable treatment option for patients with a chronic disability who fail to respond to conservative management (Phan K et al., 2017).

Classically, lumbar spinal stenosis was treated with extensive removal of posterior elements, including the lamina, spinousprocess, interspinous ligaments, and facet joints. However, this lengthy procedure will destabilize the lumbar spine and maycause secondary spondylolisthesis; hence, fusion with or without instrumentation was recommended in most cases. Because of this, several less invasive procedures, multiple laminotomies, unilateral laminotomy for bilateral decompression and, microendoscopic decompressive laminotomy, have been developed. The primary challenge is to have adequate decompression while preserving spinal stability. Knowing the common cause of lumbar spine stenosis, one can use less extensive lamina removal toprovide adequate decompression for spinal stenosis. The standard surgical procedure is still an open laminectomy with or without instrumentation, which involves deep muscle retraction and extensive removal of the posterior spinal structures. This can lead to instability and the need for additional spinal fusion (Wong Chung-Ting M. et al., 2011)

Another systemized and detailed reliable approach for bilateral decompression of narrow spinal channelis " Unilateral hemilaminectomy and bilateral flavectomy," which believed is less harmfully and useful to the standard open laminectomy regarding intraoperative visualization, postoperative stability, and degree of invasiveness. Unilateral approach for bilateral decompression of the spine in the treatment of lumbar spinal stenosis is a minimally invasive surgical method achieving satisfactory results. Low complication rate and hospitalization time, minimal blood loss and not to cause instability are advantages of this method. It appears as an advantageous and priority method in the selection of surgical treatment modalities of lumbar spinal stenosis patients in many clinical series (Kaya A, 2009).

Microscopic unilateral hemilaminectomy and bilateral flavectomy decompression:

Increasing knowledge of pathoanatomy, coupled with thedevelopment of magnetic resonance imaging, has allowed amore precise delineation of soft tissue and bony stenotic lesions. Unilateral approach preserves the facetjoints, and neural arch of the contralateral side limits postoperative destabilization and protects the nervousstructure against posterior scarring. Initially describedby Young in 1988 (Young S et al., 1988) and subsequently modified by McCulloch (McCulloch JA, 1991), a microscopic technique characterized by unilateral multifidus retraction, ipsilateral microdecompression, and contralateral microdecompression performed under the midline posterior structures. This surgical method is, and safety and currently performed from many spine surgeons widely (Çavuşoğlu H et al., 2007).

Surgical technique:

A C-arm scope checks the target level preoperatively. The incision is midline and extends over when necessary, but it is limited to the underlying re-

gion of the stenotic segment as observed on magnetic resonance imaging (MRI). A 2 to 6 cm skin incision is made for 2–4 level stenosis. Then after, a linear median fascial incision is made on the most symptomatic side of the patient. The paraspinal muscles are separated from their bony attachments on the spinous process and laminato expose the bony detail. A modified mini Taylor retractoris then used. By this way, a clear view of the ipsilateral interlaminar space is enabled to be visualized by the microscopic attachment. Ipsilateral cephalad and then caudal hemilamina is entirely resected by using a high-speed drill. The microscope is then angulated into the ipsilateral subarticular zone and, moving cephalad to caudal, soft tissue, and bony stenotic pathology is excised usinga high-speed drill and "Kerrison Rongeurs" (Fig 1). This is done sequentially until the nerve root at the operative level isseen exiting freely into the foramen. Lateral decompression was made via undermining of the hypertrophic facet joint, which was a bony stenotic pathology. The medial partthe facet joint is partially resected to decompress the lateral recess. Maximally one-third of hypertrophic facet joint was resected. A hemifacetectomy is almost never performed. Therefore, maximal preservation of the pars interarticularis and facet joint were made. If necessary, disk material is removed. Then ipsilateral ligamentum flavum is wholly resected. After complete ipsilateral microdecompression, the contralateral side is addressed. The microscope is angulated medially and, the patient tilted contralaterally, to afford visualization across the midline beneath the lowest portion of the interspinous ligament. Resection of portions or all of the interspinous ligaments and supraspinous ligaments isnot performed. The interspinous ligament is retracted mediallyusing with root retractor. A dissector is used to verify that the anterior surface of the ligamentum flavum is free from adhesion to the dura, and the medial portion of contralateral ligamentum flavum is then resected sequentially from cephalad to caudal with curved curettes and Kerrison rongeurs. The following part of the operation can not be performed without high-speed burr. A vital point in the process, to allow access for contralateral decompression, isthe adequate resection of the ''wishbone'' portion of the cephalad and caudal lamina, i.e., the junction of the lamina with the spinous process. Thus, anteroposterior diameterof the spinal canal is expanded to afford visualization across the midline beneath the lowest portion of the spinous process. Then, a dissector is used to confirm that the contralateral surface of the dura is free from adhesion to the ligamentum flavum, and the contralateral portion of ligamentum then is resected sequentially from cephalad to caudal with curved curettes and Kerrison rongeurs. The microscope then is angulated into the contralateral subarticular zone and, moving cephalad to caudal, soft tissue, andbony stenotic pathology is excised using high-speed drill and Kerrison rongeurs. This is done sequentially until nerve root at the operative level is seen exiting freely into the foramen. If necessary, contralateral disk material can be removed. Both the ipsilateral and contralateral nerve roots are well envisioned after the bilateral decompression(Fig 2). Then the same procedure is repeated for each level. When decompression is verified with directin spection under the surgical microscope, the operation is complete. All affected levels can be successfully decompressed through this unilateral approach.

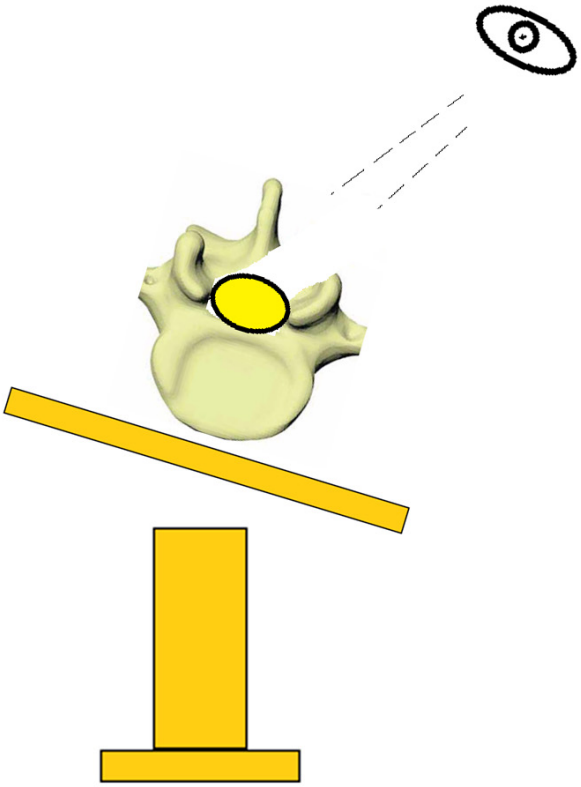

Figure 1: After ipsilateral decompression, the base of the spinous process was undercut by medial angulation of the operative microscope, the contralateral hemilaminae together with the hypertrophied medial facet were partially removed after bilateral flavectomy, and the lateral recess and neural foramina were decompressed contralaterally.

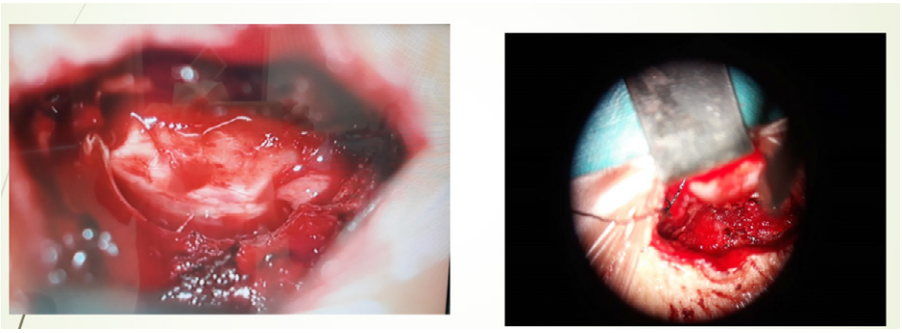

Figure 2: Preoperative microscopy images both the ipsilateral and contralateral nerve roots are well visualized after unilateral hemilaminectomy and bilateral flavectomy decompression.

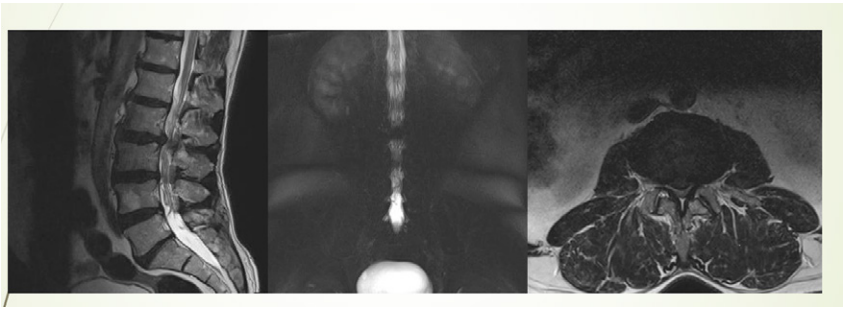

Figure 3: Preoperative MRI lumbar spinal stenosis

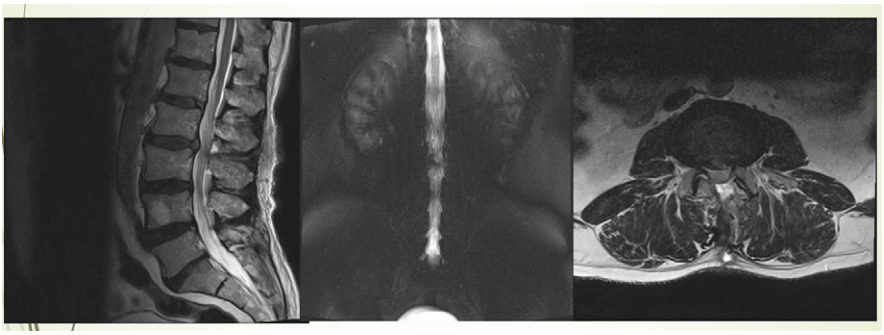

Figure 4: Postoperative MRI lumbar spinal stenosis

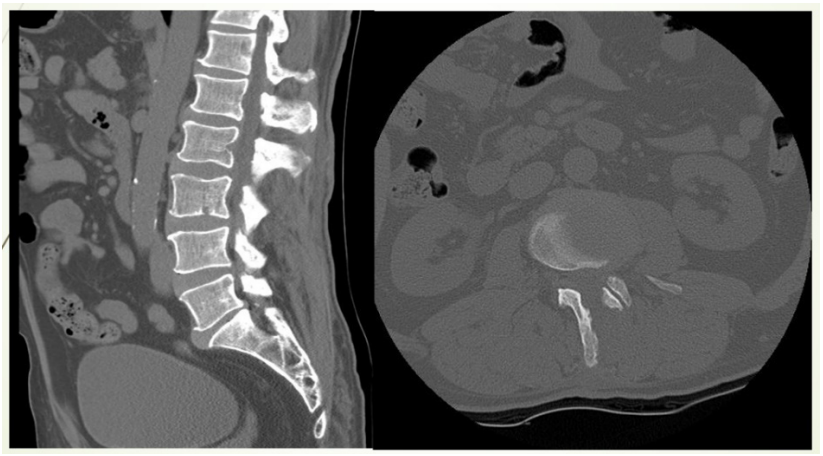

Figure 5: Postoperative CT lumbar spinal stenosis

Advantages:

- Short operation duration,
- Minimum tissue loss, and minimally blood loss,
- Having one-sided lumbar muscle column intact,
- Formation of effective spinal channel radius without using instrumentation,
- Having "interspinous ligaments and lamina" intact,
- Acceptable morbidity,
- Lower costs and short-term hospitalization
- Early mobilization of the patient (Kotil K et al., 2006)

Disadvantages:

- Incidental durotomy was the most common complication
- Progressive restenosis or residual stenosis and inadequate neural decompression at operated levels (Oertel MF et al, 2006)

Following the description of the unilateral hemilaminectomy and bilateral flavectomy technique, the authors reported good clinical results in their series in the long-term results of the microsurgical treatment.The goal of the unilateral approach to treating lumbar spinal stenosis is to achieve sufficient decompression of the neural elements. An extra benefit of a minimally invasive approach may be the potential to decrease a patient's postoperative pain

and disability as well as to decrease the length of hospital stay and thereby the treatment costs. In experienced hands, unilateral hemilaminectomy and bilateral flavectomy is an adequate microsurgical technique for decompression of lumbar spinal stenosis, that minimizes operative invasiveness and tissue trauma while maximizing preservationof the spinal integrity and stability. Secondary postoperative instability is avoided, and the excellent long-term clinical outcome could be expected.

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CHAPTER 3

EOSINOPHIL BIOLOGY, CAUSES OF EOSINOPHILIA AND EOSINOPHILIC LUNG DISEASES

Ceyda TUNAKAN DALGIÇ

Eosinophils are responsible for response to infections, tissue remodeling, and tumor surveillance. They develop and differentiate in the bone marrow by interleukin (IL)-5, IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF).In peripheral blood, an absolute eosinophil count of 0 to 500 cells/microL (<0.5 x 109/L) is normal.

Mechanisms of eosinophilia

Polyclonal expansion: It results by the overproduction of IL-5. This is called as reactive or secondary eosinophilia. IL-5, T helper cell type 2 (Th2) lymphocytes and group 2 innate lymphoid cells (ILC-2) are taking roles for this typeof expansion.

Clonal expansion: Clonal eosinophilia arises as a result of a hematopoietic stem cell proliferation.

Tissue damage: It occurs when the absolute eosinophil count exceeds 1500/microL but also, can occur with lower blood eosinophil levels.

Target organs: Skin, airways, and gastrointestinal tract are the common targets; but also cardiac and nervous system damage can occur.

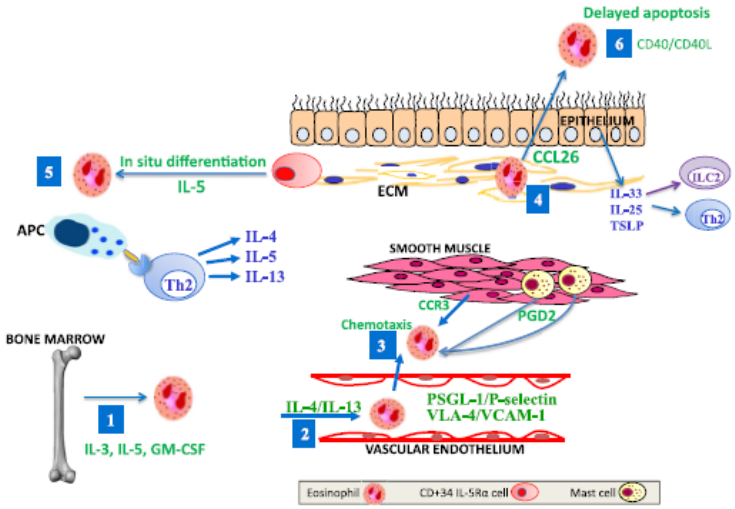

Figure 1: Selective accumulation of eosinophils. (1) Eosinophil differentiationand maturation from pluripotent CD341 stem cells. (2) Primed eosinophils bind IL-5to the IL-5R and upregulate P-selectin. (3) Eosinophil chemotaxis occurs by release of CCR3from smooth muscle and PGD2 from mast cells. (4) Eosinophil trafficking from ECM into theairspace. (5) CD341 IL-5Ra cells differentiate into eosinophils in the lung. (6) IL-5 prolongssurvival by preventing eosinophil caspase release and Fas-mediated apoptosis. APC, antigenpresenting cell; CCR, chemokine receptors; ECM, extra-cellular matrix; GM-CSF, granulocytemacrophage colony-stimulating factor; ILC, innate lymphoid cell; PGD, prostaglandin; PSGL,P-selectin glycoprotein ligand; TSLP, thymic stromal lymphopoietin; VCAM, vascular celladhesion molecule; VLA, very late antigen.*Reference: Woolnough K, Wardlaw AJ.Eosinophilia in Pulmonary Disorders.Immunol Allergy Clin North Am. 2015 Aug;35(3):477-92. doi: 10.1016/j. iac.2015.05.002. Epub 2015 Jun 18. Review.*

Definition

Eosinophilic lung diseases (ELD) are characterized bythe infiltration of the lung interstitiumand the alveolar spaces by eosinophils. ELD is represented by adramatic response to systemic corticosteroid therapy. Alveolar eosinophiliais defined by differential cell count of atleast 25% eosinophils at bronchoalveolar lavage(BAL), and typically greater than 40%.ELDs are acute- or chronic eosinophilic pneumonia, Lofflersyndrome, drug or toxin exposure and fungal infections,eosinophilic granulomatosis with polyangiitis(EGPA) , and idiopathic hypereosinophilicsyndromes.

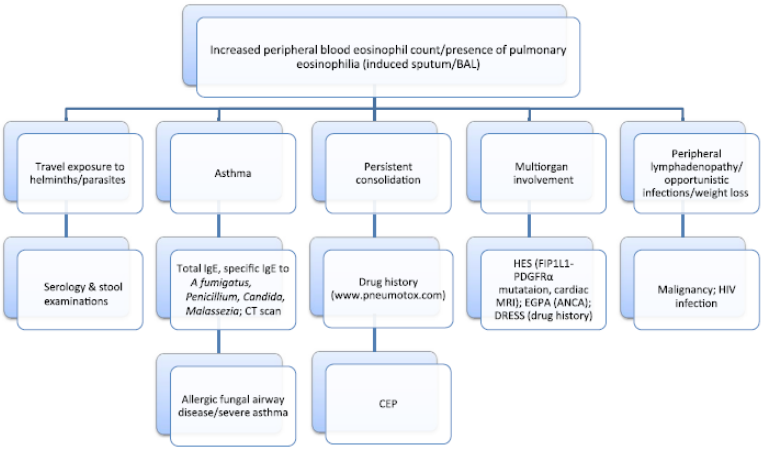

Figure 2: Diagnostic pathway for eosinophilic lung disease. CEP, chronic idiopathic eosinophilic pneumonia; DRESS, drug rash, eosinophilia, and systemicsymptoms; HIV, human immunodeficiency virus.*References:Woolnough K, Wardlaw AJ.Eosinophilia in Pulmonary Disorders.Immunol Allergy Clin North Am. 2015 Aug;35(3):477-92. doi: 10.1016/j.iac.2015.05.002. Epub 2015 Jun 18. Review.*

Classification of the eosinophilic lung diseases in clinical practice
Eosinophilic pneumonias of unknown cause
Solitary idiopathic eosinophilic pneumonias
Idiopathic chronic eosinophilic pneumonia
Idiopathic acute eosinophilic pneumonia
Eosinophilic pneumonia in systemic syndromes
Eosinophilic granulomatosis with polyangiitis
Idiopathic hypereosinophilic syndromes (lymphocytic or myeloproliferative variant)
Eosinophilic pneumonias of known cause
Allergic bronchopulmonary aspergillosis and related syndromes
Eosinophilic pneumonias of parasitic origin
Eosinophilic pneumonias of other infectious causes
Drug-induced eosinophilic pneumonias
Eosinophilic airways diseases
Eosinophilic asthma
Hypereosinophilic asthma
Idiopathic hypereosinophilic constrictive bronchiolitis
Other pulmonary syndromes with possible eosinophilia
Organizing pneumonia, idiopathic pulmonary fibrosis, Langerhans cell histiocytosis, malignancies, and so forth

Table 1: Classification of the ELD in clinical practice. *Reference: Cottin V. Eosinophilic Lung Diseases. Clin Chest Med. 2016 Sep;37(3):535-56. doi: 10.1016/j.ccm.2016.04.015. Epub 2016 Jun 25.*

Löffler Syndrome

Transient pulmonary radiographic opacities and peripheral blood eosinophilia was described by Löffler. Ascaris infection was determined to be the main cause. Patients have an irritating, nonproductive cough, burning substernal discomfort. Dyspnea, wheezing, fever, and blood-tinged sputum containing eosinophil-derived Charcot-Leyden crystals may be present.The chest radiograph shows round and oval opacities in both lung fields. The radiographic opacities are migratory. They usually clear spontaneously and completely after several weeks.Definitive diagnosis of ascariasis at the time of pulmonary symptoms requires detection of Ascaris larvae in the respiratory secretions. Stool examinations are generally negative at the time of pulmonary symptoms. Specific treatment of Löffler syndrome is generally not necessary, although anthelminthic therapy for intestinal infection after resolution of respiratory symptoms may be appropriate.

Medications and Toxins

The clinical presentation includes asymptomatic pulmonary infiltration with eosinophilia, chronic cough with or without dyspnea and fever, acute eosinophilic pneumonia, and drug reaction with eosinophilia and systemic symptoms (DRESS).Nonsteroidal anti-inflammatory drugs (NSAIDs) and antimicrobials (eg, nitrofurantoin, minocycline, sulfonamides, ampicillin, daptomycin) are the most common classes of drugs associated with pulmonary eosinophilia. DRESS should be suspected when the patient has a skin eruption, fever, facial edema, enlarged lymph nodes, and a history of initiation of a culprit medication two to six weeks prior to disease onset.

Idiopathic Acute Eosinophilic Pneumonia (AEP)

Etiology:The cause remains unknown.Outdoor activities just prior to the illness,firefighter who inhaled smoke from fireworks for three consecutive nights,recent onset of cigarette smoking, exposure to fine airborne sand or dust,and following inhalation of cocaine or heroin are thought to be etiological reasons.

Pathology: Acute and organizing diffuse alveolar damage, hyaline membranes and interstitial widening, interstitial and alveolar eosinophils were found in samples.

Clinical presentation:AEP can occur at any age, most patients are between 20 and 40 years old. They present with an acute illness of less than four weeks duration. Nonproductive cough, dyspnea, and fever are present. Malaise, myalgias, night sweats, chills, and pleuritic chest pain are also could be found.Physical examination shows fever and tachypnea. Bibasilar inspiratory crackles and rhonchi are heard upon auscultation of the chest. Hypoxemic respiratory insufficiency could be identified at presentation and often requires mechanical ventilation.

Laboratory features:No laboratory studies are specific for AEP. At presentation, the peripheral blood count may show a neutrophilic leukocytosis

without eosinophilia.

Imaging:The initial chest radiograph may show only reticular or ground glass opacities, often with Kerley B lines. As the disease progresses, bilateral diffuse mixed ground glass and reticular opacities develop. Typical findings on high resolution computed tomography (HRCT) scans include bilateral, random, and patchy ground-glass or reticular opacities and also small bilateral pleural effusions.

Pulmonary function tests (PFTs):A restrictive process may be noted (reduced forced vital capacity [FVC] and total lung capacity with a normal forced expiratory volume in one second [FEV1]/FVC); diffusing capacity for carbon monoxide (DLCO) is commonly reduced.

Diagnosis: The diagnosis of AEP is typically based upon clinical criteria that include bronchoalveolar lavage (BAL) eosinophilia with exclusion of known causes of pulmonary eosinophilia.

Diagnostic criteria:A confident diagnosis can usually made by the combination of an acute febrile illness of short duration, hypoxemic respiratory failure, diffuse pulmonary opacities on chest radiograph, and bronchoalveolar lavage eosinophilia (>25 percent), after exclusion of infection, vasculitis, or other known precipitants.

