MULTIDISCIPLINARY APPROACH IN MEDICAL SCIENCE II

EDITOR
Assoc. Prof. Dr. Hüseyin KAFADAR

İKSAD
Publishing House

MULTIDISCIPLINARY APPROACH IN MEDICAL SCIENCE II

EDITOR

Assoc. Prof. Dr. Hüseyin KAFADAR

AUTHORS

Assoc. Prof. Dr. Safak KARACAY

Assist. Prof. Dr. Fatih TAŞ

Assist. Prof. Dr. Sumeyra KOCTURK

Lec. Dr. Seyyide SARIÇAM İNCE

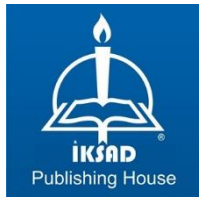
Dr. Ali Zeki BEDIR

Dr. Burcu UNER

Dr. Duygu YILMAZ

Dr. Juste BARANAUSKAI

Dr. Pankaj DWIVEDI



(The Licence Number of Publicator: 2014/31220)
TURKEY TR: +90 342 606 06 75
USA: +1 631 685 0 853
E mail: iksadyayinevi@gmail.com
www.iksadyayinevi.com

It is responsibility of the author to abide by the publishing ethics rules.
Iksad Publications – 2022©

ISBN: 978-625-6380-50-9
Cover Design: İbrahim KAYA
December / 2022
Ankara / Türkiye
Size = 16x24 cm

CONTENTS

PREFACE

Dear scientists and dear readers,

As in other fields of science, medical sciences are rapidly developing and being updated. Today, due to the direct and indirect relations of science fields, the scientific importance of multidisciplinary and even interdisciplinary applications is increasing.

Recently, serious discussions have been made on scientific platforms and scientific congresses have been held in order to emphasize the importance of multidisciplinary interdisciplinary practices.

I sincerely congratulate all writers and scientists who contributed to the book titled "**MULTIDISCIPLINARY APPROACH IN MEDICAL SCIENCE-II**" as chapter author. I would also like to thank all the publishing house managers and staff who contributed at different stages of the book.

For our valuable readers, we aimed to contribute to the scientific community by bringing together the subjects related to medical science with a multidisciplinary and interdisciplinary perspective.

Good reading.

Assoc. Prof. Dr. Hüseyin KAFADAR[1]

[1] Harran University, Medical Faculty, Department of Forensic Medicine.
Email: dr.hkafadar@gmail.com **ORCID:** 0000-0002-6844-7517

CHAPTER 1

OVERVİEW OF MYIASIS IN A SUSPECTED INTESTINAL CASE

Sumeyra KOCTURK[1]

Islam Science and Technology University School of Medicine, Department of Medical Microbiology , GAZİANTEP/TURKIYE.[1] sumeyrakocturk@yahoo.com, ORCID:0000-0001-9316-794X

INTRODUCTION

If we want to describe a mutually beneficial relationship between two organisms, as symbiosis is a relationship between microbe and a larger host organism, there are different forms. The microbe may survive on the surface of an organism (ectosymbiont), or the microbe may reside inside the host (endosymbiont). Occasionally, an organism harbors more than one symbiont, this is called a consortium. These interactions may be intermittent, cyclic or permanent. These terms refer to physical associations between organisms. Symbiont and host relationships can be classified as mutualism, cooperation, commensalism, predation, parasitism, amensalism, and competition. Myiasis is a life-form of ectoparasitism referred to as infestation of living vertebrates and humans by the larvae of certain fly species (Diptera), that feed on living or dead host tissues and body fluids (SaygıG.2009).Chart1.

Myiasis is the formation of pathological lesions by the larvae of certain flies of Arthropoda phylum and in class of Diptera by settling in living tissues and organs. Myiasis larvae, defined as larval infestations, are cutaneous, subcutaneous and cavitary depending on their location; it is often called obligatory, facultative and accidental, depending on the pathogen and the infestation it causes. Facultative and accidental types of myiasis are generally seen in humans (Dincer S.1997, Kilic K. 2011).

Myiasis is the life cycle of dipterous fly larvae on human body and other vertebrates in that are dead ,in vivo or at least for a period of time in various ways(1). Clinically, myiasis can be classified as cutaneous, atrial, wound, intestinal, or urinary, depending on the location of the fly larvae. Cases of intestinal myiasis have been reported sporadically from various countries in tropical medicine and briefly mentioned in the main textbooks of parasitology; so far no case has been reported in Turkey. Here we report a suspected case of intestinal myiasis.The large family Muscidae includes at least seven genera in which species cause myiasis (Table 19.1). Muscidae larvae (Fig. 1) develop in a variety of decaying organic matter, usually of plant or fecal origin (Mullen GR, Durden LA 2009). Gastrointestinal

myiasis caused by muscids usually results from oviposition on moist food. It may result from retroinfection through the host's anus after the fly has been attracted to foul odors or facal contamination. Genitourinary myiasis may occur in association with purulent discharge, urine-soaked clothing,and secondary microbial infections.

Myiasis, which also causes health problems and economic losses in cattle and sheep, is of great importance to veterinary medicine. It is obvious that Turkey which is located in a subtropical climate zone is exposed to the risk of myiasis. In our country, the most important species of traumatic myiasis are Lucilia sericata and Wohlfahrtia magnifica in sheep and cattle, the causes of subdermal myiasis are Hypoderma bovis and Hypoderma lineatum in cattle, and the cause of nasal myiasis is Oestrus ovis in sheep (Dik B,et al. 2012).

In this case report, a 26 year-old woman was presented who had black organisms after defecation since last year. She had received mebendazole therapy, but it was not effective. She was then pregnant, so she had not taken any other medications for control. After delivery, she was admitted to Infectious diseases outpatient clinic of Kahramanmaras Sutcu Imam University with the same complaints. In the past, she had frequently eaten out. She had no abdominal or general symptoms and her physical examination was normal, but she was anxious. Two maggot samples were taken to the microbiology laboratory for identification, along with a stool sample; her stool was normal. Our colleague and we advised her to take ivermectine therapy but the drug was not available in Turkey, so we had her to go for examined in gastroenterology. Nothing unusual was found on colonoscopic examination; they did colonic irrigation with serum physiologic and administered laxative therapy. Since then, her symptoms have resolved. In order to prevent further attacks, the patient was counseled against eating out, especially uncovered food accessible to flies.

Examination of the worms revealed several 2 cm long comma-shaped larvae, probably resembling Figure 1(Diptera info.com 2022). These species are mainly members of the families Muscidae,

Calliphoridae and Sarcophagidae, which usually deposit their eggs or larvae on sausage, cheese and other human foods and thus can be ingested, with contaminated food, pass through the intestine unharmed, and excreted in the feces (Aguilera,et al.1999). We assumed that these larvae were ectoparasitic infestations. The collected larvae were identified as Muscidae stabulans by microscopic examination in the parasitology laboratory. The clinical condition, termed accidental myiasis, was characterized by local irritation,vomiting, and diarrhea. Low oxygen levels kill the maggots but in our case they had survived, which may indicate their resistance to digestive enzymes (Sunny,et al 2016). The false stable fly (M. stabulans) (Fig. 1) is the most important species and is imainly nvolved in gastrointestinal myiasis. Occasionally its maggots appear in fetid wounds or injuries. Adults of this species look very similar to houseflies but are usually larger and more robust. They are attracted to, and feed on, plant juices, rotting fruits, and honeydew excreted by insects. Females lay their 140-200 eggs on the surface of overripe, rotting fruit. They also lay their eggs on accumulations of dead insects or feces, usually from humans, and on buried carrion. Early larvae are saprophagous but become predators as they mature. The third larval stage feeds on smaller grubs. This transition from a saprophagous to a predatory lifestyle has two advantages over species whose larvae remain saprophagous: First, the maturing larvae can store protein resources obtained from their prey for use by adults for reproduction, and, second, this lifestyle allows this species to use a wider range of low-protein resources as larval substrate. Larval development takes between 2 and 3 weeks. They usually overwinter as pupae (Durden LA,1987).