Bronchoscopy with bronchoalveolar lavage: In AEP, the BAL fluid typically shows a very high proportion (>25 percent) and total number of eosinophils.

Treatment: Initial management of AEP usually includes supportive care with supplemental oxygen and possibly mechanical ventilation, empiric antibiotics until culture results are available, and systemic glucocorticoid therapy. In the presence of severe hypoxemia or respiratory failure requiring mechanical ventilation, methylprednisolone (60 to 125 mg every six hours) is given until respiratory failure resolves. In the absence of respiratory failure (eg, pulse oxygen saturation >92 percent on low-flow supplemental oxygen), initial treatment with oral prednisone (40 to 60 mg daily) is reasonable.Oral prednisone in a dose of 40 to 60 mg per day is then continued for two weeks beyond the complete resolution of symptoms and abnormalities on the chest radiograph. At that time, the dose can be reduced by 5 mg every seven days until complete cessation of therapy. If the patient shows clinical stabilization with rapid resolution of all symptoms, then earlier glucocorticoid tapering (over 7 to 14 days) may be an acceptable treatment strategy especially for AEP patients who present with initial eosinophilia.A longer treatment course (up to four weeks) with tapering and discontinuing of prednisone over the subsequent two to four weeks may occasionally be required in patients who experienced severe respiratory failure with delayed resolution of symptoms and radiographic abnormalities.

Chronic Eosinophilic Pneumonia(CEP)

Clinical Manifestations:CEP typically affects patients in their 30s or 40s. A history of atopy is found in 60 percent. Asthma occurs in over 50 percent of cases. The disease has a gradual onset, with four to five months interval between the appearance of initial symptoms and diagnosis.Symptoms are productive cough, fever, breathlessness, weight loss, and night sweats.Peripheral blood eosinophilia is commonly present. Total immunoglobulin E (IgE) is elevated in approximately 50 percent. A high sedimentation rate, elevated C-reactive protein, iron deficiency anemia, and thrombocytosis are also common .

Laboratory:No laboratory studies are specific for CEP.

Pulmonary function tests:In CEP, PFTs may show an obstructive or restrictive pattern, or may be normal.

Imaging: Chest imaging findings of bilateral peripheral or pleural-based opacities, described as the "photographic negative" of pulmonary edema, are virtually pathognomonic for CEP.

Bronchoscopy: In CEP, the BAL almost always shows eosinophilia greater than 25%.

Diagnosis: It is typically based on the combination of clinical presentation, chest imaging showing predominantly peripheral or pleural-based, mid to upper lung zone opacities, and a bronchoalveolar lavage showing eosinophilia (≥25 percent). The differential diagnosis includes acute eosinophilic pneumonia, eosinophilic pneumonia due to drug toxicity, infection, or allergic bronchopulmonary aspergillosis, eosinophilic granulomatosis with polyangiitis (Churg Strauss), and cryptogenic organizing pneumonia.

Treatment: When the CEP has been made and alternative causes of eosinophilic infiltration is excluded, systemic glucocorticoid therapy should be initiated. For the majority of patients therapy should begin with oral prednisone, 0.5mg/kg per day. For patients with rapidly progressive CEP, high dose intravenous glucocorticoid therapy (eg, methylprednisolone 60 to 125 mg every six hours) should be used for three to five days prior to transitioning to oral therapy with prednisone. Subjective and radiographic improvement usually starts within 48 hours of initiating therapy. Prednisone sould be continued at 0.5 mg/kg per day for two weeks after the complete resolution of symptoms and plain chest radiographic abnormalities (usually four to six weeks into therapy). At that time, the dose can be decreased by one-half (0.25 mg/kg per day) and therapy continued for another eight weeks. After about 12 to 14 weeks, prednisone should be tapered by 5 mg increments every four weeks as tolerated until complete cessation of therapy or a disease flare. Most patients require prolonged treatment and up to three-fourths of patients will require ongoing therapy for several years.

Diagnostic criteria for idiopathic chronic eosinophilic pneumonia and for idiopathic acute eosinophilic pneumonia

Idiopathic chronic eosinophilic pneumonia

1. Diffuse pulmonary alveolar consolidation with air bronchogram and/or ground-glass opacities at chest imaging, especially with peripheral predominance.
2. Eosinophilia at bronchoalveolar lavage differential cell count ≥40% (or peripheral blood eosinophils ≥1000 /mm^3).
3. Respiratory symptoms present for at least 2 to 4 wk.
4. Absence of other known causes of eosinophilic lung disease (especially exposure to drug susceptible to induce pulmonary eosinophilia).

Idiopathic acute eosinophilic pneumonia

1. Acute onset with febrile respiratory manifestations (≤1 mo, and especially ≤7 d duration before medical examination.
2. Bilateral diffuse infiltrates on imaging.
3. Pao_2 on room air ≤60 mm Hg (8 kPa), or Pao_2/Fio_2 ≤300 mm Hg (40 kPa), or oxygen saturation on room air <90%.
4. Lung eosinophilia, with ≥25% eosinophils at BAL differential cell count (or eosinophilic pneumonia at lung biopsy when done).
5. Absence of determined cause of acute eosinophilic pneumonia (including infection or exposure to drugs known to induce pulmonary eosinophilia). Recent onset of tobacco smoking or exposure to inhaled dusts may be present.

Table 2: Diagnostic criteria for idiopathic chronic eosinophilic pneumonia and for idiopathic acute eosinophilic pneumonia.*Reference: Cottin V. Eosinophilic Lung Diseases. Clin Chest Med. 2016 Sep;37(3):535-56. doi: 10.1016/j.ccm.2016.04.015. Epub 2016 Jun 25.*

Distinctive features of idiopathic chronic eosinophilic pneumonia (ICEP) and idiopathic acute eosinophilic pneumonia (IAEP)

Characteristic	ICEP	IAEP
Onset	>2–4 wk	<1 mo
History of asthma	Yes	No
Smoking history	10% of smokers	2/3 smokers, often recent initiation
Respiratory failure	Rare	Usual
Initial blood eosinophilia	Yes	Often No (typically delayed)
Bronchoalveolar lavage eosinophilia	>25%	>25%
Chest imaging	Homogeneous peripheral airspace consolidation	Bilateral patchy areas of ground-glass attenuation, airspace consolidation, interlobular septal thickening, bilateral pleural effusion
Relapse	Yes	No

Table 3: Distinctive features of idiopathic chronic eosinophilic pneumonia (ICEP) and acute eosinophilic pneumonia (IAEP). *Reference: Cottin V. Eosinophilic Lung Diseases. Clin Chest Med. 2016 Sep;37(3):535-56. doi: 10.1016/j.ccm.2016.04.015. Epub 2016 Jun 25.*

Eosinophilic Granulomatosis with Polyangiitis(Churg-Strauss) (EGPA/CSS)

Eosinophilic granulomatosis with polyangiitis (Churg-Strauss), abbreviated EGPA, which was previously called the Churg-Strauss syndrome (CSS) or allergic granulomatosis and angiitis, is a multisystem disorder. It is characterized by chronic rhinosinusitis, asthma, and prominent peripheral blood eosinophilia. It is classified as a vasculitis of the small and medium sized arteries.

PHASES OF DİSEASE

Prodromal phase: The prodromal phase occurs in the second and third decades of life and is characterized by atopic disease, allergic rhinitis, and asthma.

Eosinophilic phase: This phase include peripheral blood eosinophilia and eosinophilic infiltration of multiple organs, especially the lung and gastrointestinal tract.

Vasculitic phase: In the third and fourth decades of life, a life-threatening systemic vasculitis of the medium and small vessels frequently occurs, and is associated with vascular and extravascular granulomatosis. The vasculitic phase may be with nonspecific constitutional symptoms and signs, especially fever, weight loss, malaise, and lassitude.

Asthma is the cardinal feature of EGPA and usually precedes the vasculitic phase by approximately 8 to 10 years. A peripheral neuropathy, usually mononeuritis multiplex, is seen in the patients with EGPA. Central nervous system manifestations may include subarachnoid and cerebral hemorrhage, cerebral infarction, cranial nerve palsies, and loss of visual acuity. EGPA patients have skin involvement ranging from palpable purpura to subcutaneous nodules. Cardiac involvement is one of the more serious manifestations of EGPA, accounting for approximately one-half of deaths attributable to EGPA. It should be suspected in the presence of refractory dyspnea, clinical evidence of heart failure, or cardiac rhythm abnormalities, but can also be asymptomatic. The most common echocardiographic finding in EGPA is wall motion abnormalities. Other findings include valvular abnormalities, pericardial effusion, mural thrombi, and pulmonary hypertension.

Evaluation And Diagnosis:The diagnosis is typically suspected based on the clinical findings.Although EGPA is classified as a vasculitis, only 40 to 60 percent of patients with EGPA have antineutrophil cytoplasmic antibodies (ANCA). In addition, many biopsies do not show a necrotizing vasculitis or granuloma, but rather an apparently nondestructive infiltration of vessel walls by eosinophils.

Laboratory tests: Most patients have peripheral blood eosinophilia (typically above 1500/microL), although this may be obscured by use of systemic glucocorticoids to control asthma. Antineutrophil cytoplasmic antibodies (ANCAs) are noted in 30 to 60 percent of EGPA patients. The majority of ANCAs associated with EGPA are directed against myeloperoxidase with a perinuclear staining pattern (called MPO-ANCA or P-ANCA).

Imaging: Typical findings on chest HRCT include patchy parenchymal consolidation, ground glass opacification, nodules may also be noted.

Pulmonary function tests:Spirometry typically shows variable airflow limitation (obstruction) consistent with asthma.

Bronchoalveolar lavage:BAL is typically performed to evaluate for eosin-

ophilia, infection, alveolar hemorrhage, or malignancy.

Biopsy: Surgical lung biopsyis the "gold standard" for the diagnosis. When either skin disease or peripheral neuropathy is present, biopsy of one of those sites is less invasive and often preferred to a lung biopsy.

The ACR has established six criteria for the classification of EGPA in a patient with documented vasculitis. The presence of four or more of these criteria had a sensitivity of 85% and a specificity of 99.7 %.The criteria are: Asthma, greater than 10 percent eosinophils on the differential leukocyte count, mononeuropathy or polyneuropathy, migratory or transient pulmonary opacities detected radiographically, paranasal sinus abnormality, biopsy containing a blood vessel showing the accumulation of eosinophils in extravascular areas.The main diseases to consider in the differential diagnosis of EGPA are aspirin-exacerbated respiratory disease, the eosinophilic pneumonias, allergic bronchopulmonary aspergillosis, the hypereosinophilic syndrome, granulomatosis with polyangiitis (Wegener's), and microscopic polyangiitis.

Box 1
American College of Rheumatology criteria for Churg-Strauss syndrome

1. Asthma
2. Peripheral blood eosinophilia greater than 10% of white blood cell differential count
3. Neuropathy
4. Migratory or transient pulmonary opacities
5. Paranasal sinus abnormalities
6. Tissue eosinophilia

Data from Masi AT, Hunder GG, Lie JT, et al. The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 1990;33(8):1094–100.

Table 4: American College of Rheumatology Criteria for Churg-Staruss syndrome.
Reference: Arthritis Rheum 1990;33(8):1094-100

Distinct subtypes of eosinophilic granulomatosis with polyangiitis

Characteristic	Vasculitic Phenotype	Eosinophilic Tissue Disease Phenotype
Respective frequency	~40%	~60%
ANCA	Present (mostly p-ANCA with anti-MPO specificity)	Absent
Predominant manifestations	Glomerular renal disease Peripheral neuropathy Purpura Biopsy-proven vasculitis	Cardiac involvement (eosinophilic myocarditis) Eosinophilic pneumonia Fever

Abbreviations: ANCA, antineutrophil cytoplasmic antibodies; MPO, myeloperoxidase.

Table 5: Distinct subtypes of eosinophilic granulomatosis with polyangiitis.
Reference: Cottin V. Eosinophilic Lung Diseases. Clin Chest Med. 2016 Sep;37(3):535-56. doi:10.1016/ j.ccm.2016.04.015. Epub 2016 Jun 25.

Allergic Bronchopulmonary Aspergillosis(ABPA)

ABPA is a complex hypersensitivity reaction in response to colonization of the airways with Aspergillus fumigatus that occurs almost exclusively in patients with asthma or cystic fibrosis (CF). Repeated episodes of bronchial obstruction, inflammation, and mucoid impaction can lead to bronchiectasis, fibrosis, and respiratory compromise.The prevalence of ABPA among patients with persistent asthma is estimated at 1 to 2% and among patients with cystic fibrosis, reported prevalences range from 2 to 9%.

Pathology and Pathogenesis

Aspergillus is cultured from the sputum in up to two-thirds of patients with ABPA. Although all spores that are inhaled in sufficient quantities can behave as allergens, the normally low level of IgG against fungal antigens in the circulation and the low antifungal secretory IgA in bronchoalveolar fluid suggest that healthy individuals are able to effectively eliminate fungal spores. In contrast, exposure of atopic individuals to fungal spores or mycelial fragments results in the formation of IgE and IgG antibodies.There are increases in Th2 CD4+ cell responses to Aspergillus antigens. Aspergillus-responsive T cells generate cytokines interleukin (IL)-4, IL-5, and IL-13. Proteolytic enzymes and mycotoxins released by fungi, in concert with Th2-mediated eosinophilic inflammation and IL-8-mediated neutrophilic inflammation, may result in airway damage.

Clinical Features: The clinical picture of ABPA is dominated by asthma and recurrent exacerbations. Episodes of bronchial obstruction, fever, malaise, expectoration of brownish mucus plugs, and, hemoptysis may occur. Wheezing is not always evident, and some patients present with asymptomatic pulmonary consolidation.A minority of patients with ABPA have concomitant allergic aspergillus rhinosinusitis withsymptoms of nasal congestion/obstruction, sinus pressure, and thick, dark-colored nasal discharge.

Laboratory:Laboratory abnormalities include an elevated total blood eosinophil count, elevated total serum IgE (generally >1000 IU/mL), precipitating IgG antibodies to Aspergillus, and also specific IgE and IgG antibodies to Aspergillus on immunoassay. Expectorated sputum may contain “plugs” with eosinophils, and Charcot-Leyden crystals.

Imaging: The chest radiograph may show parenchymal opacities, atelectasis due to mucoid impaction, and a number of findings characteristic of bronchiectasis;”tram line” shadows due to thickened walls of nondilated bronchi, “parallel lines” due to the presence of ectatic bronchi, “ring shadows” due to bronchial wall thickening or saccular bronchiectasis, “toothpaste shadows” due to mucoid impacted second- to fourth-order bronchi, “gloved finger shadows” due to intrabronchial exudates with bronchial wall thickening; perihilar opacities due to mucus plugging may mimic hilar adenopathy.HRCT scan may show widespread proximal cylindrical bronchiectasis with upper lobe predominance and bronchial wall thickening. In addition to bronchiectasis, other findings on HRCT include mucus plugging, tree-in-bud opacities, high atten-

uation mucus, atelectasis, peripheral airspace consolidation, or ground-glass attenuation, and possibly mosaic perfusion or air trapping.

Diagnosis:The diagnosis is usually confirmed by use of a combination of clinical, radiographic, and immunologic criteria.

International Society for Human and Animal Mycology (ISHAM) working group diagnostic criteria:

Predisposing conditions (one must be present): Asthma or cystic fibrosis (CF)

Obligatory criteria (both must be present): Aspergillus skin test positivity or detectable IgE levels against Aspergillus fumigatus, and elevated total serum IgE concentration (>1000 IU/mL).

Other criteria (at least two must be present): Precipitating serum antibodies to A.fumigatus, radiographic pulmonary opacities consistent with ABPA, total eosinophil count >500 cells/microL in glucocorticoid-naive patients.

Chest imaging: CT has become the modality of choice in evaluating patients suspected of having ABPA.

Precipitating Aspergillus antibodies: The detection of Aspergillus serum precipitins continues to have clinical utility in the diagnosis of ABPA.

Diagnosis of ABPA in cystic fibrosis: It can be difficult to establish the diagnosis of ABPA in patients with CF due to shared symptoms and often complex radiographic features, including productive cough, wheeze, and bronchiectasis.

Complications of ABPA, while uncommon, include acute invasive pulmonary aspergillosis, aspergilloma, chronic invasive aspergillosis, and problems related to bronchiectasis (eg, recurrent infections, hemoptysis).

Minimal essential diagnostic criteria of allergic bronchopulmonary aspergillosis
Patients with asthma and central bronchiectasis
1. Asthma
2. Central bronchiectasis (inner 2/3 of chest CT field)
3. Immediate cutaneous reactivity to *Aspergillus*
4. Total serum IgE concentration greater than 417 kU/L (1000 mg/mL)
5. Elevated serum IgE-*Aspergillus fumigatus* and/or IgG-*A fumigatus* (infiltrates on chest radiograph and serum precipitating antibodies to *A fumigatus* may be present but are not minimal essential diagnostic criteria)
Patients with asthma (ABPA seropositive)
Patients with the preceding criteria 1, 3, 4, and 5 (infiltrates on chest radiograph may be present but are not a minimal essential diagnostic criteria)
Patients with cystic fibrosis
1. Clinical deterioration (increased cough, wheezing, exercise intolerance, increase sputum, decrease in pulmonary function)
2. Immediate cutaneous reactivity to *Aspergillus* or presence of IgE-*A fumigatus*
3. Total serum IgE concentration ≥1000 kU/L
4. Precipitating antibodies to *A fumigatus* or serum IgG-*A fumigatus*
5. Abnormal chest radiograph (infiltrates, mucus plugging, or a change from earlier films)
Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; CT, computed tomography; Ig, immunoglobulin.

Table 6: Minimal essential diagnostic criteria of allergic bronchopulmonary aspergillosis. *Reference: Cottin V. Eosinophilic Lung Diseases. Clin Chest Med. 2016 Sep;37(3):535-56. doi: 10.1016/j.ccm.2016.04.015. Epub 2016 Jun 25.*

Hypereosinophilic syndromes(HES)

Eosinophils are derived from myeloid progenitors in the bone marrow, through the action of three hematopoietic cytokines: granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), and interleukin-5 (IL-5). Of these three, only IL-5 is specific for eosinophil differentiation.

HES, some of known and some of idiopathic etiology, are associated with marked peripheral eosinophilia and potential involvement of diverse organs such as the heart, GI tract, lungs, brain, and kidneys. Hypereosinophilia is defined as a blood eosinophil count >1500 cells/microL; a hypereosinophilic syndrome is defined by the association of hypereosinophilia with eosinophil-mediated organ damage and/or dysfunction, provided other potential causes for the damage have been excluded. Hypereosinophilic syndromes are further classified as primary (neoplastic), secondary (reactive), or idiopathic. Hypereosinophilia (HE) in the peripheral blood is defined as an absolute eosinophil count (AEC) >1.5 x 109/L (or >1500 cells/microL) on two examinations separated in time by at least one month.

Categories of HES: HES are further subclassified according to the pathogenic mechanisms resulting in eosinophil expansion: primary, secondary, or idiopathic.

Myeloproliferative variants
Etiology unknown
PDGFRA-associated HES
Chronic eosinophilic leukemia

Lymphocytic variants
Familial
Undefined
- Benign
- Complex
- Episodic

Overlap/organ-restricted eosinophilic disorders (including eosinophilic pneumonia)

Associated conditions (including CSS)

Table 7: Hypereosinophilic syndromes. *Reference: Michael E. Wechsler. Pulmonary Eosinophilic Syndromes.August 2007 Volume 27, Issue 3, Pages 477–492.DOI: https://doi.org/10.1016/j.iac.2007.07.005*

The estimated prevalence of HES is between 0.36 to 6.3 per 100,000 . Most patients are diagnosed between the ages of 20 and 50 years,

In HES, eosinophils damage the tissues that they infiltrate. Common target organs include the skin, lung, and gastrointestinal tract. Less commonly, patients can have potentially life-threatening damage to the cardiovascular system and brain.

Clinical Features: Patients may present with various combinations of symptoms and signs of end-organ damage mediated by eosinophils. Dermatological, pulmonary, gastrointestinal, cardiac and, neurological signs and symptoms can be found.Pulmonary involvement is common in HES and may result from eosinophilic infiltration of the lung with subsequent fibrosis, heart failure, or pulmonary emboli.The most common presenting symptoms were dyspnea, cough and wheezing. Abnormal chest radiography or CT findings included parenchymal infiltrates, pleural effusion, intrathoracic lymphadenopathy, and pulmonary emboli. Infiltrates were most commonly patchy ground glass infiltrates.

Laboratory Features: Patients have peripheral blood hypereosinophilia (HE) (>1.5 x 109/L) or tissue HE. Complications of eosinophilic tissue/organ infiltration may result in elevated liver enzymes, serum troponin, and rarely, increased blood urea nitrogen and creatinine levels.

Evaluation And Diagnosis: HES should be suspected when persistent eosinophilia >1.5 x 109/L in the peripheral blood on at least two occasions is present.Initial studies should include blood chemistries, including liver enzymes, creatine kinase, renal function, and troponin, electrocardiogram, echocardiogram, pulmonary function tests, chest radiograph and computed

tomography (CT), abdominal CT, tissue biopsies and additional studies as clinically indicated.

Differential Diagnosis: Leukemias and lymphomas, paraneoplastic syndromes, KIT mutation-associated systemic mastocytosis with eosinophilia, drug hypersensitivity reactions, and helminth infections should be investigated.

Feature	Acute eosinophilic pneumonia	Chronic eosinophilic pneumonia	Churg-Strauss syndrome	Hypereosinophilic syndrome
Onset	Acute (days)	Indolent (weeks/months)	Indolent (months/years)	Indolent (months/years)
Imaging	Diffuse	Peripheral	Patchy	Patchy
Fulminant respiratory failure	++	−	−	−
Asthma/allergy history	−	+	++	−
Smoking history	+	−	−	−
Vasculitis	−	−	++	−
Antineutrophil cytoplasmic antibody	−	−	+	−
Cardiac involvement	−	±	+	++
Neurologic	−	±	++	++
Requirement for therapies other than corticosteroids	−	−	+	++

Abbreviations: −, rarely occurs; +/−, occasionally occurs; +, commonly occurs; ++, occurs most of the time.

Table 8:Distinguishing features of various eosinophilic lung diseases. *Reference: Michael E. Wechsler. Pulmonary Eosinophilic Syndromes.August 2007 Volume 27, Issue 3, Pages 477–492.DOI: https://doi.org/10.1016/j.iac.2007.07.005*

Conclusion

Pulmonary eosinophilic syndromes are a heterogeneous group of disorders.It is important to diagnose quickly and to treat aggressively with corticosteroids. An important development has been done in the antieosinophil therapies, particularly those directed against the IL-5 pathway, whichis a hop efor ELD, reducing the side effects and comorbidities in these patients.