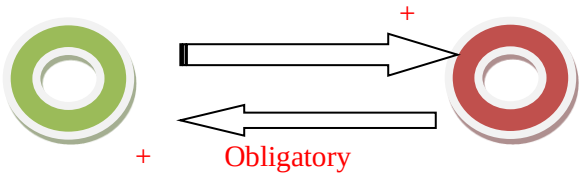
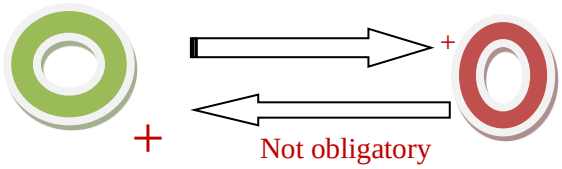
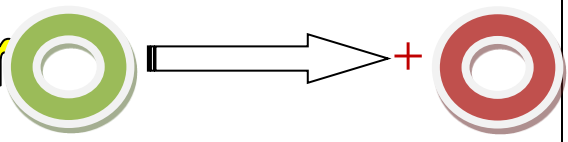
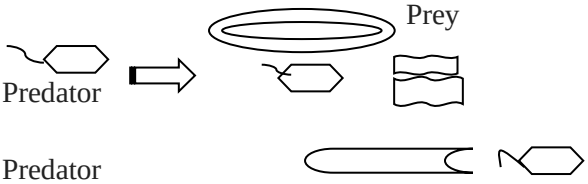
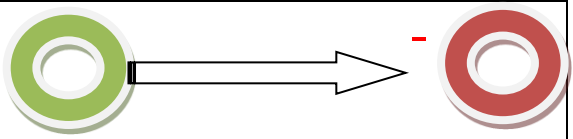
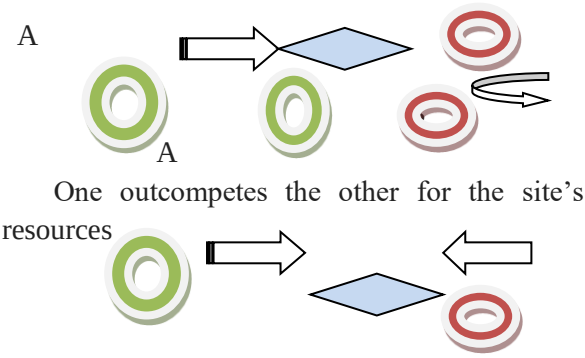
Life form	Interaction example
Mutualism	
cooperation	
commensalism	
Predation	
Amensalism	
Competition	

Diagram 1. Kocturk (2022).

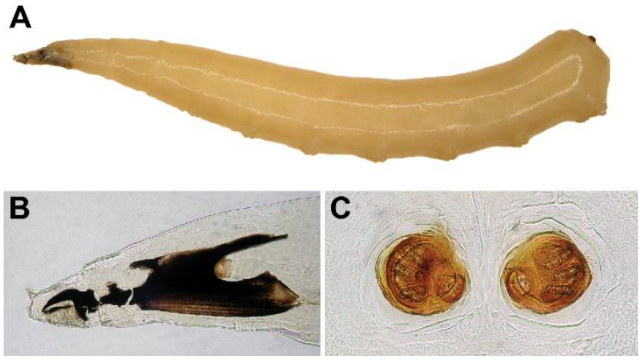


Figure 1. Larvae of Muscidae stabulans (6).

Myiasis is a disease commonly found in warm and humid climates. Since Turkey is located in the subtropical zone, there is also a risk of myiasis infestation here (Dokur M 2015, Dagci H,et al 2008). Myiasis can occur in anatomical sites, such as skin, eye, ear, nasopharynx, genitourinary tract, intestine and in wounds. Species causing wound myiasis in Turkey mainly include families such as Calliphoridae, Muscidae, Oestridae, and Sarcophagidae (Dagci H,et al 2008, Arslan F et al 2010, Yar K,et al 2011). Several myiasis cases in Turkey have been reported to be associated with Sarcophage and Calliphora species (Dagci H,et al 2008, Akcakaya AA ,et al. 2014, Demirel KF, etal 2014). Cases of intestinal myiasis caused by Musca domestica and Eristalis tenax have already been reported from the Indian subcontinent (Aguilera A,et al 1999). The cause in our case was probably one of these classes Calliphoridae, Muscidae, Oestridae, and Sarcophagidae or Eristalis tenax, which do not invade the host.

Intestinal myiasis was questioned because as it is a true pathological condition and not an infestation. Zumpt et al. did not accept intestinal infestation as true myiasis because ingested larvae do not normally ingest food while they completing their development in the intestine (Zumpt F 1963). Most cases occurred in countries where nutritional and sanitary conditions are unsatisfactory. The term "accidental" includes those that enter the living host accidentally

through contaminated food (Aguilera A,et al 1999). In our case ,we also saw a case of myiasis that was not a true invasion of the body; the low oxygen content of the gut usually kills the maggots, and most fly larvae have extremely resistant cuticles that are not damaged by digestive enzymes. Our patient showed no evidence that the maggots had entered the stool , even after defecation.