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CHAPTER 4

HISTOLOGY OF RESPIRATORY SYSTEM AND SURFACTANT PROTEIN D GENE VARIATIONS ASSOCIATED WITH SERUM PROTEIN D LEVELS IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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INTRODUCTION

The respiratory system plays a vital role in the fulfillment of many important functions in the body. This system plays an important role in delivering oxygen to the body. The respiratory system can be separated in two section; conducting section (nose to bronchioles) form a path for conduction of the inhaled gases and respiratory section (alveolar duct to alveoli) where the gas exchange takes place. Anatomically, respiratory tract is divided into upper and lower respiratory tract(1).The lungs, one of the components of the lower airway, are constantly exposed to microbes and various particles. They have important roles in detecting and distinguishing dangerous substances. The mucosal surface in contact with the external environment is known to be alveolar lining. Congenital immunity is the main feature of defense and is the first line of contact at host receptors.These receptors trigger proliferation of inflammatory events.In these receptors, the immune system plays an important role in the recognition of microbial-induced molecular models or pathogen-induced changes. Pulmonary surfactant forms the alveolar epithelium and is highly active in host defense. It performs its function by bacterial agglutination, opsonization and viral neutralization (2).Pulmonary surfactant is required to achieve normal respiratory function. The surfactant reduces the surface tension on the inner surface of the lung to prevent alveolar collapse that may occur at the end of expiration.Lipid and protein components of the surfactant are known to increase during fetal lung development. In premature infants, respiratory distress syndrome (SDR) occurs as a result of lack of surfactant. Clinical applications and active surfactant treatments are being performing for the recovery of these babies. In addition, in premature babies may develop a chronic lung disease called bronchopulmonary dysplasia (BPD)(3). Surfactant synthesized by lung type II epithelial cells in the endoplasmic reticulum is secreted in the extracellular space by exocytosis. Glycerophospholipids, dipalmitoyl phosphatidylcholine (DPPC), cholesterol and proteins, surfactant proteins (SP-A, SP-B, SP-C, SP-D) are major compo-

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nents of the surfactant(4). SP-A and SP-D surfactant proteins aglutinate viruses, bacteria and allergens and act as host defense molecules in the lung. SP-C and SP-D surfactant proteins are hydrophobic proteins and increase the surfactant film formation. They also play a role in protecting the lungs against the harmful effects of atelectasis. It is thought that there may be a significant relationship between genetic polymorphisms in genes encoding surfactant proteins and susceptibility to lung diseases such as Chronic Obstructive Pulmonary Disease (COPD) (3). In this chapter, is presented the basic structure and function of the respiratory system and it focuses on the structure of the respiratory section such as the trachea, bronchioles, bronchii, alveoli and surfactant. In addition, it will enable us to have information about the basic mechanism of COPD disease which is one of the important lung diseases.The purpose of this section is to summarize the studies conducted to investigate SP-D gene polymorphisms, which is one of the genetic factors that may play a role in the development of COPD disease.

The respiratory system and embryological development

The respiratory system consists of pathways that deliver air to the lungs. Respiratory oxygen is transported from the lungs to the cells and the carbondioxide is transported from the cells to the lungs. The breathing air is heated, moisturized and cleaned in the system, making it suitable for gas exchange (1). The respiratory system is a ridge of the ventral wall of the anterior intestine and the epithelium of the larynx, trachea, bronchus and alveoli is endodermal source. The cartilage and muscle structures are of mesodermal origin. Lung development originates from the endoderm and mesoderm during early development stages giving rise to branching morphogenesis, proximal-distal patterning of the epithelium and alveologenesis (5). Development of the respiratory system begins early in the fetus. It is a complex process that includes many structures, most of which arise from the endoderm. Towards the end of development, the fetus can be observed making breathing movements. Until birth, however, the mother provides all of the oxygen to the fetus as well as removes all of the fetal carbon dioxide via the placenta. Lung development is subdivided into three main periods; the embryonic period, the fetal period and postnatal lung development. Lung organogenesis is part of the embryonal period. While fetal lung development consists in the pseudoglandular, canalicular and saccular stages, postnatal lung development comprises the stages of classical and continued alveolarization, as well as of microvascular maturation (6). The mucus secreted from the goblet cells found in the trachea and bronchi forms a protective layer on the surface, catching the particulate material and preventing it from entering the lower respiratory tract. Serous secretion released from the glands in the connective tissue prevents the duct from drying out due to air flow (7). The presence of one way kinosilyums allows the removal of particulate material out of the respiratory tract. It also prevents the mucus secreted from the goblet cells to lower the lumen and prevent breathing in the lower respiratory tract. As the blood vessels are close to the surface throughout the system, the breathing air becomes hot. The mucosa of the respiratory system is rich in lymphoid organs as well as a large number of macrophages in the alveolus wall, thus removing air from particulate materials.The respiratory system is anatomically composed of the upper and lower respiratory system (8). The lower respiratory tract includes

larynx, trachea, bronchus, bronchioles and alveoli. This system comprising the lungs and a sequence of airways leading to the external environment. It is subdivided into: conducting portions and respiratory portions providing oxygen and eliminating carbondioxide.

Conducting portion

Conducting portion parts: nasal cavity, nasopharynx, larynx, trachea, primary bronchi, secondary (lobar) bronchi, tertiary (segmental) bronchi and terminal bronchioles. The function of this part is to clean, warm and moisten the air prior to reaching respiratory portion. It is the section where air is made available before entering the gas exchange zone and consists of pharynx, larynx, trachea, bronchi and relatively large bronchioles.

Respiratory portion

Respiratory portion is part of the gas exchange between air and blood, including the respiratory bronchioles, alveolar ducts, alveolar sacs and alveoli (9,10).

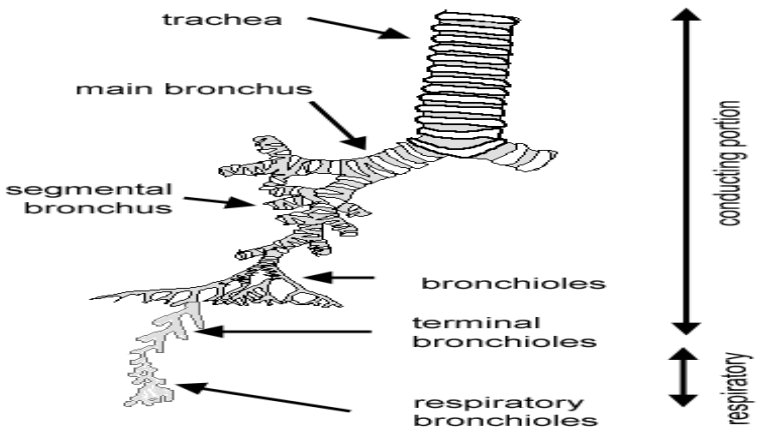

Figure1: Respiratory and conducting portion (11).

The Histology Guide. (n.d.). Respiratory | Trachea, bronchioles and bronchi. Retrived from https://www.histology.leeds.ac.uk/respiratory/conducting.php

Respiratory System

- Nasal cavities/ nasal passageways
- Pharynx- nasopharynx, oropharynx
- Larynx
- Trachea
- Extrapulmonary bronchi
- Lungs

Nasal:

A septum in the midline is divided into the right and left nasal cavities and the anterior part of the structure is supported by cartilage and the posterior part is supported by cartilage and bones. The soft palate is a muscular structure that separates the nasal cavity from the oral cavity. The soft palate moves superiorly to seal the nasal cavity during swallowing. The nasal cavity consists of three parts: vestibule, respiratory section, olfactory section.

Vestibul:

It is a part of the nose that is opened to the outer environment and it is covered with leather. The epithelium is a keratinous multi-layered flat epithelium. The connective tissue beneath the epithelium contains large amounts of hair follicles and large sebaceous glands.

Respiratory section:

The medial wall of this section has a septum with a smooth surface. Lateral walls are recessed. The epithelium laying the surface is a false multilayered prismatic epithelium. Lamina propriya located under the epithelium is a loose connective tissue rich in blood vessels. Under the epithelium, air is circulated to the lower airways by the capillary web extending parallel to the surface and large vascular plexuses located deeper, including arteriovenous anastomoses. This layer, which contains serous and mucous glands, continues with pericondria of cartilage and periosteum of bone.

Respiratory epithelium contains 5 types of cells.

Silly cells: These are prismatic shaped cells with a large number of quinolocytes on their surfaces. Because of the different size of these cells, the different nucleus levels are observed to be seen as multi-layered epithelium.

Goblet cells: These goblet-like cells have mucus secretion. Because of the glycoprotein content of the secretion products, only the PAS reaction can be stained.

Brushed cells: Brushed cells with short microvilli on their surface are sensory receptors that contact the basal faces and numerous afferent nerve fibers.

Small granular cells: Small granular cells are diffuse neuroendocrine system cells.

Basal cells: These cells, which are small, cubic shaped cells that are located on the basement membrane and do not reach the lumen, are basal storage stem cells that can be transformed into other cell types (12,13).

Olfactory section: The olfactory mucosa is composed of three primary components: epithelium, basement membrane, and lamina propria. The olfactory epithelium lamina propria (serous secreting Bowman's glands, a rich vascular plexus and many axons arising from olfactory cells of the olfactory

epithelium) is a special section at the top of the nasal cavity, containing odor receptors. A special type of pseudo-multilayered epithelium that does not contain epithelial goblet cells has a prominent basement membrane (14).

Olfactory epithelium comprises three types of cells:

- Olfactory cells are bipolar neurons whose apical aspect (dendrite) is modified to form a bulb known as olfactory vesicle.
- Sustentacular cells has a striated border composed of microvilli, and secretory granules they provide physical support, nourishment.
- Basal cells are histologically short basophilic cells. Their apical aspects do not reach the epithelial surface, they proliferate and replace both two other cells (15,16).

Pharynx

It connects the nasal and oral cavities to the larynx and esophagus. It divides into nasopharynx and oropharynx.

Larynx

It (voice box) is a modified portion of the superior of the trachea Its cartilaginous rings are connected by dense connective tissue forming a tube. It is lined by respiratory epithelium. Laryngeal cartilages (hyaline and elastic) are located in lamina propria. The cartilages connected to each other by ligaments and move with respect to one another by some striated muscles. Larynx consists of two folds superior and inferior. Superior vestibular folds are lined by respiratory epithelium. Inferior vocal folds lined by stratified squamous nonkeratinized epithelium. It works as a sphincter, transmitting air from oropharynx and nasopharynx to trachea. The larynx acts an important role in speech and swallowing (17).

Epiglottis

It is an elastic cartilage of larynx. It is lined by stratified squamous epithelium on lingual surface. Pseudostratified ciliated columnar epithelium lined is the laryngeal side. Serous and mucous glands are localized in lamina propria.

Trachea

It is a long tube that has 3 layers: mucosa, submucosa and adventitia.

Mucosa: Respiratory epithelium composed of 6 cell types located on a thick basement membrane.

- Goblet cells are about 30% of cells, produce mucinogen.
- Ciliated columnar cells about 30% of cells, are tall which have cilia and microvilli.
- Basal cells are also about 30% of cells, they are undifferentiated stem cells.

- Brush cells are just 3% of cells, they are narrow columnar cells that their function is unknown, but nerve ending associated with them.
- Serous cells are about 3% of cells, they are columnar and have serous granule.
- DNES cells, constitute about 3-4% of cells, have numerous granule in basal cytoplasm which is contain various pharmacological agents.

Lamina propria iscomposed of loose fibroelastic cartilage, contain seromucous glands and lyphoid elements, elastic lamina separate this layer from submucosa.

Submucosa is composed of dense irregular fibroelastic cartilage that houses mucous and seromucous glands, rich in blood and lymph supply.

Adventitia is a fibroelastic cartilage that houses C-shaped hyaline cartilage, at posterior aspect of cartilage, there is a dense band of smooth muscle cells known as trachealis muscle (5,18).

Bronchus

A bronchus is a passage of airway in the respiratory system that conducts air into the lungs.

Bronchial Tree

It is composed of 2 primary bronchus that enter lungs 3 lobar bronchus on right and 2 on the left. Segmentalbronchus, bronchioles, terminal bronchioles, respiratory bronchioles. Progressively airways decreases in size and cartilage, glands, goblet cells, and the height of epithelial cells. But smooth muscle cells and elastic tissue increase.

Primary Bronchi (Extrapulmonary), primary bronchi is identical to trachea, but have smaller diameter and thinner wall. Cartilage is in form of irregular plates. Smooth muscle located between lamina propria and submucosa as 2 distinct layers.

Bronchioles

It have not any cartilage or glands but have few goblet cells. In larger bronchioles epithelium is simple columnar ciliated, with occasional goblet cells. In smaller bronchioles epithelium change to simple cuboidal, with no goblet cells. Bronchioles have a smooth muscle coats surrounded by fibroelastic connective tissue.

Terminal bronchioles are terminus of conducting portion. They are lined by cuboidal cellsand Clara cells which have domed apical surface. Lamina propria is a fibroelastic cartilage tissue, 1-2 layer of smooth muscle cells separate it from adventitia. Clara cells are columnar with dome-shaped apex secretory granules.

Respiratory Bronchioles are a transitional zone between conducting

and respiratory tissues. Alveoli branching from their walls are lined by ciliated cuboidal epithelium with Clara cells that change to type I alveolar cells. Smooth muscle cells and elastic fibers underlie epithelium (19,20).

Alveolar Ducts do not have wall of their own. They are only a linear arrangements of alveoli they end as a blind out pouching known as alveolar sac. Opening of alveolus to AD controlled by a single smooth muscle cell embedded.

Alveolus has 200 micrometer in diameter and is the functional unit of respiratory system. Composed of attenuated type I and type II pneumocytes. Connective tissue between them are very scant. Air space of two adjacent alveoli communicate through an alveolar pore. Interalveolar septum is between alveoli have an extensive capillary bed.

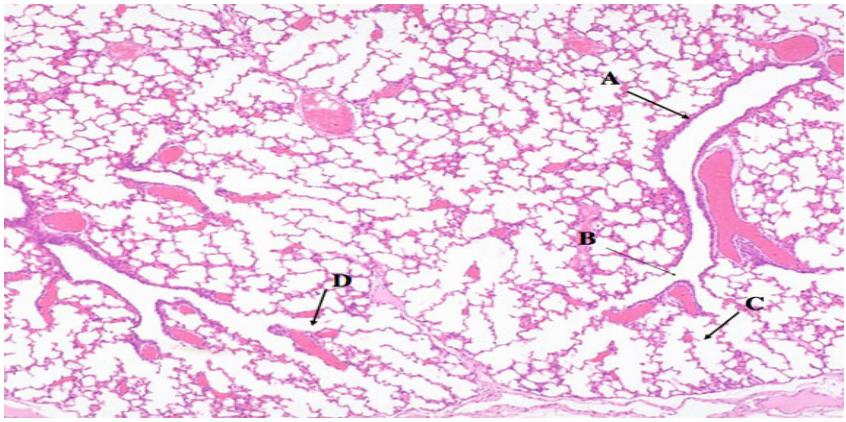

Figure2: Histological apperance: A: Terminal Bronchiole, B: Respiratory Bronchiole, C: Alveolar Sac, D: Alveolar Duct (21).
Respiratory System Lab. (n.d.). In this image, identify A, B, C, and D. Retrieved from (http://medcell.med.yale.edu/histology/respiratory_system_lab.php).

Cells of the Alveolar Septa

Endothelial cells are nonfenestrated with a thin dark nucleus, and pinocytotic vesicles.Type I squamous cells that cover most of alveolar surface area, they have pinocytotic vesicles.Type II cells are cuboidal, located on alveolar surface where septa intersect, they have foamy cytoplasm, surfactant granules (reduces surface tension to keep alveoli open during expiration). Alveolar macrophages that are known as dust cells. Interstitial cells consist of fibroblasts, mast cells and elastic, reticular fibers.

Type I pneumocytes

Type I pneumocytes are composed of 40% of alveolar wall population, 90% of alveolar surface. Flat cells with flattened nuclei and tight junctions. 95% of the alveolar surface is composed of the simple squamous cells which are known as type I pneumocytes, occluding junction attaches to other cells have basal lamina, alveolar pore formed by fusion of two adjacent type I cells.

Type II pneumocytes

Type II pneumocytes are composed of 60% of alveolar population, 10% of alveolar surface. Rounded cells located at angles of alveolar sacs. Lamellated material in their cytoplasm. They are more numerous than type I pneumocytes. It cover just 5% of the alveolar surface and located among type I cells, cuboidal with dome-shaped apical. Located where adjacent alveoli separated by septum. They have an abundance of rough endoplasmic reticulum, developed Golgi complex, their lamellar bodies contain pulmonary surfactant (22).

Alveolar Macrophages (Dust cells)

It was known as type III pneumocytes. Originate from monocytes that migrate to pulmonary interstitium. It migrate between type I cells and enter alveolar lumen. It maintain a sterile environment. Assist type II to uptake surfactant.

Surfactant and its importance for respiratory system embryology

Surfactant proteins are important for the proper structure and respiratory function of the lungs. It is a complex mixture of phospholipids, protein and glycosaminoglycan. They are special unique in composition, consisting of approximately 90% lipids, mostly phospholipids, and 8–10% surfactant-associated proteins. It forms a monolayer lining the internal alveolar surface. It reduces the alveolar surface tension. It prevents collapse of alveoli during expiration. Surfactant is required at birth and throughout postnatal life to reduce surface tension at the air-liquid interface in the alveoli. They are stored mainly in type II alveolar epithelial cells in the form of densely packed bilayers (23-27). It is known that lung diseases such as SDR, BPD and COPD can develop in the absence of the required surfactant to provide normal respiratory function (3).

Chronic Obstructive Pulmonary Disease (COPD)

COPD; is a reversible lung disease characterized by chronic obstruction of lung airflow. It is known that the disease is characterized by abnormal inflammatory responses to alveolar destruction and harmful stimuli (2). Nowadays, COPD is the fourth cause of death worldwide, but it is estimated that this disease may be the third cause of death until 2020 due to the rapid increase. To be diagnosed with COPD, there should be a history of exposure to risk factors such as cough, sputum production, shortness of breath, and smoking. Chronic cough and sputum production occur before the development of lung aerial restriction. There are discussions about the onset and severity of the disease. The most common feature for the severity and progression of the disease is the forced expiratory volume (FEV1%) at the first level. However, there is a weak relationship between FEV1 symptom and disease progression (4). There are different biomarkers in the development of COPD (28). Biomarkers for systemic inflammatory conditions in this disease are C-reactive protein, interleukin 6, blood fibrinogen, fractional exhaled nitric oxide (FEND) levels.

Biomarkers of the disease related to lung disruption are issue inhibitors of tissue matrix metalloproteinases / metalloproteinases and desmosine. Specific Clara Cell Protein 16 (CC16), a lung specific biomarker, is important in assessing the patient's therapeutic response (4). Surfactant Protein-D (SP-D), one of surfactant proteins, is believed to play an important role in the pathogenesis of COPD.

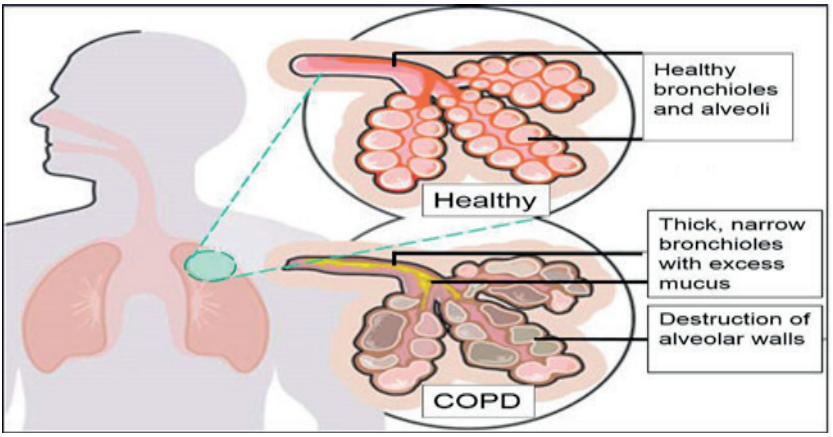

Figure 3: Healthy bronchioles, alveoli and development of COPD (29). (2017). DAFTAR OBAT PPOK YANG DIREKOMENDASIKAN. Retrieved from https://obatkupingbudeksebelah.wordpress.com/tag/kumpulan-pengobatan-ppok/

Surfactant Protein-D

Surfactant Protein-D (SP-D) is a choline subgroup of the C-type lectin family and the C-type lectin family consists of the NH_2 terminal segment. There is also a collagen-like domain, neck region and carbohydrate recognition domain. The NH_2 terminal domain contains two residues of cys, and these residues play a role in the stabilization of the four-component structure (4). It is a molecule that allows binding with various axeptors and receptors. In the presence of calcium, the carbohydrate recognition domain of SP-D is bound to defensins and carbohydrate moieties present in the virus capsid. In the absence of calcium, in the presence of myeloperoxidase, can bind C1q and glycoprotein Gp340 (30). It was determined that SP-D was present in bronchiole and terminal epithelium with various analysis methods. In addition, this protein found in vascular endothelial cells and in serum was associated with metabolic diseases such as viral response and Diabetes Mellitus (4). Type 2 Diabetes Mellitus is one of the comorbidities of COPD. SP-D serum levels and SP-D gene polymorphisms are important biomarkers for both Type 2 Diabetes Mellitus and COPD. It is thought that they can be effective in evaluating the severity of diseases (31). SP-D is also involved in the innate immune response (4). Therefore, SP-D is an important regulatory protein. It plays an important role in controlling chronic inflammation, reducing oxidative radical formation, facilitating phagocytosis and agglutination, reducing cell death, and apoptotic and necrotic cell cleaning (30). SP-D synthesis in the lung and mucosal epithelium is known to occur. There is a significant relationship between low expression of SP-D and increased risk of infection in the lung.

Significant relationships are found between local overexpression and chronic inflammatory conditions such as asthma, interstitial pulmonary fibrous and COPD. In studies performed with mice without SP-D genes, progressive emphysema has been observed. Developed emphysema is characterized by chronic inflammation, surfactant deposition, phospholipid deposition, lipid or apoptotic alveolar macrophage infiltration (32). SP-D is thought to play an important role in the pathogenesis of COPD. Apart from this disease, it also has function in oskidan production, inflammatory responses in alveolar macrophages and apoptotic cell clearance. In some cases, bronchoalveolar fluid of SP-D was found to be decreased. The cumulative effects of smoking and the development of emphysema are effective in this reduction.In some studies, a significant relationship was found between smoking and increased serum SP-D levels. It is thought that the increase in serum levels is caused by inflammation agents and not associated with bronchoalveolar lavage and local production. In COPD patients, in smokers, the disease progresses rapidly and the incidence of infection increases. The decrease in local SP-D production may explain this situation (4).

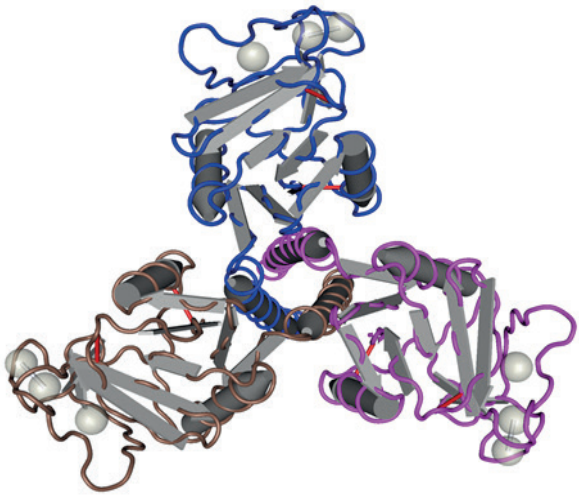

Figure 4: Structure of SP-D (33).
Dilmen, N. (2002). File:1B08 Lung Surfactant Protein D Sp-D07.png. Retrieved from https://commons.wikimedia.org/wiki/File:1B08_Lung_Surfactant_Protein_D_Sp-D07.png

SP-D Gene Polymorphisms

SP-D, which plays an important role in the pathogenesis of COPD, has proinflammatory and antiinflammatory signal functions (34). There is a significant relationship between increased serum concentrations of SP-D and risk of developing COPD (35). Increased levels of SP-D serum are thought to be important in the development of new drugs and in determining treatment strategies. Studies with animal models have shown that exposure to tobac-

co smoke is associated with increased SP-D concentrations. Structural SP-D production is known to be under genetic and environmental control (2).It is not known exactly whether the polymorphisms occurring in SP-D are genetic risk factors in the development of COPD. However, it is thought that there may be a relationship between SP-D gene polymorphisms and susceptibility to COPD. In a study, it is investigated whether there is a significant relationship between SP-D serum concentrations and three SP-D single nucleotide polymorphisms. In this study, a significant relationship was found between SP-D Met11Thr (rs721917) variant and SP-D collection, function and concentration. It was determined that there was a relationship between decreased serum SP-D concentrations and Met11Thr (rs 721917) variant.In addition, this gene polymorphism resulted in a reduction in the binding of bacterial ligands and inhibition of the oligomerized condition (36). In other studies, it was determined that the polymorphisms of SP-D were genetic risk factors in the development of COPD (2). There was also a significant relationship between high SP-D concentrations and severe COPD risk (35). Despite high SP-D concentrations in peripheral blood in COPD patients; the SP-D concentrations measured in the bronchoalveolar lavage fluid were low. This incompatibility can be explained by a mechanism. COPD patients develop inflammation in the lungs and this inflammation causes endothelial leakage of SP-D. In a study, SP-D concentration was associated with body mass index, and in another study, excessive SP-D expression was found to be related to atherosclerosis (2). In a previous study, no significant relationship was found between SP-D rs2243639, rs721917 gene polymorphisms and SP-D concentration (37). In a study conducted by Leth-Lorsen et al., it was reported that SP-D serum concentrations, polymerization and its function were affected by genetic polymorphisms in the N-terminal domain of SP-D (38). In genetic correlation studies with different populations, the significant relationships were found between the genetic polymorphisms of the SP-D gene and the risk of COPD development. In another study by Foreman et al., six single nucleotide polymorphisms were investigated in the SP-D genes of patients diagnosed with COPD.Among these polymorphisms, rs2245121 and rs911887 gene polymorphisms are intronic polymorphisms; the rs6413520 and rs721917 gene polymorphisms are non-synonymous polymorphisms. These gene polymorphisms were found to be genetic risk factors in the development of COPD. There is also a correlation between these gene polymorphisms and serum SP-D concentrations. The single nucleotide gene polymorphisms associated with COPD and SP-D concentrations, are shown to be different in the study. This condition indicates there is a variety of genetic effects for COPD and SP-D concentrations (39). In a study by Shakoori et al., it was determined that the C allele of the A allele and rs721917 gene polymorphism of the rs3088308 gene polymorphism was associated with decreased serum SP-D levels. It also was found a significant relationship between TT genotype of rs721917 gene polymorphism and COPD development risk (40). In another study by Ishii et al.,a significant relationship was found between rs721917C / rs10887199C haplotype and the risk of developing emphysema (41). In studies with different populations, SP-D serum concentrations and lung function were affected

as a result of genetic variations in SP-D. Therefore, they are found to be related to the development of COPD. Further studies are needed to determine the relationship between SP-D concentrations and genetic factors involved in the development of COPD. Single nucleotide polymorphism identifications, regions and alleles for SP-D gene polymorphisms are presented Table 1 (2).

Table 1:SNP Identifications, regionsand alleles for SP-D gene polymorphisms

SNP Identification	Region	Alleles
Rs1051246	Coding exon	A/G
Rs2245121	Intron	A/G
Rs2255601	Intron	A/G
Rs6413520	Coding exon	A/G
Rs911887	Intron	C/T
Rs721917	Coding exon	A/G

SNP, single nucleotide polymorphism; A, Adenine; G, Guanine; C, Cytosine, T, Timin (Makale 2).

Conclusion

Respiration is the process of gas exchange. It means that the release of carbon dioxide and the uptake of oxygen that occurs between red blood cell and alveoli. Gas exchange takes place in the alveoli. The respiratory system consists of two divisions with different structural parts that reflect their unique function. The purpose of epithelia in the respiratory system is cleaning, warming and moisturizing air. Type II pneumocytes and Clara cells are both involved in surfactant production. The lung tissue histology of such an infant would appear with collapsed alveoli. Premature children do not produce adequate amounts of pulmonary surfactant.In conclusion, a living thing must breathe to survive. The lung surfactant consists of lipids and proteins that cover the alveolar surface of the lung. This material reduces the surface tension and prevents alveolar collapse at the end of expiration. The surfactant components are known to be produced by alveolar type II epithelial cells. While, the majority of the surfactant consists of lipids, a small portion of surfactant consists of surfactant proteins. Among these proteins, it is known that the maximum amount of protein is SP-A.SP-A and SP-D proteins are hydrophilic proteins and combine with other surfactant components in the alveolar lumen. It is determined that the polymorphisms in SP-D gene, serum SP-D levels were related to the development of lung diseases such as COPD. Different results were obtained between SP-D gene polymorphisms and COPD development risk, between SP-D serum concentrations and COPD development risk in the studies to investigate the relationship. In these studies, it is thought that obtaining different results is due to patient and control selection criteria, different study design or different populations. Determination of SP-D gene polymorphisms and SP-D serum concentration levels in patients with COPD,

understanding the underlying mechanism of COPD is very important in terms of developing new drugs and new treatment of strategies for the treatment of the disease.

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CHAPTER 5

THE CHARACTERISTICS OF HORSES USED IN THERAPY

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INTRODUCTION

Horses have been close to humans until today due to their proper morphological and physiological structures, as well as their submissiveness, loyalty, bravity and skillfulness, in addition to deep sensations and emotions. Horses have always had great effect on human's life due to these properties. Nowadays, horses are used for therapy and support for solving some diseases and problems (Anonymous, 2017a).

Horses' being social and inward like humans, having different personalities, attitudes and emotional status are the main reasons for their usage for therapeutic purposes. Horses are used for therapy due to their morphological, physiological, emotional, mental, cognitive and social benefits (Meregillano, 2004; Violette and Wilmarth, 2014).

Horses are used as a tool in psychology to develop the skill for problem solving, to establish relationships, honest communication, trust, leadership, patience, initiation, and love, due to the common properties with humans (Kersten, 2014; Trask, 2014). Horses are also used as a tool to improve a strong character, work ethics, responsibility, respect and integrity (Kersten,

The mechanisms of action in the treatment of physical diseases or problems include horses' 1,5-2°C higher body temperature than humans and their rhythmic movements that have healing effects on loco-motor and central nervous system of the patients. Gait style and steps of the horses have great specialties; the steps' being symmetrical and rhythmic enable emotional and motor input for the patients. Therefore, the success of the therapy is based on the gait (Anonymous, 2017b).

As the horses' body temperatures have height, it increases the blood circulation in the muscles of the patients and creates physical therapy on the muscles. This effect may be used in many diseases. Furthermore, the internal organ activity of the patient is stimulated and certain muscle reflexes become active through its positive therapy effect in other disorders, and a unique therapy can be provided in various system diseases after paralysis (Anonymous, 2017c). Horses are used for therapy in many diseases. Most of these diseases are specifically based on statistical analyses performed on more than 30,000 patients (Anonymous, 2017c; Anonymous, 2017d). Different therapy

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methods and procedures are also available in horses used as a treatment tool. These include horse-supported activity, therapy, learning, psychotherapy, and facilitated horse-supported learning (Anonymous, 2014e; Anonymous, 2014f).

Horses used for therapy are selected according to the prior purposes and the requirements of the patients. Selection of the therapy horses is of great importance due to the sensitivity of the planned procedure. The correct and proper horse should be selected for safety, effectiveness and success of the therapy. The procedure that is applied by using an improper horse would not yield the expected outcome, even if all factors are proper. Therapic horses are selected according to racial and individual properties (Kersten, 2014; Köseman, and Şeker, 2015b). This review is presented to provide information about the properties that the therapy horses should have.

The Properties in Selecting Therapy Horses: Therapy horses have properties that bring patients together and the disabled, volunteers and medical professionals together (Moisa at al, 2012). Horses are in the focus of therapy. Therapy horses should have some additional properties beside spesific species of general properties (Anonymous, 2017g). Selecting therapy horses according to these properties has great importance for success in the therapy.

Race: The first aim of specifying therapy horses is to select the proper race among approximately 500 horse races (Hendricks, 1995). The horses that are selected as therapy horses are usually of hot-blooded horses that are also used for riding, light-weight-pulling and sports. These horses have a light heavy and thin body structure and active temperament. Arabian and Thoroughbred horses are the main common and popular hot-blooded horses (Arpacık, 1996).

A great number of different horse races are used for therapy worldwide. While Light Horses, Quarter Horses, Arabian horses, Morgan, Standardbred, Thoroughbred, Gaited Horses, American Saddlebred, Missouri Fox Trotter, Tennessee Walking Horse, Draft Horses, Clydesdale, Appaloosa, Palomino, Pinto, Shetland Pony, Welsh Pony and Pony are the most raised horses in USA where horse therapy is the most common and most developed (Anonymous, 2017h), Quarter horses are the most preferred race for therapy (Anonymous, 2017i). Hucul horses (30%), Crossbred horses (18%), Polish Koniks-like ponies (13%), Fjord horses (10%), Polish halfbreed horses (9%), Felinski ponies (6%), Wielkopolski horses (5%), Haflingers (4%) and the other races (4%) were reported to be used for hippotherapy in a study from Poland (Pawelec et al, 2014) In another study in horse therapy centers in Poland, Wielkopolski (25%) and Hutsuls (21%) were reported to be preferred most and besides, 15% of the horses were from an unknown race (Cieśla, 2007).

In Turkey, mainly Arabian, Thoroughbred and native horses are raised. Arabian horses and Haflinger horses are the most frequently used for therapy in Turkey (Köseman and Şeker, 2015a; Köseman and Şeker, 2015b). Coloured horses are used for therapeutic purposes in several centers in Erzurum and

Malatya provinces (Köseman and Şeker, 2015a; Köseman and Şeker, 2016).

The mean height of withers has been reported as 137.9 cm and the mean hip height of Coloured horse has been reported as 138.1 cm (Yılmaz and Ertuğrul, 2011). These horses, which are quite and submissive and morphologically proper, are reported to be very appropriate for therapy (Köseman and Şeker, 2015a; Köseman and Şeker, 2015b). Coloured horse is a race that is also known to be available in other countries and they are registered as Pinto horse in USA (Köseman and Şeker, 2016).

Gender: Gender-specific physiological and morphological differences play a role in selection of proper horses. The more developed muscle and bone structure of stallions compared to mares leads to a physical advantage. The smaller and narrower thorax leads to a lower lung volume in mares. While stronger muscles, bones, larger and deeper thorax put the male horses in the foreground in high performance works, but, the requirement for calmness, softness and kindness in the therapy causes the limited use of male horses for therapy (Köseman and Şeker, 2015b; Köseman and Şeker, 2016).

In a study, it was reported that gender had an important effect on behaviors and related factors, and that gender-related differences in the prevalence of emotional disorders arise from the differences in hormones (Domonkos et al, 2017).

In a study from Poland gelds (63%) and females (35%) were reported to be used for hippotherapy and males were reported to be used very seldom (Pawelec et al, 2014). In another study conducted with 34 therapy horses in 5 hippotherapy centers in Poland, 71% of the horses were reported gelds, and 29% were reported to be female (Cieśla, 2007).

Age: Horses that live approaximately 39 years are (Özbeyaz and Akçapınar, 2010) categorized as young (3-8 age), middle aged (9-18 age) and elderly (>18 age). They should be in different ages in respect to their usage (Claes et al. 2017).

It was reported in various studies that the age had significant influence on the race performance (Chrzanowski and Koeboke,1993; Ueno et al, 1991) and the age is also a significant factor in theraphy horses. These types of horses are not preferred in therapy because very young of them have extreme mobility and lack of experience, and elderly may have inadequacies due to old age. In the therapy, horses are mostly used at the age of 5-20 years, and horses of 8-16 years old are preferred (Anonymous, 2017f).

In a study in Poland, it was reported that horses used in hippotheraphy were in 2.5-23 ages that have average of 10.4 age and 15% of those are in 16 and over aged (Pawelec et al, 2014). In another study conducted in Poland, it was determined that the youngest horse used in therapy is 3 years old, the oldest horse is 20 years old and the average of those used is 8.4 (Cieśla, 2007).

Foot Health, Leg Structure, Gait Properties: Gait type is of great importance for the success and level of therapy. Many horse races have different

walking types defined as walk, trot, pace, canter and gallop. There are also some spesific gait types for only some races (Yılmaz and Ertuğrul 2013). All horses are not expected to be present in all gait types. Horses may exhibit some gait disturbances due to various reasons. Congenital or acquired disability, disease, fatigue, weakness, use of improper horseshoe, improper nail cut, incorrect sitting of the horse rider and incorrect bridle use may lead to gait disturbances (Yılmaz and Ertuğrul 2013). The ability to perform gait types depends on the race, bloodline, training level, body structure and nutrition of the horse (Batu, 1938). Horses that have extremely straight and strong legs, short and strong cannons also have well swiftness, strength and durability (Gökçe, 2016).

In a study, that expresses horses' walking characteristics as multidimentional, changeable, rhythmic and repetitive, therapy horses are reported to contribute to healing through creating a three-dimensional movement effect on the pelvis of the horse rider as the horses' body movement (Anonymous, 2017f).

Gait type of the horse is quite important for patients who have stiff muscle tone. Swiftness and tempo of the therapy horse is adjusted according to the status and muscle tone of the patient in order to develop balance, bio-mechanic adaptation and postural control (Anonymous, 2017g).

Under the light of these data, therapy horses' having straight legs, faster than trot but slower than gallop walk, having soft and clearly steps and not having foot or tread disturbances are necessary for the effectiveness and success of the therapy (Köseman and Şeker 2015b).

Physical Characteristics and Height of Withers: Height of withers is an important horse selection criterion depending on the patient's being a child or an adult, and an important racial characteristic defining the distance between the ground and the withers of a horse, which regularly stands on its four legs on a flat floor. Each race has a certain genetically inherited height of withers (Özbeyaz and Akçapınar, 2010). In general, horses that have 1.48-1.55 cm height of withers is "low," 156-1.65 cm height of withers is "moderate" and 1.66 cm is"high" (Batu, 1938).

Horses with short and strong bones have thick muscles. These types of horses are slower but stronger. A well-developed rump also has a great effect on the efficiency of the horse. Horses with a short rump are not proper for use in services that require velocity (Violette and Wilmarth, 2014). While horses with the height of withers of between 135-150 cm (low) are preferred for hippotherapy and therapeutic riding in order to enable intervention and control of the disabled person, horses with a height of withers of 160 cm or above (moderate and high) are used for taller and heavier patients (Anonymous, 2017b; Anonymous, 2017f).

In a study from Poland, horses used in therapy centers were determined to have withers between 130-167 cm, thorax circumference between 156-210 cm, and have body structures proper for riding and general use (Cieśla, 2007).

Level of Training: Therapy horses should be well trained. These horses' being submissive under every condition is among the main selection criterion of the horses (Anonymous, 2017b; Anonymous, 2017f).

Horses should also be adjusted to toys, equipments used for the therapy program, ramp or sidewalk for wheelchairs before being integrated in therapy schedule (Anonymous, 2017g).

Horses should not be allowed to lose their attention due to objects, human beings and events around, and should be focused on the orders and the work. They should stand still during the process of patient's getting on the horse and getting down, should approach the ramp smoothly and should have the desire for the work (Anonymous, 2017b; Anonymous, 2017f).

The behaviours of the horses during the therapy are very important for assessment of its relevance and they should have great patience, compassion and motivation for the work (Anonymous, 2017g).

Therapy horses should reassure and tolerate any sudden changes and environmental factors during the therapy. The horses' being social, quiet, compatible and controlled against the therapy team, environment, place, equipment, patients, and the other therapy horses are the main training markers of them (Anonymous, 2017b; Anonymous, 2017f).

In a study, keeping 1-2 year old horses together with older horses was reported to be useful for learning new behaviors, developing social cooperation, increasing positive social behaviors, and reducing agnostic interactions (Bourjade et al, 2008).

Psychological Status and Temperament: Movements and behaviors of horses are not related to absolute instinct, but they have mechanical movements aiming at meeting a desire or termination of an unwanted condition, automatic movements arising from balance and direction centers, and movements directed by sixth sense. Reflex behaviors are managed by different centers in horses and valuation-requiring behaviors are realized with intelligence as a result of causality and valuation (Tesio, 2003). Brave, enterprising and submissive horses display all their effort and force even if they are tired (Anonymous, 2017j). In a study investigating the influence of genetic and environmental factors on different characters and personalities, genetic factors such as paternity and race were determined to be dominant on neofobia reactions, and environmental factors such as breeding aims were detected to be dominant on social differentiation and learning (Hausberger et al, 2004). Another study has revealed that the skills of the horses arise from their natural, biological, physiological and psychological properties, and that each horse has a different and personality and they also have feelings like love and like (Anonymous, 2017f). Bad temper is the expression of nervousness and may result from mistreatment or bad training; stubbornness may usually result from genetic factors. Bad temper in horses usually cannot be corrected later on (Oytun, 1943).

Temperament, personality and their reactivity are considered for selec-

tion of the horses for therapy. Temperament features are characterized by fear reactions, social motivation, reacting against humans, and perseverance/distraction and locomotors activity (Hausberger et al, 2008; Lansade, 2005). Some researchers have defined the temperament of horses as control, anxiety, excitability, preserving, socializing and curiosity (Sian-Loyd et al, 2008).

Questionnaires filled out with the groom, trainer and horse owner, and more objective, simple and rapid behavior tests are used for defining the temperament and personality features of the horses (Momozawa et al, 2005). Reactivity of the horses includes both behavioral components (avoidance or fear reactions, vocalizations, defecation, etc) and physiological events (heart rate, blood pressure, respiration rate etc). Reactivity is measured through emotionality scores, isolation at separate paddocks and exposure to new stimuli (Lansade et al, 2008). Therapy horses should be strong, quiet, submissive, patient and calm for maintenance of the therapy, and for safety of the patient and the therapy team. Cranky, stubborn, excessively excited, fearful horses whose behaviors cannot be predicted and exhibit negative behaviors like biting or double throw, should not be included in the therapy (Anonymous, 2017f).

Care and Health: Eligible horses that are selected in accordance with the pre-specified criteria should undergo an adaptation period so as to include the adaptation process and used for therapy after the required health procedures. They should undergo regular exercises, maintained at maximum condition, and their care, training and management should be correctly carried out. A comprehensive health report including a detailed analysis about zoonotic and contagious diseases and also about the previous diseases, treatments and vaccination schedule should be obtained on admission. Horses who were vaccinated against internal and external-parasites, other micro-organisms, whose tooth and foot cares have been completed, should be used as therapy horses (Anonymous, 2017f).

Conclusion: Horses taking part in every field of human life and being used for therapy purposes have a special significance today. Specialized human power, sufficient equipment and proper infrastructure are required for success in this field. Selection of the most proper therapy horses is the main factor for maximum success and safety in gradually increasing and developing hippotherapy procedures. Race, gender, foot health and gait characteristics, physical characteristics, particularly height of withers, training level, psychological status, temperament, care and health issues are the main factors for selection of therapy horses.

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CHAPTER 6

GENERAL CHARACTERISTICS AND CURRENT CLASSIFICATION OF MYELODYSPLASTIC SYNDROME

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Introduction

Myelodysplastic syndrome (MDS) is a clonal, acquired stem cell disorder of the hematopoietic system. MDS causes varying clinical pictures such as peripheral blood cytopenias in some patients and pancytopenia in some other patients. In addition to dysplasia in bone marrow, the cellularity of the bone marrow is usually increased and there is a risk of acute myeloblastic transformation with abnormal cell morphologies which may cause increased myeloblast in bone marrow as well. While MDS may show a slow clinical course in some patients, it may also transform into leukemia at early stages. MDS is rarely seen in children, it often occurs at later stages of life. While MDS may develop de novo, it may also develop secondary to previous chemotherapy and radiotherapy treatments. Each of the granulocytic, erythrocytic, and megakaryocytic lineages in bone marrow may be affected. MDS is more common in men and primarily occurs after 65 years of age. Its incidence increases with age. The annual incidence of MDS is believed to be approximately 4/100000; which goes up to 20-30/100000 after 65 years of age and 65-100/100000 after 80 years of age.[1,2,3]

Etiopathogenesis

Etiology cannot be identified in most cases of MDS. Patients are under high risk of MDS or secondary AML development 5-10 years after exposure to alkylating agents. These cytotoxic agents may cause deletions in bone marrow, complex karyotypic abnormalities, and chromosomal abnormalities. Etiopathogenesis is better identified in MDS cases developing after topoisomerase II inhibitors such as anthracycline and etoposide. In addition to previous chemotherapy and radiotherapy; exposure to chemical agents, viral infections, and pesticides may lead to MDS development as well.[4]

Chromosomal structural abnormalities in hematopoietic stem cell lead to a malignant clone. This clonal disorder accompanied by increased blastic cell count and infiltration leads to transformation into leukemia. This process is accompanied by cytopenia pictures in patients. Other immunological abnormalities, which increase the susceptibility to apoptosis (programmed cell death), caused by this malignant clone are blamed in the etiopathogenesis as well.[5,6]

Clinical studies have shown that the most common clonal chromosom-

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al abnormalities are del (5q), trisomy 8, monosomy 7, inv (11), del 5 q, del (20q), -Y, del (12p), del (13q). Cytogenetic findings play an important role in supporting MDS diagnosis and determining its prognosis. MDS is identified as primary or secondary depending on whether the patient has been exposed to previous chemotherapy, radiotherapy, and other toxic agents. The rate of cytogenetic abnormalities is much higher in secondary MDS compared to primary MDS. Del(5q) should be separately discussed. This syndrome has a different clinical picture due to refractory anemia characterized by deletion in the long arm of the 5th chromosome. This syndrome is more commonly seen at later stages of life and in women, responds quite well to treatment, and has good prognostic characteristics. Patients often present with anemia with thrombocytosis in the peripheral blood. No increase in myeloblast count is observed in bone marrow.[7,8]

Diagnosis

MDS should be considered primarily in cases of unexplained cytopenia or monocytosis presence, especially in elderly patients. Exposure to toxic agents, previous chemotherapy or radiotherapy, alcoholism, and drug abuse should be carefully examined in patient's history. Peripheral smear (PS) and bone marrow aspiration and biopsy are gold standards in MDS diagnosis.[9] MDS patients are often asymptomatic at the time of diagnosis. Diagnosis is made after cytopenia picture observed in routine complete blood count. Physical examination may not show typical findings as well. Petechia and ecchymosis leading to suspicion of thrombocytopenia associated with deep cytopenias and severe thrombocytopenia-associated bleeding are observed. Pneumonia associated with neutropenia and granulocyte dysfunction, fever secondary to urinary system infection are usually seen due to complications in later stages of the disorder. Patients with cytopenia picture most commonly present with anemia. Splenomegaly, hepatomegaly, and lymphadenopathy are rarely seen. Splenomegaly may occur as a MDS-myeloproliferative disorder.[10]