In an Indian study patients with the same condition were treated with colonic irrigation, using oral polyethylene glycol (PEG), 137.15 g dissolved in 2 l of water, a laxative that is also available in our country trade name Golytel, but we could not find it. Patients in the Indian study were asked to drink it within 2 hours. This resulted in 4 to 5 bowel movements in the next few hours (UdgaonkarUS2012).

Access to entomologists knowledgable in dipteran classification is usually difficult, especially in developing regions, where myiasis can be a real public health problem (Francesconi F, Lupi O,2012).

REFERENCES

Aguilera A., et al. Intestinal Myiasis Caused by Eristalis tenax. Journal of Clinical Microbiology, 0095-1137/99. Sept. 1999, p. 3082. Vol. 37, No. 9.

Akçakaya AA, Sargın F, Aslan ZI, Sevimli N, Sadikov F (2014) External ophthalmomyiasis seen in a patient from Istanbul, Turkey. Acta Parasitologica Turcica 38(3):205–207.

Arslan F, Mete B, Ozturk R, Samasti M (2010) External Ophthalmomyiasis caused by Oestrus ovis in Istanbul. Trop Doct 40(3):186–187.

Dagci H, Zeyrek F, Gerzile YK, Sahin SB, Yagci S, Uner A (2008) A case of myiasis in a patient with psoriasis from Turkey. Parasitol Int 57(2):239–241.

Demirel Kaya F, Orkun O, Çakmak A, Inkaya AC, Erguven S (2014) Cutaneous myiasis caused by Sarcophaga spp larvae in a diabetic patient. Microbiol Bul 48(2):356–361.

Dinçer Ş. İnsan ve Hayvanlarda Myiasis. Özcel MA, Daldal N, edi-tors. Parazitolojide Artropod Hastalıkları ve Vektörler. Türkiye Parazitol Dern Yay No: 13, İzmir, 1997, p. 169.

Dik B., et al. Myiasis in Animals and Humanbeings in Turkey. Kafkas Univ Vet Fak Journal/ 18 (1): 37-42, 2012.

"Diptera info.com" internet site.2022.

Dokur M., et al. Two different myiasis cases in southeast of Turkey: ophthalmomyiasis and cutaneous myiasis. Short communication. Parasitol Res (2015) 114:2767–2770 DOI 10.1007/s00436-015-4455-6.

Durden L.A. Predator-Prey interactions between Ectoparasites. Parasitology Today, vol 3, No 10 (1987), p:306.

Francesconi F, Lupi O (2012) Myiasis. Clin Microbiol Rev 25(1):79–105

Kılıç K., et al. Kars'ta bir kadında Lucilia sericata (Diptera: Calliphoridae)'nın neden olduğu postoperatif yara myiasisi. Lucilia sericata in a women in Kars. Turkiye Parazitol Derg 2011; 35: 43.

Mullen GR, Durden LA. Medical and veterinary entomology.(2009) p: 397.

Saygı G. Parazitoloji terimleri. Logos yayıncılık (2009) shf 15.

Sunny B., et al. Maggot infestation: various treatment modalities. Journal of the American College of Clinical Wound Specialists (2016) 8, 51–3.

Udgaonkar US.,et al. Intestinal myiasis. Indian Journal of Medical Microbiology, (2012) 30(3): 332-7

Yar K, Ozcan AA, Koltas IS (2011) External ophthalmomyiasis: case reports. Acta Parasitologica Turcica 35(4):224–226.

Zumpt F. The problem of Intestinal Myiasis in humans. S Afr Med J 1963;37:305-7.

CHAPTER 2

PYLORIC STENOSIS

Dr. Duygu YILMAZ[1] , Assoc. Prof. Dr. Safak KARACAY[2]

[1] Yeditepe University, Faculty of Medicine, Department of Pediatrics, Istanbul, Türkiye. md.duyguyilmaz@gmail.com, Orcid ID: 0000-0003-3252-3466.
[2] Yeditepe University, Faculty of Medicine, Department of Pediatric Surgery, Istanbul, Türkiye. safakkaracay@gmail.com, Orcid ID: 0000-0001-8615-7727.

INTRODUCTION

Pyloric stenosis, also known as infantile hypertrophic pyloric stenosis (IHPS), is a rare condition in infants that causes gastric outlet obstruction characterized by abnormal thickening of the gastric pyloric muscles. Clinically, newborns are normal at birth. Later, projectile vomiting is observed, usually at 3 to 6 weeks of age, after disruption of the continuity of the gastric tract due to pyloric thickening. There is a serious loss of fluid and electrolytes due to vomiting. In infants who are very sensitive to dehydration, this situation presents with a severe clinical condition with electrolyte imbalances. The most typical clinic for IHPS is undoubtedly projectile non-bilious vomiting. This vomiting results in hypochloremic hypokalemic metabolic alkalosis. Weight loss with vomiting, malnutrition and jaundice are other common findings. On ultrasonography, it is diagnostic if the pyloric muscle thickness is over 4 mm and its length is over 14-16 mm. Surgical treatment is often required. It requires a multidisciplinary approach consisting of a pediatrician, radiologist and pediatric surgeon.

1. ANATOMY AND EMBRYOLOGY

The stomach is an organ of the digestive system consisting of muscles and therefore expendable. It is located between the esophagus and the duodenum. It lies on the left upper quadrant of the abdomen. The stomach breaks down the food that enters it physically, owing to peristalsis, and chemically, with the aid of acid, protease enzymes and pepsin. The fundus and corpus form the proximal part of the stomach which are responsible for storage. The most distal part is called the antrum. Here, the foods are mixed and ground. As a result, chyme, a semi-liquid material with the consistency of liquid mixture, is formed. There are two sphincters, one at the proximal and the second at the distal part of the stomach. Of these, the lower esophageal sphincter prevents the stomach contents and most importantly stomach acid from escaping back into the esophagus, while the pyloric sphincter allows the chyme to flow slowly from the stomach to the duodenum.

The stomach appears as a fusiform enlargement of the foregut at the 4th week of fertilization. In the following weeks, the shape and the position of the stomach change significantly as a result of the different growth rates in different parts of its wall and the changes in the positions of the surrounding organs. The stomach rotates around its axis. The pylorus is in the gastroduodenal region, in this region the stomach and sphincter complex intertwine and occur in the antral part of the stomach from the increase of muscle fibers. Unfortunately, detailed information about the embryology and fetal development of the pylorus is not known. It can be visualized by ultrasound (USG) from the 9^{th} gestational week. After the 14^{th} week of gestation, measurements of the fetal stomach can be made. However, there are some limitations to this measurement before the second trimester. Therefore, gastric outlet measurements are difficult to make and there is no consensus among researchers on the sensitivity and specificity of these measurements. During fetal life, some developmental anomalies can be observed. The most common among these is pyloric stenosis, followed by prepyloric septum and pyloric atresia. Congenital hypertrophic pyloric stenosis (CHPS) is thought to result from abnormalities in pyloric innervation, extracellular matrix proteins, and smooth muscle cells. In the diagnosis of pyloric stenosis, It is important to evaluate the muscle thickness, length and diameter of the pyloric canal by USG for the diagnosis. (Koyuncu, Malas, Colonel, Çankara, Karahan, 2009).

The incidence of pyloric stenosis is 0.2-0.5% per year. It is the second most common pediatric surgical disease after inguinal hernias in the first months of life. It is 4 times more common in boys than in girls. The incidence among the first children in families is higher. In order of frequency, its incidence in populations is 0.24% in Caucasians, 0.18% in Hispanics, 0.07% in Blacks, and 0.06% in Asians. (Galea, Said, 2018),(Abdellatif, Ghozy, Kamel, Elawady, Ghorab, Attia, Le, Duy, Hirayama, Huy, 2019). It is more common in O and B blood groups than in

group A. It is reported that the frequency is relatively high in patients with phenylketonuria, esophageal atresia and Turner syndrome.

2. ETIOLOGY

Although it has been known, investigated and treated since the early 1900s, its etiology is not known for certain. It is thought to be multifactorial. Some environmental and genetic risk factors are known, but the mechanism is not yet clear. In some studies, it has been shown that the incidence of IHPS increases in infants using macrolide antibiotics, especially erythromycin. Other risk factors include bottle feeding, inadequate breast milk intake, prematurity, cesarean delivery and firstborn babies. Heavy maternal smoking during pregnancy increases the risk of hypertrophic pyloric stenosis 1.5 to 2.0 times (Galea, Said, 2018). In addition to genetics and environmental factors, pyloric stimulation deficiencies, abnormalities in nitric oxide metabolism, and hormonal differences also play a role. Despite the known risk factor, a clear cause-effect link regarding etiopathogenesis has not been established yet.