Classification

Myelodysplastic syndromes were defined as Dysmyelopoetic Syndrome by British, French, and American hematologists for the first time in 1976. Since this classification could not predict transformation into acute leukemia, the international FAB classification was proposed in 1982. The FAB classification consists of 5 groups: refractory anemia (RA), refractory anemia with ring sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess of blasts in transformation (RAEBt), and chronic myelomonocytic anemia (CMML). The WHO classification was introduced by WHO (World Health Organization) in 2008 due to due to inadequacies of the the FAB classification. Different from the FAB classification, RAEB-T was removed from this new classification and a blast ratio >20% was defined as AML. The RAEB group was divided into two sub-groups as RAEB-I and RAEB-II depending on myeloblast count of 1-5% and 11-19%, respectively. RARS and RA were divided into two sub-groups, too, depending on presence of dysplasia in bone marrow lineages. The (5q-) syndrome, described above in detail, was recog-

nized as a separate entity. Cases that do not fit these groups were referred to as unclassified MDS.[11]

ın 2016, WHO introduced a new classification (Table 1). This classification involves the following:

- MDS with single lineage dysplasia (MDS-SLD),
- MDS with ring sideroblasts (MDS-RS),
- MDS with multilineage dysplasia (MDS-MLD),
- MDS with excess blast of 5-9% in bone marrow (MDS-EB-1),
- MDS with excess blast of 10-19% in bone marrow (MDS-EB-2),
- Unclassifiable MDS (MDS-U),
- MDS with 5q deletion,
- MDS Provisional entity: Refractory cytopenia of childhood Myeloid neoplasms with germ line predisposition Terms such as "refractory anemia" or "refractory cytopenia" were replaced with dysplasia.12

Prognosis

In addition to hemogram and bone marrow assessments, cytogenetic evaluation was established to be significantly effective in the prognosis of MDS. Thus, the International Prognosis Scoring System (IPSS) was developed by adding cytogenetic evaluation, blast count in bone marrow, and degree of cytopenia to prognostic measurements. Each of the 3 variables is scored from 0 to 2 and the total IPSS score is calculated by combining the scores for these variables (Table 2). The potential for survival and evolution to leukemia is predicted according to IPSS (Table 3).[13]

Table 2: International Prognosis Scoring System (IPSS)[13]

Score value	0	0.5	1	1.5	2
Bone marrow blasts (%)	<5	5-10		11-20	21-30
Karyotype (*)	Good	Intermediate	Poor		
Cytopenia (**)	0-1	2-3			

(*) Good: Normal, −Y, del (5q) del (20q), Poor: Poor, complex (≥3 anomalies) or chromosome 7 abnormalities. Intermediate: Other abnormalities. **(**)** Hemoglobin <10 gr/dL, absolute neutrophil count: <1.8 x 10^9/L, trombosit<100 x 10^9/L

Table 3: Overall survival and leukemia evolution potential according to IPSS[13]

Risk group	Score	Median survival (years)	Estimated time until AML evolution (years)
Low risk	0	5.7	9.4
Intermediate-1	0.5-1.0	3.5	3.3
Intermediate-2	1.5-2.0	1.1	1.1
High risk	≤2.5	0.4	0.2

After the IPSS was introduced, the importance of some cytogenetic markers in clinical follow-up of patients was better understood. This new prognostic scoring system was named "revised IPSS (R-IPSS)." Table 4, 5, and 6 show the details of R-IPSS, risk classification, expected survival time, and leukemic evolution risks.[12]

Table 4: R-IPSS[13]

Prognostic variable/ score	0	0.5	1	1.5	2	3	4
Cytogenetics (x)	Very good	-	Good	-	Intermediate	Poor	Very poor
Blast (%)	≤ 2	-	>2-<5	-	5-10	>10	-
Hb	≥10	-	8-<10	<8	-	-	-
Platelets	≥ 100	50-<100	<50	-	-	-	-
Absolute Neutrophil Count	> 0.8	≤ 0.8	-	-	-	-	

Greenberg PL, Tuechler H, Schanz J et al. Revised International Prognostic Scoring System for myelodysplastic syndromes. Blood 2012; 120: 2454-2465.

Table 5: R-IPSS risk scores[13]

Risk category	**Risk score**
Very low	≤ 1.5
Low	> 1.5 - 3
Intermediate	> 3 - 4.5
High	> 4.5 - 6
Very high	> 6

Table 6: Expected survival and AML evolution times according to R-IPSS risk category[13]

Risk group	Patient (%)	Median survival (years)	AML evolution (years)
Very low	19	8.8	Not Reached
Low	38	5.3	10.8
Intermediate	20	3	3.2
High	13	1.6	1.4
Very high	10	0.8	0.73

In addition to prognostic scoring systems described above; accompanying chronic kidney, heart, lung, and liver diseases stand out as other factors which affect the mortality. Further, the presence of mutations associated with bad prognosis (point mutations such as TP53, EZH2, ETV6, RUNX1, NRAS, and ASXL1) is another factor which affects the mortality directly.[13]

There are treatment options which are implemented according to MDS sub-type and may be relatively effective. The only curative option among these is allogeneic hematopoietic stem cell transplantation. However, this option seems to be applicable for patients with high risk of acute leukemia evolution and high IPSS and R-IPSS scores. The main treatment approach is to take the underlying clonal disorder under control, delay acute leukemia evolution, increase the life expectancy, and reduce the transfusion requirement. Since MDS patients are usually the elderly, it should be remembered that supportive replacement therapy is one of the methods that preserve the patient's quality of life.[14]

In conclusion, the predictive properties of IPSS and R-IPSS, the classification and prognostic scoring systems used for MDS today, are important for MDS patients. However, the undeniable effect of cytogenetic outcomes on the prognosis of MDS leads to the idea that treatment will involve target-specific methods in the future. Understanding the accompanying cytogenetic abnormalities and their outcomes will provide a better understanding of the disorder.

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CHAPTER 7

ISOLATED FETAL CEREBRAL VENTRICULOMEGALY

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INTRODUCTION

The term "fetal venticulomegaly" is used when the enlargement of fetal cerebral ventricles is seen antenatally. The diagnosis is made usually by obstetric ultrasonography or rarely by fetal magnetic resonance imaging (MRI). Although its incidence is approximately 2/1000 newborns, it is encountered relatively more frequently on prenatal ultrasound evaluations (Laurence et al., 1968; Gaglioti et al., 2009). In this chapter, the focus will be on the isolated fetal cerebral ventriculomegaly where no other accompanying imaging abnormalities could be detected antenatally.

Anatomy and Embryology of Cerebral Ventricles

Cerebral ventricular system includes two lateral ventricles, third ventricle and the fourth ventricle. Right lateral ventricle is located in the right cerebral hemisphere and the left lateral ventricle is located in the left cerebral hemisphere. Cerebrospinal fluid (CSF) is produced by choroid plexus, which is located in the ventricles (Damkier et al., 2013). CSF production is begun as early as 6th week of embryonic life (Kousi et al., 2016). CSF provides a cushion mechanism to protect neural tissues from trauma and it reduces tracking forces on brain stem. It also allows proper chemical stability for neural tissues. By means of foramina Monro and aqueduct of Sylvius, CSF flows into the third and fourth ventricle, respectively. Foramina Luschka and foramina Magendie provide exit points for the flow of CSF through cisterna magna. Lateral ventricles have unique structural shapes are called "horns". Depending on their projection sites, these horns called frontal, occipital and temporal horns. The convergence point of occipital and temporal horns is called "atrium".

Hydrocephalus and Ventriculomegaly

Even though the terms of hydrocephalus and ventriculomegaly are used interchangeably in many medical sources, they have different meanings. Hydrocephalus indicates an increased intracranial pressure and in the majority of cases enlarged ventricles are present (Tully et al., 2014). On the other hand, when an obstetric ultrasound refers to a fetal ventriculomegaly diagnosis, intracranial pressure increase may not necessarily accompany it (Pisapia&Sinha et al., 2017). Fetal cerebral ventriculomegaly can develop from reasons other than intraventricular increased pressure such as periventricular white matter abnormalities and cerebral atrophy.

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Naturally, obstruction of ventricular passage system in developing brain with different reasons such as developmental defects, hemorrhage, infection or tumor, may cause the enlargement of the ventricle system as in hydrocephalus.

FETAL VENTRICULOMEGALY

Enlargement of the fetal cerebral ventricles as commonly detected by antenatal obstetric ultrasonography (Guibaud et al., 2015) creates anxiety both for family and for the healthcare providers. If ventricle enlargement is only on one side, it is called unilateral ventriculomegaly. If both ventricles are affected it is called bilateral (symmetric) ventriculomegaly.

Even though the definition is not uniform across the medical literature, the most common criteria used to diagnose fetal ventriculomegaly is the measurement of ventricle diameter as 10 mm or larger. Measurement is made at the level of atrium of the cerebral lateral ventricle (ISUOG 2007; Pisapia&Rozycki et al., 2017). To provide uniformity among researches and scientific reports, use of a standardized technique for measuring fetal ventricle diameter has been recommended (Figure 1).

Figure 1*.

Correct measurement of fetal cerebral lateral ventricles by antenatal ultrasonography (ISUOG)
1. Transventricular plane: Skull intactness, bone density, falx cerebri occipital/posterior horn of lower lateral ventricle, frontal horns of both lateral ventricles. 2. In the symmetric axial view at optimal zoom, atrium of the lateral ventricle is measured at the level of the glomus of the choroid plexus, opposite to the parieto-occipital sulcus. 3. Calipers placement: the inner edge of the ventricle wall perpendicularly ➢ Measurement of lateral ventricle width ≥10 mm is used for upper limit of normal.

*ISUOG 2017 recommendations for standard measurement of lateral ventricles

There is also another subclassification that can be come across in medical literature: mild, moderate and severe ventriculomegaly based on the measured diameter of the lateral ventricle (10-12 mm, 13-15 mm and >15 mm, respectively) (Griffiths et al., 2010).

Etiology

The etiology of fetal cerebral ventriculomegaly is diverse. Intrauterine infections such as toxoplasmosis, cytomegalovirus, enterovirus, zika virus, chromosomal anomalies, genomic copy number variations, genetic syndromes such as X-linked hydrocephalus, Di-George syndrome and Pettigrew syndrome, intraventricular hemorrhage, cerebral hypoxia, teratogens such as isotretionoin, cerebral atrophy and masses may cause fetal cerebral ventriculomegaly and/or hydrocephalus (Kousi et al., 2016; Pisapia&Sinha et al., 2017; Melo et al., 2017).

MANAGEMENT OF ISOLATED FETAL CEREBRAL VENTRICULOMEGALY

The diagnosis of fetal cerebral ventriculomegaly is usually straightforward. By using standard measurement criteria, 10 mm or larger ventricle width is adequate for the diagnosis (ISUOG 2007). However, ventriculomegaly is usually a sign of a disease, a genetic abnormality or a developmental defect rather than an ultimate diagnosis (Darouich et al., 2017).

When fetal ventriculomegaly is the only abnormality that could be detected during antenatal ultrasonography, in other words, no other fetal organ system could be shown to have another structural abnormality, "isolated ventriculomegaly" term is used (Pisapia&Sinha et al., 2017). Sometimes, the use of fetal MRI may detect additional subtle central nervous system (CNS) anomalies that were overlooked previously (Griffiths et al., 2017). Detection of additional CNS or other system anomalies may help in the diagnosis of a known syndrome or a sequence. Therefore, every effort must be made to detect possible additional structural abnormalities, starting with a thorough ultrasound evaluation of CNS and other organ systems. Fetal MRI is strongly suggested in severe cases with lateral ventricle diameter 15 mm or larger. Even though use of fetal MRI on mild ventriculomegaly cases is rather controversial, many authors recommend its use (Mehlhorn et al., 2017). Because when ultrasound appearance is seemingly isolated, neonatal MRI may detect additional 6-7% cerebral anomalies in cases with euploid fetal mild cerebral ventriculomegaly (Baffero et al., 2015; Pagani et al., 2014). In another recent study, with the help of fetal MRI, additional abnormalities were detected in approximately 12% of cases with seemingly mild ventriculomegaly (Kandula et al., 2015). Furthermore, with the worsening ventricle enlargement in-utero over time, the possibility that fetal MRI would detect additional CNS anomalies is increased (19%) (Baffero et al., 2015).

Antenatal ultrasonography may have some inherent obstacles in providing clear images in certain clinic situations such as in maternal obesity and improper fetal position. Also, echogenic shadows and artifacts may sometimes obscure images. Therefore, fetal MRI may overcome this obstacle and catch additional anomalies that could be overlooked by antenatal ultrasound. Studies suggest that the most common fetal cerebral anomalies that were detected by fetal MRI in cases with seemingly isolated ventriculomegaly were

corpus callosum abnormalities (Griffiths et al., 2010). Other anomalies that fetal MRI usually helpful to detect are fetal cerebral parenchymal abnormalities, hemorrhage and gyration disorders (Baffero et al., 2015).

When antenatally an isolated ventriculomegaly is detected, it might be a good idea to repeat ultrasound evaluation monthly, because a previously undetected anomaly can be more discernible by advancing gestation. Repeat obstetric ultrasonography may also help to follow up on the progression of ventricular dilatation. Approximately 14% of cases with isolated mild ventriculomegaly worsen with advancing gestation (Kelly et al., 2001).

As imaging studies can provide valuable information for the infectious etiology such as in the findings of organ calcifications, ascites and organomegaly, maternal serum and amniotic fluid ELISA and PCR studies for suspected infectious agents can also be helpful in differential diagnosis.

A detailed family history can give important clues regarding an underlying familial genetic disease. Therefore, genetic counseling can be considered in cases with fetal cerebral ventriculomegaly even when they look like isolated. For example, L1CAM mutation analysis may be required in male cases with positive family history or in cases with severe ventriculomegaly (>15 mm) (Pisapia&Sinha et al., 2017).

In approximately 5% of cases with isolated mild fetal cerebral ventriculomegaly, a karyotype abnormality is found (Pagani et al., 2014). Isolated ventriculomegaly may sometimes be associated with submicroscopic chromosomal rearrangements such as microdeletions and duplications. Namely, when conventional karyotyping could not identify any numerical or structural chromosomal abnormalities, a chromosomal microarray study may detect genomic copy number variations in the additional 3-8% cases (Schumann et al., 2016; Li et al., 2017; Hu et al., 2017; Wang et al., 2018).

Differentiation of cases with isolated ventriculomegaly from the cases with ventriculomegaly accompanied by additional CNS abnormalities is important not only for the correct diagnosis, but also for family counseling and management. Cases with true isolated fetal cerebral ventriculomegaly have approximately 5-7% risk of neurodevelopmental delay (Pagani et al., 2014; Scala et al., 2017). When additional abnormalities are present, neurodevelopmental outcome may be more severe depending to the severity of the problem or to the type of syndrome. A proportion of these pregnancies deliver in preterm gestations. A recent observational research studied the neurodevelopmental outcomes of premature neonates with ventriculomegaly. When they have removed the cases with prematurity-related intraventricular hemorrhage from the statistical analysis, they found increased risks for neourodevelopmental impairement, cognitive impairement and cerebral palsy in extremely preterm neonates (odds ratios: 3.07, 3.23, and 3.68, respectively) (Pappas et al., 2017).

SUMMARY

In conclusion, antenatally detected fetal ventriculomegaly is a relatively common finding. In management, it is important to look for additional CNS or other system anomalies as it can help to identify etiology. In situations where antenatal obstetric ultrasonography fails to detect additional anomalies, fetal MRI can be useful. True isolated fetal ventriculomegaly may be associated with adverse neurodevelopmental outcomes even though the risk may be lower than that of severe (>15mm) cases with additional CNS abnormal findings. Besides the routine look up for infectious etiology by imaging studies for a marker, maternal serum/amniotic fluid evaluation for a specific infectious agent may be helpful. Chromosomal microarray studies can identify additional submicroscopic genomic alterations in a small subset cases with isolated fetal cerebral ventriculomegaly when karyotype results are normal. Pregnant patients with isolated fetal cerebral ventriculomegaly should be managed by a team of experts including a maternal-fetal specialist, neonatologist, and a radiologist who is experienced in fetal MRI studies.

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CHAPTER 8

ORAL ANTIDIABETIC DRUGS

Osman KUKULA[1]

Introduction

Diabetes is a chronic disease characterized by the inability of the pancreas to produce enough insulin or use insulin. The disease causes the socioeconomic burden due to the resulting acute-chronic complications, disability and decreased quality of life (1). Blood glucose levels are high in patients with diabetes and they cannot use the glucose in the blood. Its initial emergence is usually sudden and dramatic. Frequent urination, excessive thirst, recurrent infections, unexplained weight loss and fatigue are common symptoms.

Type 1 Diabetes

In type 1 diabetes, insulin is not enough or there is no production of insulin. In this type of diabetes insulin is quite a necessary medication. Of the people with diabetes, 5-10% has type 1 diabetes (7).

Type 2 Diabetes

Insulin production is present in type 2 diabetes, but patients cannot use insulin effectively. This type of diabetes is more common. Of the patients with diabetes, 90% consist of such patients (7).

Symptoms of Diabetes:

- Sensation of thirst and excessive increase in fluid intake
- Frequent and excessive urination
- Increased appetite
- Frequent and extreme hunger
- Weight loss
- Fatigue and weakness
- Blurred vision
- Numbness in feet, tingling

If the family has a tendency to diabetes, care should be taken in terms of lifestyle. Ideal weight should be maintained and necessary exercises should be performed. Exercise should be performed at least 3-4 times a week for 45 minutes (1). Risk factors should be avoided. One should be careful about

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nutritional habits and precautions should be taken against risk factors such as hypertension and cholesterol. Blood sugar level should be monitored carefully. High blood sugar accelerates atherosclerosis, causing plaques that clog the blood vessels (5). As a result, large vessels are also affected negatively by this process. Renal failure as a result of damage to the kidney vessels, and cataracts and hemorrhages occur due to damage in retinal veins. The damage to the nerves causes numbness and burning. Memory problems occur if brain vessels are affected, and cause vital problems such as stroke and hypertension (5).

Nutrition recommendations for patients with diabetes:

- Balanced and adequate nutrition
- Preservation of appropriate body weight
- Not skipping meal
- Consideration of drug intake time and dosage
- Adherence to the recommended exercises
- It is advisable to avoid smoking.

Oral antidiabetic drugs:

Insulin is a drug that cannot be taken orally. For this reason, drugs that can be taken orally, but act like insulin have been developed (2,3,10). They are used in non-insulin dependent patients with diabetes and only if blood glucose cannot be controlled through nutrition. The best-treated patients are the patients with diabetes mellitus for less than 5 years and occurred after 40 years of age (7,9). However, these drugs are not effective in childhood type diabetes. They can be used orally in type 2 diabetes. They can be divided into 4 groups according to their mechanism of action (Table 1).

Table 1: Classification of oral antidiabetics according to their mechanism of action

Insulin-releasingdrugs (Secretagogues)	Sulfonylureas Meglitinides
Drugs that increase insulin sensitivity	Biguanides Thiazolidinedione derivatives
Drugs that slow down glucose absorption	Alpha glucosidase inhibitors
Incretinmimetics	Glucagon-like peptide 1 agonists Dipeptidyl peptidase 4 inhibitors

Oral antidiabetic drugs:

- Sulfonylureas
- Glinides

- Biguanides
- Thiazolidinediones (Glitazones)
- Alpha glucosidase inhibitors
- Incretin-mimetics

Sulfonylurea Compounds:

The main mechanism of action is on the pancreas. They increase the release of insulin from the pancreas. Therefore, they have no effect on childhood diabetes, because there is an absolute lack of insulin (4,7,8). Insulin is produced in type 2 diabetes, but there is an insensitivity against the insulin. They may cause hypoglycemia also in normal individuals without diabetes. They can slightly suppress the glucagon release. They increase the binding of insulin, potentiating the effect of insulin (7,12).

The sulfonylureas reduce the conductivity of potassium channels that are sensitive to ATP, blocking potassium channels. Thus, they depolarize the membrane and cause calcium influx from the voltage sensitive calcium channels. They cause secretion of insulin by depolarizing the beta cells like glucose (6,7).

It is absorbed rapidly in the digestive system. If taken together with food, their absorption slows down. They bind to 90% of plasma albumin. Therefore, they can interact with drugs that show weak acid properties. They are metabolized primarily in the liver. Metabolites are excreted in urine. Therefore, it should be used with caution in patients with renal or hepatic disease (7).

The sulfonylureas are divided into two groups: first generation sulfonylureas and second generation sulfonylureas:

They are drugs discovered at different times. They are different from each other in terms of their effectiveness. The second generation is more effective, and the first generation is less effective. For this reason, second-generation drugs are given to patients with fewer doses.

First Generation Sulfonylureas:

The effect of tolbutamide from the first generation drugs lasts 6-10 hours.

Chlorpropamide: The half-life is 24-48 hours. It has antidiuretic effect.

Acetohexamide: It has diuretic and uricosuric effect.

Tolazamid: Has a diuretic effect.

Second Generation Sulfonylureas:

Gliburide (Glibenclamide): It has a diuretic effect.

Glipizide: The half-life is very short and 2-4 hours.

Gliclazide

Glimepiride

Side-effects of sulfonylureas:

- Hypoglycemia related reactions
- Allergic rashes on the skin
- Cholestatic jaundice. It is rarely seen only in the use of chlorpropamide.
- Hyponatremia
- Hematological toxicity: Agranulocytosis, Aplastic/haemolytic anemia. It's very rare.
- In particular, chlorpropamide creates a disulfiram-like effect when taken together with alcohol. It has a flushing effect. Manifestation is characterized by the inhibition of aldehyde dehydrogenase.
- Tolerance may develop. They may not be effective after 6-12 months of administration.

Glinides:

They are used orally and are insulin secretagogues.

Repaglinide is one of the drugs in this group. It is structurally different from sulfonylureas. It stimulates insulin release from pancreatic beta cells like sulfonylureas by closing ATP-sensitive potassium channels. It is rapidly absorbed through the stomach intestinal tract. The half-life is up to 1 hour. It undergoes metabolic reactions in the liver. Caution must be exercised in patients with liver and renal failure. The main side-effect is hypoglycaemia, as in of sulfonylureas (7,13).

Nateglinide is one of the drugs in this group. Its mechanism of action is similar to that of repaglinide and sulfonylureas. Reduces post-meal hyperglycemia in patients with type 2 diabetes. It causes less hypoglycemia (7,13). Basically it is metabolized in the liver.

Biguanide Compounds:

Their mechanisms of action and lack of hypoglycaemia are among their differences. They have no effect on the insulin secretion from beta cells of pancreas. They are drugs with non-pancreatic effects. They directly stimulate the anaerobic glycolysis, increase the use of glucose, remove glucose from the blood, reduce gluconeogenesis in the liver, reduce glucose absorption from the small intestine and reduce plasma glucagon level (7,11).

They increase glucose utilization and accelerate lactic acid formation. They can reduce glucagon levels in blood plasma. We can state that they do not cause hypoglycemia, different from sulfonylureas (7,11).

Fenformin: Due to lactic acidosis, it has been removed from the market in many countries.

Metphormine: It may be used singly or together with insulin and/or a sulfonylurea compound medication. It is mainly absorbed from the small intestine. It does not bind to proteins in plasma. It is discharged unchanged with urine. It has a half-life of about 2 hours.

Side-effects:

- In chronic use, it reduces the absorption of folic acid and vitamin B12 from the intestine.
- Tolerance development
- Lactic acidosis
- Diarrhea, abdominal cramps, nausea, vomiting,

Contraindications: heart failure, chronic hypoxic lung disease, hepatic failure, renal insufficiency.