3. PATHOPHYSIOLOGY

The pathognomonic feature of pyloric stenosis is hypertrophy of the circular and longitudinal muscles of the pylorus (Rosenthal, Chodick, Grossman, Shalev, Koren, 2019). The pylorus is located in the antrum of the stomach. This thickening causes the lumen to narrow and lengthen. When the contraction is severe, the gastric outlet is blocked. Vomiting of extra-biliary bullets is observed. In case of complete occlusion, the stomach volume expands. Thus, a feeling of early satiety occurs.

4. HISTORY AND PHYSICAL EXAMINATION

The prominent clinical sign in infants with pyloric stenosis is projectile, non-bilious vomiting. IHPS cases, which start to appear towards the end of the neonatal period and are usually diagnosed within 4-8 weeks, present with different clinical severity. Vomiting can start as early as the first week or as late as 3 months. The period of vomiting may vary depending on the degree of stenosis and nutritional status.

Some patients vomit intermittently, while others vomit after each feeding. While it does not present with prominent projectile vomiting in the early phase of the disease, after the pyloric hypertrophy becomes evident, it evolves into severe projectile vomiting that is encountered in almost every feeding. Vomit does not contain bile. In approximately 60% to 80% of infants with IHPS, when the stomach is empty and the child is calm, a fixed, non-tender, rigid pyloric muscle 1 to 2 cm in diameter at the junction of the costal margin and the right rectus muscle, classically described as the "olive sign" palpable. On careful inspection after feeding, reverse peristaltic waves can also be observed over the stomach.

While the clinic is more stable in cases with early diagnosis, severe dehydration and changes in consciousness can be detected in cases with late diagnosis. Depending on the degree of hypertrophy and vomiting, and the amount of food that can reach the intestine, there are different degrees of constipation, dehydration, and weight loss. The main signs of dehydration in infants are sunken fontanels, dry mucous membranes, decreased tears, poor skin turgor, decreased urine output, and lethargy.

Electrolyte imbalance can be seen in pyloric stenosis due to vomiting. In particular, it most commonly occurs as hypochloremic, hypokalemic metabolic alkalosis. Thanks to the more widespread and practical use of ultrasonography, the rates of early diagnosis have increased. Therefore, electrolyte imbalances are now present in less than 50% of cases. In delayed cases, fasting may hide metabolic alkalosis as it will cause metabolic acidosis. Hypernatremia may occur due to dehydration, as well as hyponatremia. Both conditions can cause prerenal kidney failure. Hyperbilirubinemia is present in 2-10% of cases and is mostly indirect. Glucuronyl transferase deficiency and starvation are thought to cause this condition (Bašković, Župančić, Lesjak, Vukasović, 2016).

5. DIAGNOSIS

Clinical evaluation and a detailed history are usually sufficient for diagnosis. Ultrasonography, a method that supports the definitive diagnosis, is accessible, easy to perform, reliable, sensitive, and highly specific. Diagnosis is made by demonstrating a thick, hypoechoic pyloric muscle mass just medial to the right kidney. A pyloric wall thickness of 3 mm or more and a pyloric canal length of 15 mm or more are considered abnormal and support the diagnosis. Delayed gastric emptying and target sign are other signs that we can see on ultrasound.

Barium radiological examination can be performed in conditions where ultrasonography cannot be performed or ultrasonography cannot be evaluated clearly. Elongation and narrowing can be observed in the distal antrum and the pylorus, and this string mark is called the double-track mark or the beak mark.

Very rarely, endoscopy can be performed when the diagnosis can not be made with other imaging techniques.

Although the sensitivity and specificity of abdominal X-ray is low, its availability is high. In the x-ray, it can be seen that there is no dense gas in the stomach or gas passage to the colon secondary to gastric obstruction. The enlarged appearance of the stomach due to excess gas is called the caterpillar symptom.

Severe hypochloremia and hypokalemia and secondary metabolic alkalosis may develop in infants who vomit frequently due to pyloric stenosis. (Bašković, Župančić, Lesjak, Vukasović, 2016).

6. DIFFERANTIAL DIAGNOSIS

In the differential diagnosis of vomiting, dietary mistakes, gastroesophageal reflux, viral gastroenteritis, disorders related to increased intracranial pressure, achalasia, midgut volvulus, sepsis, and urinary system infections should be considered. Pyloric spasm is a condition observed in some restless, hyperactive infants and causes persistent vomiting. Physical examination findings are normal in infants with pyloric spasm, and dehydration or weight loss is not

observed. Vomiting is reduced with phenobarbital and atropine. No pathology is detected ultrasonographically or radiologically.

Midgut volvulus is a complication of malrotation caused by the rotation of the mesenteric root around the superior mesenteric artery. Although it is more common in infants, it can also be seen in adults. Because the symptoms are similar, it may be confused for pyloric stenosis. In the upper serial X-ray studies of the gastrointestinal tract, a "corkscrew" appearance may be observed (Goldman, Gross, Novak, Poletto, Kim, Son, Levin, 2019)

Gastroesophageal reflux, gastroenteritis, urinary tract infection, sepsis, constipation, cow's milk intolerance, and necrotizing enterocolitis may be common causes of vomiting in infants. In addition, other causes of obstruction such as duodenal web, intussusception, gastric duplication, hernia, and Hirschsprung's disease should definitely be considered.

8. TREATMENT

8.1. Fluıd Management

Medical treatment consisting of rehydration and correction of electrolyte imbalances is required as the first step. The patient's degree of dehydration and electrolyte disturbances are the main factors determining the timing of the surgery. Hypochloremic hypokalemic metabolic alkalosis is the first problem that needs to be resolved. Deficits should be eliminated with parenteral therapy and urine and blood pH should be monitored at intervals. If there is no sign of dehydration or if it is mild, administration of 0.25% NaCl with 5% dextrose and a maintenance fluid with KCl is required. Given its effect on potential hypoventilation, bicarbonate levels should be corrected and monitored. We suggest the following targets for fluid and electrolyte therapy prior to surgery, as recommended by an expert panel: pH $\leq$7.45 and/or base excess $\leq$3.5, bicarbonate <26 mEq/L, sodium $\geq$132 mEq/L, potassium $\geq$3.5 mEq/L, chloride $\geq$100 mEq/L, glucose $\geq$72 mg/dL (4.0 mmol/L).

Infants with significant alkalosis at the time of surgery are at increased risk of postoperative apnea. Patients with severe hypokalemia (<2.5 mEq/L) or hyponatremia (<120 mEq/L) are at particularly high risk for perioperative complications and should be managed carefully, with expert consultation if needed.

8.2. Anesthesia

Aspiration during the induction of anesthesia in patients with pyloric stenosis is one of the complications of most concern. To reduce this risk and minimize residual gastric content, it is recommended to empty the stomach with an orogastric or nasogastric tube just prior to the operation. The patient is placed supine, a 10 or 14 french catheter is inserted into the stomach, and aspiration is performed in the left lateral decubitus and right lateral decubitus positions to maximize gastric emptying (Cook, Liacouras, Previte, 1997). Gastric volumes may be large in patients with preoperative pyloric stenosis, and therefore, aspiration just before surgery may not completely empty the stomach.

Some clinicians routinely administer anticholinergics (glycopyrrolate or atropine) prior to gastric aspiration and anesthesia induction to reduce the risk of bradycardia. Others argue that anticholinergic should be given only if there is a risk of bradycardia.

Rapid sequential induction and intubation (RSII) is a technique designed to intubate as quickly as possible to protect the airway from aspiration. RSII requires preoxygenation followed by simultaneous administration of an IV induction agent and a neuromuscular blocking agent (NMBA), avoidance of mask ventilation, and intubation as soon as neuromuscular block is established. Propofol or ketamine is generally preferred for induction, and succinylcholine or rocuronium is preferred for neuromuscular blockade.

Infants have chest wall elasticity and wide bellies, resulting in reduced functional residual capacity compared to older children and adults. They also have significantly higher oxygen consumption. These factors can cause deep oxygen desaturation even in short-term apneas despite adequate preoxygenation. Low-pressure mask ventilation (peak pressure <12 cm H2O) is used to prevent oxygen desaturation and

minimize the risk of gastric insufflation. The benefit of cricoid pressure is uncertain, can compress the infant trachea, and make mask ventilation more difficult. (Park, Rattana, Peyton, 2021),(Kamata, Cartabuke, Tobias, 2015).

The suggested advantages of awake intubation include preservation of airway reflexes, reduced risk of aspiration, and safety in the event of potentially undiagnosed airway abnormalities. However, awake intubation has been associated with soft tissue trauma, bradycardia, breath-holding, hypoxemia, and laryngospasm (Kamata, Cartabuke, Tobias, 2015).