Thiazolidinediones (Glitazones):

They are also called insulin sensitizers. They make the affected cells more sensitive to insulin.

Troglitazone is one of the drugs of this group. It has been withdrawn from the market since it causes severe hepatic necrosis.

Rosiglitazone and pioglitazone are drugs again of this group and have lesser effect on liver.

In particular, they bind and activate receptor-gama receptors that activate the peroxisome proliferator in adipose tissue cells. They have an agonist effect to these receptors. For the effectiveness, there should be insulin and resistance development in the environment (7).

They reduce insulin resistance in the periphery. They reduce the formation of glucose in the liver. It increases the synthesis of specific glucose-binding proteins, increases their translocation, and increases glucose passage into striated muscles and adipose tissue. They can activate genes associated with free fatty acid metabolism in the periphery (7,11).

Increase in body weight, water retention in the body, anemia, edema may occur as a side-effect.

Alpha Glucosidase Inhibitors:

They inhibit the alpha glucosidase enzyme in the intestine. Thus, they slow the absorption of carbohydrates. They reduce postprandial blood glucose. They can be used in combination with other oral medications. They can be used with insulin. They must be taken before meals (7).

Miglitol and acarbose are the drugs of this group.

During the first use, they cause quite discomfort for patients. They cause diarrhea, malabsorption, abdominal pain, gas discomfort.

Incretin-mimetics:

The release of insulin in the body is associated with glucose levels. Some studies have shown that the glucose delivered by intravenous and orally given glucose does not induce insulin secretion at the same rate. Glucose given orally has been found to further stimulate insulin secretion from the pancreas. Incretin hormones makes this difference. Incretins are secreted in the gastrointestinal tract after oral glucose intake and stimulate insulin release from the pancreas (7).

The effect of incretin is impaired in patients with type 2 diabetes and this is important in the adjustment of blood glucose level. Some of the agents that increase endogenous insulin secretion via incretin hormones and their inhibitors in the treatment of type 2 diabetes are now included in the treatment of diabetes (7).

These agents are as follows:

Incretinmimetics: Glucagon-like peptide 1 agonists (liraglutid, axathid)

Dipeptidyl peptidase 4 inhibitors (sitagliptin, vildagliptin)

Glucagon-like peptide 1 agonists are secreted from L cells in the intestinal mucosa. They have insulinotropic effect. They are effective in hyperglycemia after meals. Increases the insulin secretory effect of glucose. They have blood sugar-dependent effects, so hypoglycemia is not expected. Increases insulin release and production. Suppresses glucagon secretion. Its effect that increases the insulin secretion from pancreas and suppressing the glucagon release is a glucose-dependent effect. Prevents apoptosis, increases the proliferation of beta cells. It increases the feeling of fullness. Regulates the speed of gastric emptying. Causes weight loss. It is used subcutaneously. Their most important side-effect is nausea. (7).

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CHAPTER 9

PHAGE DISPLAY AND METHODS TO PRESENT THE TARGETS TO THE PHAGE DISPLAY LIBRARY: WHOLE CELL BIOPANNING

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Phage Display

The 2018 Nobel Prize in Chemistry is shared between two evolution-related topics; one half awarded to Frances H. Arnold "for the directed evolution of enzymes", and the other half jointly to George P. Smith and Gregory P. Winter "for the phage display of peptides and antibodies" [URL1]. Phage display, the basis of this review, was first discovered by George Smith in 1985 for the display of peptides on phage coat proteins and later modified by Gregory P. Winter for the display of antibody parts on phage coat proteins. The technique is one of the leading methods for selecting target specific peptides and monoclonal antibodies. It is a widely and effectively used method in which large peptide/antibody libraries are screened for a given sample target, in order to select target specific peptides or antibody fragments [Marr *et al*.,2011].

Targets for Phage Display Selection

Phage display has been used in many studies to select thousands of peptides/antibody fragments for different organic or inorganic target samples to evaluate molecular interactions, including identifying cellular receptors, screening epitopes or mimotopes, confirming peptides targeted specific cellular or tissue types [Rojas *et al*., 2014]. Here are the examples of countless studies reported in literature; studying protein-protein interactions [Kiewitz *et al*., 1997; Fuh *et al*., 2000; Hertveldt *et al*., 2009; James *et al*., 2009; Voss *et al*., 2009; Caberoy *et al*., 2010], enzyme-substrate interactions, catalytic region and activator-inhibitor determination in enzymology [Benhar *et al*., 2001; Kay *et al*., 2001; Kehoe *et al*., 2005; Diamond *et al*., 2007], determination of targeting antibodies for proteomics, drug release and intracellular processes [Smith *et al*., 1997; Benhar *et al*., 2001; Hoogenboom *et al*., 2005; Bratkovic *et al*., 2010], the detection of agonists and antagonists for the cell surface receptor structure [Deshayes *et al*., 2002; Koolpe *et al*., 2005], selection of peptides capable of binding to inorganic surfaces [Whaley *et al*., 2000; Huang *et al*., 2005; Tamerler *et al*., 2007], selection of human stem cell specific peptides [Bignone *et al*., 2016], gastric cancer cell specific peptides [Sahin *et al*., 2018], and neuroprotective agent neoechinulin A specific peptides [Kamisuki *et al*., 2018].

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Adalimumab (Humira) is one of the best examples of phage display-derived antibodies. It is a human IgG antibody that binds tumor necrosis factor (TNF) and blocks its activation of TNF receptors. Humira became the first phage display-derived antibody granted a marketing approval in 2002 and it is currently the best selling antibody drug on the market with over 18 billion dollars predicted sale in 2020 [Urquhart *et al*., 2018]. Numerous phage display-derived antibodies are currently under advanced investigation [Frenzela *et al*., 2016].

Phage Display Screening Methodology

Phage Display is based on the connection between the phenotype and the genotype of the bacteriophage used. The random peptide sequences encoded in the bacteriophage genome are expressed on specific proteins on the phage surface. In short, the change in genotype will be observed directly in the phenotype. The bacteriophages used are included in filamentous phage family and use different gram-negative bacteria as hosts. Commonly used M13 bacteriophage is a rod-like filamentous virus with 6 nm. in diameter and ~ 900 nm in length. The genome is single-stranded DNA and is packaged with 5 different types ofprotein. Approximately 2800 pVIII major coat proteins encircle the virus from one end to the other, while five copies of pIII and pVI proteins terminate one end, and pVII and pIX proteins close the other end [Deutscher *et al*., 2010; Pande *et al*., 2010) (Figure1).

Phage display libraries can be prepared to display short peptides (7-12 mino acids long short peptides) or relatively larger antibody fragments. Peptides have many advantages over monoclonal antibodies and large protein ligands, including small size, ease of synthesis, ease of tumor screening, and high biocompatibility [Borghouts *et al*., 2005; Qiu *et al*., 2007; Thayer, 2011; Sun *et al*., 2012]. In addition, the affinity, charge, hydrophobicity, and stability of the peptide can be chemically modifed. In this regard, the peptides may also be optimized for use *in vivo*. On the other hand, higher specificity can be achieved with longer antibody fragments having more definite three dimensional structures, and and antibody fragments can be directly used to obtain full size antibody molecules.

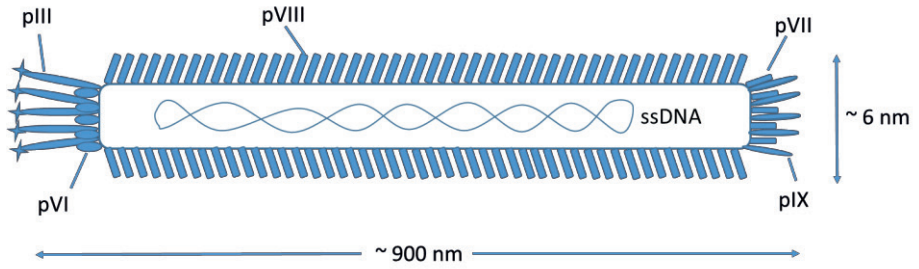

Figure 1: Schematic diagram of the M13 bacteriophage; protein coat composed of 2800 copies of pVIII major coat protein and 5 copies of each minor coat proteins, pIII, pVI proteins at one end and pVII and pIX proteins at the other end, encircling 6407 nucleotides long circular ssDNA.

Biopanning Procedure

For the isolation of target specific peptides based on their binding affinity to a given target, phage library is incubated with the target molecule for 3-5 cycles called biopanning (also called panning) or affinity enrichment [Parmley *et al.*, 1988; Alfaleh *et al.*, 2017] (Figure 2). First, the library is exposed to the desired target sample (antigen, cell, tissue, etc.) to capture specific phage binders. Then, non-selective or low affinity binders are eliminated by repeated washing steps and remaining strong binders are eluted and finally recovered phages are amplified by using suitable host bacteria. This process is a biopanning cycle and the final amplified phage pool is used as the phage library for the second round of panning. After each biopanning round, the diversity of phage is reduced while the proportion of selective strong affinity binders to the target is increased (enrichment) [Vieira *et al.*, 1987, Winter *et al.*, 1994, Deutscher *et al.*, 2010; Pande *et al.*, 2010].

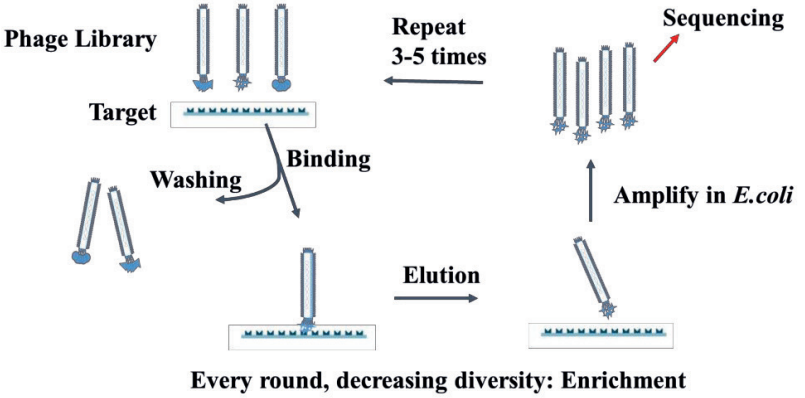

Figure 2: Biopanning procedure of a phage display library to select phages binding to desired target.

Presenting Target To The Phage Display Library

One of the most important factors in the panning procedure is how to present the target to the peptide/antibody library to ensure that it is displayed in its correct conformation (Figure 3). If the target antigen can be purified and immobilized onto plastic surface, the library can be applied directly onto the immobilized antigen. Although having a relatively simple and homogeneous surface (just the target layer as the binding surface) will decrase the level of background non-specific binding, immobilization may cause a conformational change of the target antigen which may change the binding level of selected peptides on the target *in vivo*.

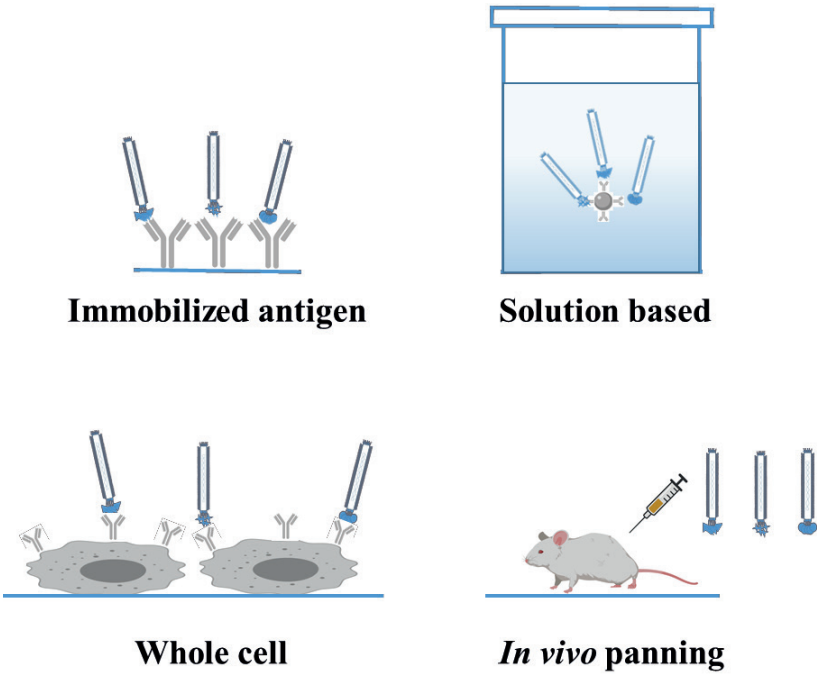

Figure 3: Presenting the library to the phage display library; immobilized antigen, solution based antigen presentation, whole cell panning and *in vivo* panning.

As an alternative, solution-based panning using biotinylated antigens [Fellouse *et al*., 2017] or calmodulin binding peptide-tagged antigen [Mukherjee *et al*., 2015] can be used to present the antigen to the library without immobilizing the antigen onto a surface. Streptavidin coated beads and calmodulin beads are used to capture the biotinylated or calmodulin binding peptide tagged antigens, respectively. In this way, the effect of immobilization on the conformation of antigen will be eliminated, but still the antigen must be purified and tagged properly with a tagmolecule.

Immobilized or solution-based methods use a specified target antigen molecule and search for specific peptides for antigens by using panning procedures. When there is no information about the antigen molecule, or the antigen molecule on the membrane is unavailable to be purified, whole cell can be used as a target molecule to select a specific peptide/antibody. Here, the method is actually an *in vitro* cell based panning. Target cells are attached on cell culture flasks or targeted in suspension form. [Rader *et al*., 1997; Siva *et al*., 2008; Krag *et al*., 2006; Dantas-Barbosa *et al*.,2012].

Whole cell or cell-based biopanning is one of the commonly used phage display panning method to screen for peptides or antibodies against the antigens in their natural conformation. Cell-surface antigens may have complex conformational constraints, such as multiple membrane-spanning regions or protein interaction domains, in addition to glycosylation and other post-trans-

lational modifications. Using the cell directly for the biopanning process instead of purified and immobilized antigen ensures the isolated binder peptides/antibodies to access epitopes *in vivo*. Additionally, whole-cell panning identifies targets that are expressed in sufficient quantities on the cell surface which enables the detection of binding with standard flow cytometry [Cai and Garen, 1997; Palmer *et al*., 1997; Liu *et al*., 2004; Popkov *etal*.,2004]. Themethodcanalsobeusedfortheselectionofpeptides/antibodieswhichcan discriminate between different cellular states, and it will be useful when the antigen of interest is not available in pure form [Rader *et al*., 1997].

The largest group of drug targets for the selection of peptides/antibodies for diagnostic or therapeutic purposes are membrane proteins such as receptor tyrosine kinases, immunoglobulin-like receptors, G protein-coupled receptors and ligand-gated ion channels. These proteins account for 20 to 30% of all proteins in most living entities and represent 44% of human drug targets, playing key roles in transportation and signal trunsduction [Alfaleh *et al*., 2017]. Normal activity levels of some membrane receptors change in some diseases such as cancer. For instance, human epidermal growth factor receptor 2 (Her2) is overexpressed in breast cancer cells. Her2 receptor is a possible target membrane protein for the selection of breast cancer specific peptides or antibodies [Slamon *et al*., 1989] which can be targeted by whole cell phage display panning in its natural conformation [Alfaleh *et al*., 2017]. In biopanning procedure, the library is first depleted against non-cancerous cells which is called subtractive panning and then incubated with cancer cells for the selection of peptides specific for oveexpressed membrane proteins.

Several studies in literature carried out whole cell panning against cancer cells. In these studies, the cells are mostly attached on cell culture flasks and phage display peptide/antibody library is applied directly on the attached cells after a subtractive panning step for each round. Subtractive panning aims to deplete the library of non-specific binders by incubating the starting phage library first with normal cells and non-specific binders for normal cells are taken away to use as phage library for the cancer cells [Bakshinejad *et al*, 2018]. Using subtractive panning in each biopanning step decreases background binding and significantly improves the chance of obtaining specific phages with high binding selectivity towards target cancer cells. Bakhshinejad and Nasri, (2018) applied three rounds of biopanning against human non-small cell lung carcinoma (A549) (along with other cancer cell types) and screened a phage display library of 7-mer random peptides in-vitro. Human normal lung epithelial cells SAEC and human normal fibroblast cells were used as control cells for the subtractive panning steps. About 170-fold increase in phage recovery efficiency was achieved after three rounds of panning and LCP1 (Lung Cancer Peptide1-AWRTHTP) was identified as the most effective binder which selectively binds on cancercells.

In our previous study [Sahin *et al*., 2018], we applied 5 rounds of biopanning against gastric cancer cells (MKN45) and identified one experimental and one designed peptide binders (designed by using the amino acid frequen-

cies of experimental binders) which selectively bind on cancer cells but not on normal HFE-145 stomach cells.

***In vivo* Biopanning:** Instead of using target cells in suspension or attached on to surface, the panning can be applied *in vivo*, targeting the tissue directly. There are currently different *in vivo* panning techniques such as bacteriophage, aptamer libraries and viruses as platforms which use peptides, DNA/RNA and viruses, respectively, as library type [Gustafson *et al*., 2018]. To screen phage display libraries for specific binders *in vivo*, the library is injected into organism and incubation is performed *in vivo* condition. To obtain the specific binders, target tissue is isolated and binding phages are amplified by using host *E.coli* cells. In principle, the technique is similar with *in vitro* whole cell panning with the target antigens in their nativeconformations.

Similarly, the cells in whole cell panning will be in their intact form and having a preserved cell surface topography and conformation of cellular receptors similar to *in vivo* panning conditions [McGuire *et al*., 2014]. On the other hand, in *in vivo* panning, phage particles can be absorbed largely by the reticuloendothelial system present in organs such as liver before reaching target tumor tissues [Bakshinejad *et al*, 2018; Aina *et al*., 2002; Molenaar *et al*., 2002] which will be followed by clearance of phages from the blood and the whole body. Also, capillary vessels of the vascular system form a barier for phages to pass through and most phages are cleared through adhering to endothelial cells before reaching the tumor site of interest causing selection of vascular endothelium binding peptides instead of tumor (target) specific peptides [Pasqualini *et al*., 1996; Rajotte *et al*., 1998; Arap *et al*., 2002].

Conclusion

Phage display is a widely used method for drug discovery, studying protein-protein interactions, in vitro protein evolution, target specific peptide and antibody selection, detection of agonists and antagonists, and many other therapeutic and diagnostic purposes. The cycles for the enrichment of the phage display library against a desired target is called biopanning and presenting the target antigen to the library is one of the most important factors of the panning procedure. Among with other techniques, whole cell biopanning, a technique of presenting target antigen to the phage library, makes the membrane proteins available in their natural conformations which are mostly used targets for drugdiscovery.

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CHAPTER 10

BACTEREMIA IN DENTISTRY AND ORTHODONTICSY

Yasin AKBULUT[1]

Bacteremia and Infective Endocarditis

The bacteria that normally live in the skin, bowels, urinary tract, mouth and the upper respiratory system can enter the bloodstream, and this is called bacteremia (1). The entry of a small number of bacteria, which is generally lower than 10 CFU/ml, into the systemic bloodstream, which is regarded sterile, through an entryway in the body, and the presence of these bacteria in the circulation for as long as 30 minutes is called transient bacteremia (2, 3). Following the entry of the bacteria into the systemic bloodstream, they form three different bacteremia as temporary, intermittent and persistent bacteremia. Temporary bacteremia may occur even after tooth brushing while intermittent bacteremia enters into the bloodstream from a source such as cellulitis or peritonitis. Persistent bacteremia occurs due to the direct entry of the bacteria through a catheter (4-7). Temporary bacteremia may occur as a result of a minor trauma or hemorrhage following medical procedures conducted in mucous membranes containing a high number of bacteria such as an oral cavity, urogenital system or gastrointestinal system. Briefly, the infections of the inner surface of the heart, the endocardium layer, or the cardiac valves are called infective endocarditis. In individuals with congenital cardiac anomaly or acquired cardiac diseases, pathogen microorganism such as the bacteria and fungi contaminating the blood increase the risk of infective endocarditis, which is serious and sometimes fatal.

Phases of Bacterial Endocarditis Formation

1. Accumulation of platelet or fibrin that causes non-bacterial thrombotic vegetation on the surface of the damaged endocardium.

2. Entry of bacteria into the systemic bloodstream through any area in the body, causing the temporary bacteremia

3. Sticking of bacteria to the non-bacterial thrombotic vegetation

4. Reproduction of bacteria inside the vegetation

5. Emerge of systemic and local sequelae due to bacterial endocarditis (8, 9).

The bacteria that enter into the systemic bloodstream exist without exhibiting clinical symptoms (10-12). In most individuals, the blood circulation becomes sterile within 20 minutes (13). In healthy individuals, the microor-

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ganisms that enter into the systemic bloodstream are cleaned by the reticuloendothelial system while in patients with congenital cardiac anomaly or acquired cardiac diseases, it is possible for endocarditis to occur. Because the cause of endocarditis is mostly bacteria, it is also called bacterial endocarditis (14). Endothelial damage may occur even due to blood flow into a problematic area of cardiac valves or the heart, and endothelial damage is a significant factor in the formation of bacterial endocarditis. The thrombocyte and fibrin accumulated in the damaged area cause non-bacterial thrombotic endocardial lesion (vegetation). In the case of bacteremia, bacteria settle into this vegetation through the systemic blood circulation, causing bacterial endocarditis (15-17). In a study, it was reported that body piercing, substance use or excessive alcohol consumption of patients with cardiac diseases increased the probability of the occurrence of bacterial endocarditis (18).

Embolic occurrences with unknown causes, lack of appetite, loss of weight, incipient murmur and fever are among the main signs of bacterial endocarditis (19). The diagnoses of bacterial endocarditis are made with the help of vegetation in echocardiography, orifices in abscess and prosthetic cardiac valves (20,21), positivity in blood culture, immunological tests and molecular biology techniques (22-24), and with Modified Duke Criteria (25).

Modified Duke Criteria (Diagnosis, Prevention and Treatment of Bacterial Endocarditis / 2009 update) (25)

<table>
<tr><td colspan="2">MAJOR CRITERIA</td></tr>
<tr><td colspan="2">Positive Blood Culture in terms of Bacterial Endocarditis
• Typical microorganisms compatible with bacterial endocarditis in two distinct blood cultures:
Viridans streptococci, Streptococcus bovis, HACEK group, Staphylococcus aureus or
Enterococci acquired from the society provided that they are not the primary focus
• Microorganisms compatible with bacterial endocarditis in persistent positive blood cultures:
• Positive results obtained in at least two blood cultures taken 12 hours apart
• Positive results obtained in all of the three separate blood cultures or in most of the four separate blood cultures (provided that the first and last samples were taken at least 1 hour apart)
• Single positive blood culture for Coxiella burneteii or phase I IgG antibody titer > 1:800</td></tr>
<tr><td colspan="2">Evidence of endocardial involvement
• Positive echocardiography for bacterial endocarditis
• Vegetation-abscess-incipient partial dehiscence of prosthetic valve
• Emergent valvular deficiency</td></tr>
<tr><td colspan="2">MINOR CRITERIA</td></tr>
<tr><td colspan="2">• Predisposition: Predisposing heart condition, intravenous drug abuse
• Fever: body temperature of >38°C
• Vascular phenomena: glomerulonephritis, Osler’s nodes, Roth’s spots, Rheumatoid factor
• Microbiological evidence: positive blood cultures but major criteria are not met or serological evidence of active infection with microorganism compatible with bacterial endocarditis</td></tr>
<tr><td>Definitive bacterial endocarditis diagnosis if the following are present:
• 2 major criteria or
• 1 major and 3 minor criteria or
• 5 minor criteria</td><td>Possible bacterial endocarditis diagnosis if the following are present:
• 1 major and 1 minor criteria or
• 3 minor criteria</td></tr>
</table>

Bacteremia Originating from Oral Flora

The entry of the bacteria found in normal oral flora into the systemic bloodstream following dental procedures conducted in the mouth can cause infective endocarditis, infection of distant areas and brain abscess, which can cause serious morbidity and mortality (26). While these bacteria are cleaned within minutes by the reticuloendothelial system upon entering into the bloodstream of a healthy person, there is a risk of infective endocarditis occurrence following

bacteremia in individuals with congenital cardiac anomaly or acquired cardiac disease. Along with differences between groups evaluated in the society, it is encountered at an annual rate of 3-11.6 in a hundred thousand individuals (27-30). Bacterial endocarditis is encountered more in adults compared to children (31). According to a study conducted in France, it was determined that bacterial endocarditis was encountered at high rates after the age of 50, and this rate peaked around the age of 70 (29). Thus, it is suggested that attention should be paid in dental procedures and antibiotic prophylaxis should be conducted for these patients prior to these procedures (32-35).

Poor oral hygiene was pointed out as the most significant cause of infective endocarditis (36). In 1909, it was reported by Horder (37) that microorganisms found in the oral flora had a rather significant role in the occurrence of bacterial endocarditis. Following this statement, oral flora microorganisms were found in the blood cultures taken from patients with bacterial endocarditis diagnosis (37).

Researchers revealed that a significant rate of bacterial endocarditis cases was due to dental procedures. Rajasuo et al. (38) reported that 14-20% of the bacterial endocarditis patients were due to dental procedures while Droz et al. (39) reported that 30% of them were due to dental procedures. Drangsholt (40) reported that 8-10% of the patients with bacterial endocarditis were due to dental procedures without bleeding. Roberts et al. (41) reported that bacteremia was observed even after rubber dam and matrix band without bleeding. In the study conducted by Akbulut et al. (42) in 2018, it was reported that bacteremia occurred following debonding procedures without bleeding.

In dental procedures, all of the procedures that cause bleeding cause bacteremia. While it may occur following any type of surgical procedure, it may also occur following non-interventional procedures such as crown preparation, dental plaque removal and orthodontic band placement (43). All of the procedures that result in gingival bleeding cause bacteremia at a serious rate (44). In a study, it was reported that even daily oral care procedure caused bacteremia (45).

Although the number of bacteria in the systemic blood for the occurrence of bacterial endocarditis is not entirely known, the number of bacteria originating from oral flora was determined as $1x10^2$ CFU/ml. The number of bacteria required for the experimental formation of bacterial endocarditis in studies conducted with animals was determined between $1x10^6$ and $20x10^6$ CFU/ml. When the values presented are compared, it is observed that even the existence of lesser amounts of bacteria is enough for bacterial endocarditis in humans (46).

Epidemiology of Bacteremia Originating from Oral Flora

Periodontal probing caused bacteremia in 40% of the patients with periodontitis. While rubber dam and matrix band placements caused bacteremia in 31% of the healthy individuals, bacteremia was observed in 32% of the individuals following restoration wedge. In blood cultures taken from children following various dental interventions, 38 different bacteria were observed (47).

In the study conducted Debelian et al. (48) in 1995, it was reported that during and after endodontic treatment of asymptomatic teeth with apical periodontitis, bacteremia was detected in 34-54% of the individuals. Following local anesthesia conducted with the vestibule infiltration technique, bacteremia was observed in 16% of the patients while it was observed in 97% of the patients following local anesthesia conducted with intraligamentary technique, and bacteremia occurred in 50% of the patients following the anesthesia implemented in the attached gingiva.

In a study conducted by Coulter et al. (49) with children in 1990, it was determined that temporary bacteremia occurred following tooth extraction. Along with this, it was reported that the incidence of bacteremia was reduced from 63% to 35% by antibiotic prophylaxis.

Orthodontics and Bacteremia

In the orthodontics literature, the studies conducted until today reported that bacteremia could occur during molar band placement and removal (50-53). It is known that fixed orthodontic appliances increase the incidence of gingival enlargement, and patients cannot completely provide oral hygiene while these appliances are in the mouth. Schlein et al. (54) detected bacteremia at a rate of 25% in the blood cultures taken after tooth brushing from patients who underwent fixed orthodontic treatment.

Bacteremia frequently occurs following various dental procedures. This state causes bacterial endocarditis in cardiac disease patients with certain predisposing factors. These predisposing factors may include congenital or acquired cardiac malformations, previous infective endocarditis, rheumatoid arthritis, acquired valvular dysfunctions, hypertrophic cardiomyopathy, mitral valve prolapse with valvular regurgitation, prosthetic cardiac valves and surgically placed pulmonary shunts (52).

In the literature review, researchers were observed to focus on orthodontic band placement and orthodontic band removal in studies conducted on bacteremia (50-53, 55, 56).

In the study conducted by McLaughlin et al. (24) in 1996, bacteremia investigations were conducted in 30 patients before and after the band implementation. Two different blood samples were taken from the patients, one being just before the band implementation and the other being 60 seconds later than the band placement. Bacteria were observed in one of the samples taken prior to the band implementation and in three of the samples taken following the band implementation. In both cases, the bacteria, *Streptococcus parasanguinis* and *Streptococcus mitis* were observed.

In the study conducted by Burden et al. (27) in 2004, bacteremia investigations were conducted in 30 patients following bracket removal along with a band. In their study, two different blood samples were taken, one being before the removal and the other being after the cleaning procedures along with the removal. Bacteremia was observed in 1 patient with the blood sample taken

before the removal and in 4 patients with the blood sample taken after the removal. *Streptococcus viridans* was predominately detected in these 4 patients.

In the study conducted by Erverdi et al. (25) in 2000, bacteremia occurrence was investigated in 30 patients before and after both band removal and debonding. In this study, bacteremia was observed in the samples taken before and after both debonding and band removal. While *Streptococcus salivarius* and *Streptococcus* parasanguinis were observed in the blood samples taken at first, *Streptococcus parasanguinis* and *Streptococcus mitis* were observed in the blood samples taken following the procedures of debonding and band removal.

In the study conducted by Gürel et al. (57) investigating the bacteremia following rapid maxillary expansion (RME) appliance, *Streptococcus parasanguinis* and *Staphylococcus aereus* were observed, and they suggested prophylaxis before the removal of orthodontic RME appliance.

Furthermore, for orthodontic stripping, mini-screw insertion and removal procedures, fixed treatments were reported to produce the bacteremia (58, 59). In both studies, researchers detected *Streptococcus sanguinis*.

Akbulut et al. (42) investigated bacteremia following orthodontic debonding procedures. In this study, the band was not used, and only the patients with brackets and molar tubes were chosen. Even though no blooding occurred during the procedures, bacteremia was detected. In this study, *Streptococcus viridans, Streptococcus mitis, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus oralis Staphylococcus aereus, Actinomyces oris, Actinomyces naeslundii* and *Klebsiella pnömonie* were also detected. As a conclusion of this study, it was reported that bacteremia could occur by causing bacteria entry into the blood even after debonding procedures without bleeding, and it was argued that prophylaxis implementation could be beneficial for patients in the risk group.

Microorganisms of Bacterial Endocarditis

The oral cavity is a rich environment that harbors a large number of microorganism. Because different microorganisms are present in certain areas in the oral cavity, the microflora of each area is different (60-62). Providing that oral hygiene is decent, the total number of the microorganisms in the oral cavity decreases, and a rich microflora is formed by the aerobes (63). It was reported that a majority of the bacteria obtained in the blood cultures taken after dental procedures were anaerobic (38). In the study conducted by Okabe et al. (64) in 1995, it was reported that only 2% of the bacteria, which caused bacteremia, were aerobic.

In several studies, it was reported that in blood cultures taken from individuals who performed daily hygiene procedures and who underwent dental treatment, 275 types of bacteria entered into blood (65, 66). In a study, it was reported that more than 500 types of bacteria existed in the oral flora (67) and more than 700 types of bacteria were reported in another study

(68); and it was also reported that it was possible to isolate each one of these types of bacteria (69-71). In such an intense bacterial environment, bacteremia occurs repeatedly during the day in individuals especially with gingivitis and periodontitis (72). In an individual with periodontitis, the dentogingival pocket surface area is approximately 8-20 cm^2, and the ulcerated parts of the pocket create a perfect environment for the bacteria to enter into the bloodstream (73). Tooth surface, salivary gland and gingival sulcus contain more of certain microorganisms (60, 62, 63, 71). Even a decrease in the salivation or a deterioration in its structure cause changes in the microbial count (74).

Causes of bacterial endocarditis are bacteria, certain fungi and certain microorganisms (75). Among these microorganisms, the most common cause of bacterial endocarditis is regarded as the Viridans group streptococci. However, it is known that other types of bacteria also cause bacterial endocarditis (38). Types of streptococci, especially *Streptococcus viridans,* are the most important microorganism of oral flora causing bacterial endocarditis (76, 77).

The most commonly observed bacteria in the blood cultures obtained from surgical procedures conducted in oral tissues are *Streptococcus viridans* (78). They could be present solely or together with other bacteria. Because the microorganisms causing bacterial endocarditis are that diverse, a multidisciplinary approach is necessary for its treatment (79, 80). The incidence and severity of bacteremia are proportional to the procedures conducted in periodontal tissues and the intensity of the trauma (81).

In recent years, the staphylococcus type of bacteria was shown to be the responsible pathogen for half of bacterial endocarditis patients. In approximately 70% of the cases with bacterial endocarditis developed due to IV drug use, the responsible type of microorganism was also shown to be staphylococcus (81).

In the study conducted by Okell and Eliot (82) in 1935, it was reported that streptococcus types of microorganisms were effective in the 64% of patients with bacteremia out of 138 patients who had a tooth extraction. Lipopolysaccharides, which are among the components of bacteria that cause oral infection, can cause disruptions in blood coagulation mechanism, prostaglandin release and platelet formation by causing atherosclerosis, leading to cerebral or myocardial infarction (83).

Epidemiology of Bacterial Endocarditis

According to a study, 14-20% of the causes for patients with bacterial endocarditis were oral flora (84). Researchers revealed that a significant portion of the bacterial endocarditis cases was due to dental procedures. Rajasuo et al. (38) reported that 14-20% of the bacterial endocarditis patients were due to dental procedures while Droz et al. (39) reported that 30% of them were due to dental procedures. The results of several studies vary depending on the individuals' status of oral hygiene and antibiotics prophylaxis (84-86). Various types of bacteria are detected in the blood cultures taken following interventional dental procedures. Bleeding and trauma caused mainly as a re-

sult of procedures conducted on gingiva are shown to be reasons for bacteremia. The epidemiology of bacteremia depends on how invasive the conducted intervention is. Due to this information, among dental procedures, tooth extraction is believed to be the one with the highest possibility of causing bacteremia. It was reported that even tooth brushing, dental floss use and chewing any object could cause bacteremia at a rate of 15-80%. The prevalence rate of bacteremia in children who underwent tooth extraction was determined to be 65%. The main source of oral flora-based bacteremia, whose presence is detected in the bloodstream, is gingival sulcus (87-89). All these elements point to the importance of a decent oral hygiene and dental examination for bacteremia and bacterial endocarditis (90). Following bacteremia, in healthy individuals with no heart anomaly, the number of bacteria in the systemic bloodstream rapidly drops within 10-30 minutes, rendering the blood sterile again (38).

Prevalence of bacteremia occurring following dental procedures and hygiene procedures (81)

Oral/Dental Procedures	**Prevalence of Bacteremia**
Single Tooth Extraction	51%
Multiple Teeth Extraction	68-100%
Root canal treatment where canal instrument does not reach to the tip of the root	~31%
Root canal treatment where canal instrument reaches to the tip of the root	~54%
Periodontal surgical procedures where the flap is removed	36-88%
Gingivectomy	83%
Scaling and root planning	8-88%
Periodontal prophylaxis	~40%
Tooth brushing	~26%
Dental floss use	20-58%
Interproximal tooth brushing	20-40%
Dentogingival irrigation	7-50%
Chewing	17-51%

The rate of infective endocarditis occurring due to dental treatment was reported to be 15% (51). In a study conducted with 20 patients who underwent fixed orthodontic treatment, it was reported that bacteremia occurred at a rate of 25% in the blood taken after tooth brushing (91).

In previous studies, it was proven that antibiotics administered prophylactically reduced the prevalence and severity of bacteremia (26, 77). In several studies, antibiotics' mechanisms of preventing bacteremia were explained by the elimination of the microorganisms approaching endocardium via the preventing microorganisms from sticking to the cardiac valves (92, 93).

In the study conducted by Roberts (9) in 1999, it was suggested that bacteremia occurred even in the absence of apparent bleeding, and bleeding was

not absolutely necessary for the occurrence of bacteremia. In this case, the occurrence mechanism of bacteremia was explained by the notion that bacteria were drawn inside the blood vessel by the negative blood pressure occurring due to microscopic damages in the blood vessels following dentogingival manipulations in the capillary. Bacteremia occurrence was observed due to the intermittent negative and positive pressure cycle occurring in gingival blood vessels due to these manipulations. Within this cycle, the movement of any tooth inside the socket was deemed sufficient.

Bacterial endocarditis could pose life-threatening circumstances for patients with congenital or acquired cardiac diseases (94). Streptococci of oral flora bacteria were frequently held responsible for bacterial endocarditis. Thus, prophylaxis is a significant factor to pay attention, especially in surgical and dental procedures regarding nasopharynx, esophagus, neck and mouth (95).

Prophylaxis

Antibiotics implementation before procedures in order to prevent microorganism colonization independent of any infection and reduce the possibilities of complications following the operation to a minimum is called prophylaxis (96). Prophylaxis is suggested for bacterial endocarditis due to high morbidity and mortality (26). The aim of prophylaxis is to reduce the bacterial endocarditis risk to a minimum by preventing bacteremia via antibiotics or to prevent bacterial endocarditis by preventing bacteria from sticking to the endothelium by changing their properties (93). Although the effect of antibiotics on bacterial endocarditis is not completely known, a study reported that antibiotics prevented bacteria from sticking to the cardiac valves, eliminating the bacteria that reached endocardium (97). In many studies, it was determined that antibiotics reduced the prevalence and the severity of bacteremia (26, 32-34, 49, 92, 93, 95, 97-99). According to a study conducted with rats, antibiotics were effective at the last step of the formation mechanism of bacterial endocarditis (97).

In time, belief and implementations regarding antibiotics prophylaxis have gone through changes. Results of novel studies conducted with animals, results of pharmacokinetic tests, antimyogram tests, antibiotics efficacy tests and studies conducted on bacteremia have caused these changes (100-106).

The necessity of antibiotics prophylaxis is still a topic of discussion today. In studying discussing the necessity and protective efficiency of prophylaxis premedication in patients of risk group, conducted various evaluations (9, 107-109). Prophylaxis protocols are planned according to the standards and suggestions of several medical institutions such as American Heart Association (AHA), American Academy of Orthopedic Surgeons (AAOS), American Dental Association (ADA) and British Dental Association (BDA) (111). Apart from these suggestions, various suggestive protocols also exist on a country basis (111, 112). Even though different scientific associations have adopted different antibiotic prophylaxis protocols, three distinct factors play roles in

the determination of these:

1. Cardiac pathologies with bacterial endocarditis risk
2. Dental procedures which have a possibility to cause bacterial endocarditis
3. Type and dosage of the antibiotic which is considered to be used to prevent bacterial endocarditis

The most commonly used protocol for antibiotic prophylaxis is the one suggested by AHA (113-116), and this protocol is exactly adopted and used by ADA, CDA and BDA (117). Infective endocarditis was a serious disease, which was globally fatal before the use of sulfonamide agents in the late 1930s and especially before the use of penicillin in the mid-1940s. It is still a significant cause of mortality and morbidity. Today, infective endocarditis still affects the health of 1.58 million patients (118). AHA has been making suggestions to prevent infective endocarditis since 1955. Firstly, in 1995, the definition of various forms of infective endocarditis was made, and suggestions were made for the diagnosis and the treatment of *Streptococcus viridans* infection. These suggestions are continuously updated on a periodic basis. AHA, which provided continuous updates by presenting various protocols in time, has made the latest update in 2015 (119).

In the last decade, the changes occurred in the epidemiology of infective endocarditis and in the characteristics of the disease along with the emergence of *Staphylococcus aereus*, which is an etiologic agent of infective endocarditis, the importance of infective endocarditis has increased further. Some of these changes are the decrease in the number of patients with rheumatic heart disease, increase in the average age of patients, increase in the number of patients with cardiac valve, increase in the rate of patients with infective endocarditis, who underwent cardiac surgery and increase in the rate of using other cardiac devices (120-122).

The detailed explanations regarding the characteristics of infective endocarditis in children were specified in the scientific statement, "Infective Endocarditis in Childhood: 2015 Update", by AHA Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee (123). In this statement, a significant amount of emphasis was placed on the general oral hygiene by the author group. The major reason for this is the studies that demonstrated that bacteremia occurring due to daily oral activities form a more frequent and larger bacterial load compared to dental procedures (65).

Patients with Prophylaxis Risk Factors

AHA and ADA have listed the patient groups below, which could cause bacterial endocarditis by developing bacteremia during dental procedures. High and intermediate-risk groups are the cases for which antibiotic prophylaxis should be implemented. For the low-risk group, it was reported that the prevalence of bacterial endocarditis is not different from the general population, and antibiotic prophylaxis is not necessary (114, 115).

Conditions where Endocarditis Prophylaxis is suggested

According to the classification of conditions under endocarditis risk published by AHA, the patients that require prophylaxis implementation are classified into two groups as high and intermediate-risk groups (113-115).

High-Risk Group

- Patients with prosthetic cardiac valves or the presence of prosthetic material used in cardiac valve repair, patients with bioprosthetics and hemograft,
- Patients with previous bacterial endocarditis,
- Patients with cardiac transplantations who developed cardiac valvulopathy,
- Patients with complex cyanotic congenital heart disease (such as tetralogy of Fallot, single ventricle conditions, transposition of large arteries),
- Patients with surgically placed systemic pulmonary shunt or canals (113-115, 119, 124).

Intermediate-Risk Group

- Congenital cardiac malformation apart from those listed above and listed in the low-risk group (PDA, ASD, VSD, coarctation of the aorta, bicuspid aortic valve),
- Those who underwent valve repair without using prosthetic material,
- Acquired valvular dysfunction (rheumatic fever, Kawasaki disease, collagen tissue diseases (SLE, rheumatoid arthritis, ankylosing spondylitis)),
- Hypertrophic cardiomyopathy,
- Valvular dysfunction/valve regurgitation or mitral valve prolapse together with a thickened valve (113-115, 119, 124).

Conditions where Endocarditis Prophylaxis is not suggested

Low-Risk Group

This group does not carry higher risks than the general population. According to the AHA's classification of the conditions under the endocarditis risk, the group that does not need prophylaxis implementation is classified as the low-risk group (113-115, 119, 124). The individuals in this group are listed as the following:

- Patients with isolated secundum atrial septal defect,
- Patients with surgical repair due to atrial septal defect, ventricular septal defect or patent ductus arteriosus and who do not develop an opening for longer than 6 months,
- Patients who previously underwent coronary artery bypass graft

surgery,

- Patients with a physiological, functional and innocent heart murmur,
- Patients with previous Kawasaki disease without valve dysfunction,
- Patients with previous rheumatic fever without valve dysfunction,
- Patients who carry a cardiac pacemaker and an implanted defibrillator.

Diseases that require prophylaxis apart from cardiac diseases

Certain patients require prophylaxis apart from those with cardiac diseases (113-115, 119, 124). These are as the following:

- Uncontrolled Type I Diabetes Mellitus,
- Patients who undergo chemotherapy and radiotherapy,
- Dialysis patients with a permanent vascular catheter,
- Diseases that cause immunosuppression (AIDS, leukemia, multiple myeloma, aplastic anemia etc.),
- Those who use immunosuppressive drugs (organ and bone marrow transplantation, SLE, rheumatoid arthritis, Behçet disease etc.),
- Patients who carry joint prosthesis (except for pins, plates and screws) require antibiotic prophylaxis in healthy individuals for two years following the operation,
- Hemophilia disease,
- First four week following coronary stent implementation,
- Those who carry cerebrospinal shunt due to hydrocephalus,
- Patients with previous prosthetic joint infection.

Dental Procedures that require antibiotic prophylaxis

- Dental procedures that will result in serious bleeding conducted in intraoral soft and hard tissues,
- All of the surgical procedures including tooth extraction,
- Dental implant implementation,
- Intraligamentary anesthesia,
- Subgingival strip or fiber placement,
- Endodontic treatment that has a possibility to go beyond the apex,
- All of the periodontal treatments including scaling, curettage, root planning, periodontal pocket measurement,
- Re-implantation of an avulsed tooth,
- In case it is believed that bleeding will occur in tooth or implant cleaning,
- Orthodontic band placement and removal (115, 124).

Dental Procedures that do not require antibiotic prophylaxis

- Restorative treatment in which bleeding is not expected

- Endodontic treatments which are inside the canal
- Post placement
- Measuring sizes
- Postoperative suturing
- Topical fluoride implementation,
- Local anesthesia implementation (except for intraligamentary),
- Taking radiographs,
- Rubber-dam implementation,
- Fluoride implementation,
- Placement of removable orthodontic and prosthetic appliances,
- Placement and removal of orthodontic appliances and brackets* (except for band) (115, 124).

* Although it is stated that prophylaxis is not required in the removal of brackets in these references, Akbulut et al. (42) stated in their study that bacteremia occurred following the removal of brackets.

For patients with cardiac disease and who enter into the high and intermediate-risk groups, preoperative prophylaxis implementation should be conducted if at least of the dental procedures that require prophylaxis will be conducted. Considering the procedures that will be conducted in some of the dental procedures, separate clinical evaluations should be carried out for each patient (115)

Prophylaxis Guide

Although the success of antibiotic prophylaxis on bacterial endocarditis is not entirely clear, it is a fact that antibiotics play a role in the phases of bacterial endocarditis formation (125). In previous studies, it was reported that antibiotics prevented bacteria from sticking to the cardiac valves, eliminating the bacteria that reached endocardium (97). Antibiotics decrease the severity and the prevalence of bacteria (126).

In a study conducted in 1985, upon observing erythromycin's gastrointestinal problems and stomachache in 530 patients, AHA suggested clindamycin use instead of erythromycin in patients with penicillin allergy (127). However, despite this suggestion, several researchers still suggest erythromycin use (128).

Despite all these suggestions, the hypersensitivity and anaphylactic shock that can be developed against penicillin in patients should not be ignored. The anaphylactic shock's incidence rate against penicillin is 0.04-0.11%, and this rate increases by receiving penicillin via the parenteral way (129).

Along with all these information, another point that should not be ignored is the fact that unnecessary use of antibiotics causes anaphylactic shocks and increases microorganisms' resistance to antibiotics (130). To set an example on *Staphylococcus aereus*, which is one of the most commonly observed microorganisms in blood cultures, despite these bacteria gained resistance against methicillin in USA at a rate of 25% and in Canada at a rate of less than

5%, it was reported that they gained resistances of 30-50% around Latin America and above 30% in Turkey (133, 134).

AHA Infective Endocarditis Prophylaxis Guide (115, 124)

Situation	Antibiotic	Regimen: Single Dose* 30-60 minutes before the procedure	
		Adults	Children
Oral	Amoxicillin	2 g	50 mg/kg
Unable to take oral medication	Ampicillin	2 g IM/IV	50 mg/kg IM/IV
	Cefazolin or Ceftriaxone	1 g IM/IV	50 mg/kg IM/IV
Allergic to penicillin or ampicillin	Cephalexin**	2 g	50 mg/kg
	Clindamycin	600 mg	20 mg/kg
	Azithromycin or clarithromycin	500 mg	15 mg/kg
Allergic to penicillin or ampicillin and unable to take oral medication	Cefazolin or Ceftriaxone***	1 g IM/IV	50 mg/kg IM/IV
	Clindamycin	600 mg IM/IV	20 mg/kg IM/IV

* Total dose for children should not exceed the dose for adults.
**or at adult and pediatric doses, 1st and 2nd generation other oral cephalosporins ,
*** Cephalosporins should not be used in individuals with a history of anaphylaxis, angioedema, or urticaria with penicillins or ampicillin.

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CHAPTER 11

SOME OBSERVATIONS ON THE THIRD PERSON CONTRIBUTION AS SOCIAL INFLUENCE ON THE ARTICULATION RATE OF THE PEOPLE WHO CLUTTER

Ahmet TARCAN[1]

Introduction

Cluttering is one of the fields that is the least studied and the least published about communication, language and speech disorders. While a part of the studies in this field addressed the relationship between cluttering and brain functions (Ward et al. 2015), an important part of them compared these cases with stammering within the scope of fluency disorders (Op't&Uys 1974, Van Zaalen, Wijnen. Jonckere 2009). No study has been encountered that questions whether the social environment has an impact on cluttering cases.

Cluttering is a disorder which is hard to diagnose as it may exist together with other disorders . While most of the authors in this field consider cluttering a language and speech disorder, there are some authors who consider cluttering a communication disorder. "The speculative literature available indicates that clutterers are most likely to be confused with stutterers, and other speech and language problems, such as misarticulations, are quite common in the disorder" (Myers& St. Louis 1986:38). "Stuttering and cluttering are both fluency disorders, albeit having largely opposite characteristics. The common thread however is the contribution of the environment towards the two disorders" (Kelkar& Mukundan 2016:10).

The literature review by Kenneth O. St. Louis demonstrated that researchers reported at least 65 different indications for cluttering cases. St. Louis who analyzed opinions of the authors of six different sources written between 1964-1986 emphasized that the "rapid speech rate" was the most significant indicator that the researchers (5 out of 6) agreed upon. For the authors reviewed by St. Louis, "rapid speech rate" ranks first, "articulation disorders" ranks second and "bad handwriting" ranks third (Myers&St. Louis 1986).

Purpose of Study:

As described above, high speech rate and thus high articulation rate is referred to as the most significant indicator that distinguishes cluttering from other disorders. Moreover, the highest priority target of the therapy process in cluttering is to lower the high speech rate (Percevault 2015:84). This study questions if the high articulation rate changes under social influence in cluttering cases. In other words, the impact of the social environment on development and severity - in terms of articulation rate - of the disorder is the subject of this study.

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Cluttering and Social Influence

An individual's behaviours are sometimes limited and sometimes encouraged by the social environment starting from the early childhood. Language acquisition starts under such social encouragement and thus social influence. In adulthood, social norms and the social environment maintain their gradually increasing impact on the individual's language and speaking action. In healthy individuals, the social environment influences the use of the individual language on the level of sound, word and syntax. On the other hand, every environment where social interaction occurs (family, friends, education etc.) can affect speech disorders. There are studies which report that stammering, one of fluency disorders, is affected by the socio-cultural environment. For example, Johnson (1955) has reported that "Shoshone locals in the US do not have stammering" (as cited in Özgür 2003:74). Starkweather et al. who sought for the reasons for stammering explained the influence of the social environment in the Demands and Capacities theory. This theory asserts that children are exposed to the demands of the social environment above their own capacities in the language development process. "When a child's capacities do not keep pace with environmental demands, the stage is set for stuttering to develop." (as cited in Siegel 2000:322).

Today's people who live gradually more rapidly in large cities have an important time problem. The necessity of completing daily work in city centres' requires individuals to be faster. Eating fast, walking fast, fast transport, fast communication (speaking, listening, writing, reading) etc. Therefore, urban life, differently from rural life, prompts the individual to speak faster. Boutet reported that people in urban areas of France speak faster than those living in the rural (Boutet 1997:18). Of course, urban life might not be the sole reason for cluttering. However, it is not possible to conclude that "speech rate" which is one of the important components of cluttering is not affected by the metropolitan lifestyle and time stress. A statistical study into cluttering in urban and rural areas would provide more concrete data. At this point, however, it can be estimated that metropolitan life might negatively affect the speech rate in certain individuals with cluttering tendency. Time problem created by urban life can lead to weaker communication even in small social groups such as family. A person who feels required to say more in less time under the time pressure will be forced to generate more words in unit time, which might lead to accelerated speaking rate as well as articulation disorders. In this respect, some patients can attribute their problem to wrong attitudes of the individuals in their social environment and not providing the patient with sufficient time.

Even though there is no study available which directly examines into the effects of the social environment on cluttering, it is understood between the lines of publications that social interaction has an impact on cluttering. For example, a study conducted by Langova on 57 cluttering cases reported that individuals with cluttering correct their speech while talking to superiors and have impaired speech while talking to those in their inner circle (as cited in Op't &Uys 1974:1626). The influence of the social environment emphasized

in Langova's work has also been affirmed by further authors. For example, Desportes & Meyer reported that patients can control themselves in formal communication environments (e.g. with superiors and in test conditions) (Desportes & Meyer 2014:14).

Methods

The study was conducted based on voice records of an adult who reside in Diyarbakır city and have cluttering symptoms. Written permission was obtained for voice and image recording to be used in "linguistic publications". Diagnosis was made with the triple test method recommended by Myers and St. Louis (1986) and the Predictive Cluttering Inventory (PCI) developed by David A. Daly (2006) and participant was considered to be possible cluttering cases. This pre-diagnosis was confirmed by the assessment of the voice recordings by the speech therapists.

The participant was 20 years old, university student. Interviews with the participant were maintained around 15 minutes, and interviews were made on the same hour of the day. The participant is native speaker of Turkish. It was observed that the participant of the study did not have a concrete indication of stammering, and sometimes had problems with fluent and understandable speech. Considering the hand writing of the participant, it was hard to read at some points.

Procedure

In the study, the social influence is designed and observed in two different sessions: In the first session (15 minutes), the researcher and the participant talk to each other. The researcher asks the participant a series of questions about the participant's profession or education. In this second session (a week later-15 minutes), a third person is included in the talk to create the social influence. The third person does not talk at all and only participates in the interview as a listener to measure the social influence in a controlled way on a minimum level. Therefore, the impact of this person on the participant through behaviors such as speaking, asking, interrupting etc. is precluded and it is tried only to measure the effect of the presence of the third person. The third person is a 42 year old male master's degree student residing in the same city.

Articulation rate

As discussed above, the articulation rate is calculated by cutting out the gaps (longer than 250 split seconds) in the speech. In recent studies, it is preferred to calculate the articulation rate rather than the speech rate (Van Zaalen et al. 2009-Chon,Sawyer,G.Ambrose 2012). This study measured articulation rates of the participant before and after the social influence[2] . Data obtained from this measurement was provided in numbers. However, this study is mainly a qualitative study. Numeric data is provided in tables for the study

2 In this study, the term "social influence" means adding a third person in the speaking process.

to be clear, understandable and concrete. As the sentence length has an effect on the articulation rate (Siegel 2000:322), it is important to keep the length of the sentences analyzed in the study within a certain standard for objectivity of the study. In this study, intelligible utterances with 40-90 syllables were preferred.

However, individual differences and instant emotional conditions affect the speech rate[3]. For example, while anger, enthusiasm, fear and happiness lead to accelerated speech, discomfort, sadness, grief and repulsion lead to slowing down (Trouvain 2003:15). Daily tiredness[4] also may have an effect on the speech rate.

In such studies, even though external factors (age, gender etc.) could be equaled, it is very hard to completely equal internal factors (the person's emotional state, emotional state during recording, excitement etc.). Therefore, it is not possible to generalize the articulation rate in this study. It is important to state that the results are only specific to the participant in this study and are limited to this study.

Acoustic analysis

The selected sentences were analyzed in Praat (Boersma & Weenink-version 5412) software, and articulation rates were calculated. While calculating the articulation rates of the sentences, silent and filled pauses longer than 250 millisecond were cut out. Cutting out the pauses requires attention; it is important not to cause an artificial impairment (overcut, wrong cut etc.) or telescoping Sentences analyzed from the speech texts and articulation rates are provided in the table below (Table 1).

first session			second session		
Time (sec)	Number of syllables	Articulation rate	Time (sec)	Number of syllables	Articulation rate
5.4	46	8,51	8.17	65	7,95
8.5	63	7,41	7.43	70	9,42
12	99	8,25	5.76	45	7,81
10	76	7,6	10.55	88	8,34
10.32	82	7,94	9.38	75	7,99
Average	7,94		Average	8,30	

While the difference between the lowest and the highest articulation rates of the participant in the first session is 14,84 %, this difference was 20.61 % in the second session of the participant when there was a third person and the social influence was measured (The lowest articulation rate 7,81 and the highest articulation rate 9,42). It means that the articulation rate of the

3 Studies conducted in different languages in the world to measure the speech rate demonstrate that every language naturally has a certain normal speed. For example, this rate is 4.34 syllables per second in English and 5.73 syllables per second in French (Trouvain 2003:8). Different results can be obtained in different dialects of a language in different regions (Schwab et al. 2012:525).

4 Some sources report tiredness increases cluttering (Percevault 2015:10). As different times of the day have an impact on tiredness, the interviews were held between 12.30 and 13.30 in the afternoon.

participant was negatively affected by the third person in the group and increased accordingly (Table 1).

Within the scope of this study it can be concluded that the third person in the group caused an increase in the articulation rate. The third person involved in the speaking process can be expected to increase the speech rate also in normal healthy individuals. Nevertheless, this increase almost never brings a problem of understandability. The important thing for this study is to determine the influence of the third person and thus the group size in those with cluttering tendency in whom the speech rate leads to disorder. Since the speech rate is one of the main indicators of this disorder and lowering the rate is among the main and priority targets of the treatment process. Therefore, the impacts of the third person factor on the speech rates of normal persons and clutterers are completely different in terms of consequences.

Discussion

The social environment can be expected to exert control on the individual and approximate the speech to standard values. For example, a person can select the words more carefully and pay more attention to the language in a group under the social influence. As mentioned above, within the framework of this study, we draw attention to the fact that the social environment might have a negative rather than positive impact in terms of control of the speech rate.

Identifying the impacts of the social environment on cluttering will lead to reassessment of the techniques used in the treatment process and considering the social influence in the therapy process. Even though the social environment does not solely cause this disorder, identifying the impacts which might trigger, develop or prevent this disorder can create significant potential results for preventing and treating cluttering.

It was explained in previous paragraphs that there are many factors which influence the speech rate. Therefore, this study does not aim to reach a final judgment that the sole reason for the increase in the speech rates of the participant is the social influence. Because, many factors such as emotional (excitement) states of the participant on that day or in that hour, content of the subject discussed, command of the speaker on the subject, length of the sentence etc., might have caused an increase in the speech rate.

On the other hand, considering the study results alone with the observations on the participant, it can be concluded that the articulation rate and severity of the disorder are directly proportional. The participant with higher articulation rate has a more severe understandability problem and has more parameters such as omissions, filling word frequency, unnecessary word repetitions.

In this study, it was tried to discuss the effect of the social influence in the simplest way possible. The social influence, undoubtedly, is a phenomenon

which is a deeper way and has different dimensions from those discussed in this article. In future researches, the study can be designed in three sessions, and it can be discussed if the articulation rate in the third sessions when there is no third person recedes back to the value in the first session.

The study can be repeated by increasing the group size and adding 4th, 5th, 6th persons in the group. Therefore, it can be observed more clearly whether there is a correlation between the group size and the articulation rate gradually. Moreover, it can be examined whether the third person or other persons in the group increase other traits of the disorder than those of the articulation rate, such as omissions, phonetic changes, filling sounds, repetitions etc.

It can be assumed that the quality as well as the quantity of the group the individual lives in may have an impact on the articulation rate. Although the verification of this hypothesis is something difficult/troublesome, it can be possible through controlled experiments conducted with differentiated groups in terms of features such as education, gender, age, etc.

Conclusion

"The deceleration of articulation rate" which is one of the most important and prior components of therapy process is a rather hard period with regard to both therapist and clutterer. The negative effect of high articulation rate can be avoided in two ways: trying to reduce the existing high articulation rate through various techniques, and intervening in conditions causing high articulation rate. Thus, knowing the positive and negative impacts of social circle on articulation rate in detail, and taking precaution against it accordingly may have significant contributions to the therapy of the disorder.

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CHAPTER 11

ANTIEMETIC DRUGS

Osman KUKULA[1]

Introduction

Nausea and vomiting are among the most common clinic symptoms. Nausea and vomiting can occur due to both the medication and non-medication reasons.

Vomiting occurs by contraction of the diaphragm and abdominal muscles, and stomach contents are excreted through the mouth. Before vomiting, a discomfort sensation is felt, and then nausea and vomiting occurs. Both nausea and vomiting are called emesis. Emesis is accompanied by yellowing on face, increased salivation and sweating (1).

From a neurophysiological point of view, vomiting is a complex reflex and is managed by the vomiting center. Nausea and vomiting are signs of some diseases, not a disease alone. The center that coordinates the vomiting is located in the brain near the chemoreceptor trigger site, which is located at the base of the 4th ventricle (2,4,9).

There are different receptors in the chemoreceptor-stimulating site and in the gastrointestinal tract: For example, dopaminergic receptors, histaminergic receptors, cholinergic receptors, opiate receptors, etc. The substances that stimulate vomiting stimulate serotonin release in the gastrointestinal tract. Secreted serotonin induces nausea by stimulating the chemoreceptor triggering site through serotonergic 5-HT3 receptors (9).

Other nausea-forming agents, especially drugs used in cancer treatment, stimulate vomiting through these receptors. Antiemetic drugs prevent nausea and vomiting.

Depending on the medication, or without medication, emesis may occur due to different reasons. Nausea and vomiting occur in many conditions such as inflammatory conditions in the gastrointestinal tract, myocardial infarction, myocardial insufficiency, migraine, anxiety disorders, diabetes mellitus and pregnancy (8,11).

Nausea and vomiting may occur as a side-effect due to drug use. It may occur due to the use of cancer drugs or long-term antibiotic use. Emesis caused by cancer drugs is associated with the amount of serotonin in intestinal mucosa and the brain stem (9).

Nausea and vomiting may develop due to vertigo, depending on the infections, traumatic or psychogenic conditions. It may also occur idiopathically.

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Scopolamine or antihistamine may be used in these patients. Scopolamine can be used for nausea related to motion sickness (7).

Nausea and vomiting are experienced in the early stages of pregnancy. Nausea and vomiting during the pregnancy may be mild to severe enough to affect daily life. 5-HT3 blockers may be used, dopamine antagonists may be used, or corticosteroids may be used (9). If there is hyperemesis, ondansetron is administered (9).

We can group antiemetic drugs as follows:

Antiemetic drugs

1. Scopolamine
2. Antihistamines
3. Neuroleptic drugs
4. Metoclopramide and the like
5. Domperidone
6. Trimetobenzamide
7. Antiserotonergic drugs
8. Cannabinoids
9. Glucocorticoids
10. Benzodiazepines
11. Substance P receptor antagonists

1. Scopolamine:

It has spasm relieving effect on smooth muscles in the gastrointestinal tract, bile ducts and urinary canals. Scopolamine does not enter the central nervous system. Therefore, it does not cause anticholinergic side-effects in the central nervous system. Peripheral anticholinergic effects depend on the blocking effect on the visceral ganglia and its antimuscarinic effect (12).

It has parasympatholytic effect. It inhibits cholinergic synapses on the vestibular pathway in the brain stem. It is used for motion sickness. It has a short duration of action, antihistamines are preferred over long journeys (9,12).

2. Antihistamines:

Antiemeticly used: Diphenhydramine, dimenhydrinate, hydroxyzine, sinarizine, buclizine, meclizine, promethazine (9).

-In cases of motion sickness and other nausea-vomiting of vestibular origin (Meniere syndrome, labyrinthitis, etc.).

-In pregnancy vomiting (hydroxyzine is not used)

-In vomiting due to toxins, radiation and cancer drugs (especially meclizine and promethazine)

-In postoperative vomiting (promethazine and hydroxyzine may be used).

-As side-effects, they can cause parasympatholytic side-effects (blurred vision, dry mouth and constipation effects) and drowsiness. Teratogenic effect is not shown in pregnant women, hydroxyzine is not recommended (9).

3. Neuroleptic drugs:

Phenothiazine derivatives:

- Fluphenazine
- Prochlorperazine
- Perphenazine
- Thiethylperazine
- Trifluoperazine
- Promazine
- Chlorpromazine

Butyrophenone derivatives:

- Haloperidol

They act by blocking dopaminergic receptors in the chemoreceptor trigger site (3,9).

Emesis indications:

- Emesis due to urea and other exogenous toxins
- Emesis due to radiation sickness
- Chemotherapy-induced emesis
- Postoperative vomiting
- Pregnancy vomiting (not used for a long time).

Side-effects:

- Autonomic side-effects
- Weight gain
- Sedation
- Extrapyramidal side-effects
- Sexual dysfunction
- Allergic reactions

- Hepatotoxic effect

4. Metoclopramide and the like:

They have a motility-enhancing effect on the gastrointestinal tract. They inhibit central and peripheral effects of apomorphine. They are central effective antiemetic drugs. They have a motility-enhancing effect on the upper part of the digestive system. They make tissues sensitive to the effect of acetylcholine. They increase the severity and tone of contractions, especially in the antrum section of the stomach. They increase the movements in the duodenum and jejunum section of the small intestine, and have a relaxant effect on the pyloric sphincter. Thus, they accelerate the emptying of the stomach, while accelerating the passage of food from the intestines. They do not affect the movement of the colon and gallbladder. They have no effect on the pancreas, bile and gastric secretions. They block D2 receptors, block 5-HT3 receptors and activate 5-HT4 receptors (6,9).

Indications:

- In postoperative vomiting,
- In vomiting occurred in chemotherapy and radiation therapy,
- In vomiting due to toxins,
- In vomiting due to morphine and other narcotics.

They can be used in pregnancy vomiting, but are not recommended.

5. Domperidone:

It is similar to metoclopramide in terms of medication effect. It acts by blocking dopaminergic D2 receptors. It has no serotonergic or antiserotonergic effect. It enters very little into the central nervous system and therefore has little side-effects. It's a drug with antiemetic indications similar to metoclopramide (9).

Domperidone is a dopamine antagonist and is a drug with antiemetic properties. It cannot pass the blood brain barrier easily. Extrapyramidal side-effects are rarely seen in domperidone users, but domperidone increases prolactin secretion from the pituitary gland. The anti-emetic effect depends on the gastrokinetic effects and the antagonism of the dopamine receptors in the chemoreceptor trigger site. Animal studies have shown that domperidone is effective on dopamine receptors especially in the periphery (9,10).

If taken on an empty stomach orally, its plasma level will peak in 30-60 minutes. The bioavailability is low when taken orally. Low bioavailability depends on the first pass metabolism in the liver. The absorption of domperidone is impaired in the case of decreased gastric acid (3,9,10).

6. Trimetobenzamide:

It weakly blocks the dopaminergic D2 receptors. Its antiemetic effect is low. It is a drug with little side-effects. It has anticholinergic properties. Its mechanism of action is not fully known, but it is believed to be effective on the chemoreceptor trigger site. The emetic stimuli are transmitted to the vomiting center via this site in the medulla oblongata. Trimethobenzamide is believed to directly affect the chemoreceptor trigger site. It does not directly affect the vomiting center (5).

Clinical use:

It is used in vomiting in the gastroenteritis and postprandial vomiting. Trimetobenzamide is not recommended in pregnancy vomiting. The use of trimethobenzamide should be avoided in patients with acute vomiting. Rarely, Parkinson's symptoms and hypersensitivity reactions can be seen as side-effects. Vertigo, headache, blurred vision, dizziness, coma, depression, disorientation, drowsiness may occur (5,9).

7. Antiserotonergic drugs:

Ondansetron, granisetron and tropisetron are in this group. They block 5-HT3 receptors. They're expensive drugs. Their efficacy increases if combined with dexamethasone. Ondansetron is a potent and selective 5-HT3 receptor antagonist drug. The mechanism of action in nausea and vomiting is not fully known. Chemotherapeutic agents and radiotherapy initiate the vomiting reflex via 5-HT3 receptors by secretion of serotonin in the small intestine. Ondansetron plays a role in blocking the onset of this reflex (9).

Granisetron acts as a selective 5-HT3 receptor antagonist. Granisetron has no or little effect on other serotonin receptors (9). The 5-HT3 type receptors are located in the peripheral vagal nerve terminals, and centrally in the chemoreceptor trigger site. In the chemotherapy, mucosal enterochromafine cells secrete serotonin and as a result stimulate 5-HT3 receptors. In this way, vomiting is induced by vagal route. Granisetron inhibits serotonin secretion with 5-HT3 receptors and prevents nausea-vomiting (9).

Tropisetron is a potent and selective antagonist of 5-HT3 receptors. Tropisetron selectively blocks the stimulation of presynaptic 5-HT3 receptors of peripheral neurons. Tropisetron can also directly affect 5-HT3 receptors in the central nervous system (9).

Clinical use:

- In chemotherapy-induced emesis
- In radiotherapy-induced emesis
- In postoperative emesis
- Headache and constipation are the most common and frequent side-effects.

8. Cannabinoids:

Dronabinol and nabilon are used as antiemetic. Dronabinol is a drug derived from marijuana.

They activate cannabinoid CB1 receptors in the brain in the vomiting center and surrounding neurons (9).

Clinical use:

- In chemotherapy-induced emesis
- In anorexia
- In AIDS

Side-effects:

Central sympathomimetic effect on central nervous system

Marihuana-like effect

Withdrawal symptoms when treatment is discontinued

9. Glucocorticoids:

The mechanism of action in antiemetic use is unclear. They are believed to act through inhibition of prostaglandin synthesis or preventing the permeability increase of the blood-cerebrospinal fluid barrier by cytotoxic drugs. Dexamethasone, methylprednisolone can be used for this purpose (9).

Clinical use:

In chemotherapy-induced emesis (especially dexamethasone is used)

They act by inhibiting prostaglandin synthesis. They prevent the cytotoxic drugs from increasing the permeability of the blood-brain barrier.

As side-effects, they can lead to osteoporosis that suppresses calcium absorption ability of the body. They may increase cholesterol and triglycerides. They increase the risk of ulcer and gastritis. Delay wound healing. They suppress the immune system and make it more susceptible to infections. Use of glucocorticoids may cause cushing syndrome (9).

10. Benzodiazepines:

They have no specific antiemetic properties. Their amnestic, sedative, anxiolytic effects contribute to the treatment of nausea-vomiting (9).

11. Substance P receptor antagonists:

The Substance P has been shown to be effective in delayed emesis occurred in antineoplastic treatment. (9).

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