Inhalation induction in pediatric patients has a low overall risk of perioperative aspiration, with no good data suggesting that one type of induction is superior to another.

8.3. Surgial Management

After the baby is rehydrated and the electrolyte imbalance is treated, surgery is planned. Pyloromyotomy (Fredet-Ramstedt surgery), which involves a longitudinal incision of the hypertrophic pylorus with blunt dissection to the submucosa level, is preferred. This method relieves the narrowing and ensures the normal passage of stomach contents into the duodenum. Feeding should be stopped as soon as the patient is diagnosed, but routine nasogastric tube use should be avoided. In current approaches, the use of nasogastric tubes is recommended in patients with aspiration risk. Depending on the surgeon, the surgery can be performed with open, laparoscopic, or as endoscopic balloon dilatation.

In laparoscopic pyloromyotomy, the Ramstedt procedure is performed in a minimally invasive manner. Where experienced surgeons are available, laparoscopic pyloromyotomy is the first choice. A laparoscopic pyloromyotomy is associated with a lower incidence of postoperative vomiting, less need for analgesia, and shorter hospital stay. However, it can sometimes cause incomplete pyloromyotomy or mucosal perforation (Staerkle, Lunger, Fink, 2021). A transumbilical approach can also be used,

but the operative time is longer (Kim, Lau, Lee, 2005). The surgery is curative and has very low morbidity.

There are studies showing that intravenous and/or oral atropine sulfate relaxes the pyloric muscles and regresses stenosis. However, this approach typically requires a prolonged hospital stay alongside weeks or months of atropine with parenteral nutrition or postpyloric enteral nutrition (Danko, Evans, Upperman, 2022).

There are observational studies of the conservative treatment of infants with IHPS. This approach typically involves a trial of continuous nasoduodenal feeding, usually lasting several months, until the gastric outlet obstruction decreases as the infant grows (Yamashiro, Mayama, Yamamoto, 1981).

Another treatment method is to reach the stenosis with the endoscope and dilating it with a balloon. However, balloon dilation does not reliably disrupt the seromuscular ring of the pylorus but is associated with a significant risk of pyloric perforation (Ogawa, Higashimoto, Nishijima, 1996).

8.4. Postoperative Management

Blood gas and electrolyte samples should be repeated every 6 hours and fluid adjustments should be made, especially according to the blood bicarbonate levels. In general, he aim is to completely correct the liquid electrolyte imbalances within the 48 hours.

Opioid-free analgesics are usually sufficient for postoperative pain. The patient's liver disease should be questioned before using acetaminophen. NSAIDs are not preferred in infants due to bleeding and the risk of impaired renal function. It is common to apply a local anesthetic to the incision and laparoscopic entry sites. The surgeon's application of the long-acting local anesthetics bupivacaine or ropivacaine to the surgical site can provide effective postoperative analgesia a few hours after the surgery.

Opioids may increase the risk of postoperative apnea in patients who may already be at high risk due to prematurity or residual metabolic alkalosis. It can also cause decreased intestinal motility,

leading to feeding intolerance (Squillaro, Ourshalimian, McLaughlin, 2020).

Single-injection rectus sheath or transversus abdominus nerve blocks have also been used primarily for open procedures (Kumar, Wilson, Engelhardt, 2014).

In the post-operative pyloric feeding regimen, the ng tube is left in place and 2/3 maintenance volume of iv fluid is started. If the breastfed baby is awake, then can be breastfed 2-4 hours after the operation. Small feeds are given initially and gradually increase to normal feeds depending on tolerance. Milk can be given every 2 hours. Nutrition is gradually increased to 75-100-150 ml/kg/day. When the 75 ml/kg/day dose is tolerated, the intravenous fluid is reduced. Ng tube can be removed according to the feeding tolerance. Vomiting may continue after surgery, but is usually self-limited and not harmful. Severe vomiting is rare after surgery. If vomiting persists for more than five days, radiological evaluation should be performed. The study may be difficult to perform due to post-operative swelling. (Paramalingam, Hallows, Sheth, Lazner, Chadwick, 2019).

Because of the young age of infants with IHPS, the risk of apnea after the surgery is relatively high. It is recommended to monitor apnea for at least 24 hours. Postoperative bleeding, perforation, vomiting, inadequate pyloromyotomy, wound infection and gastroesophageal reflux may occur. Patients should be followed in the hospital. Infants' hydration and nutritional tolerance should be evaluated.

9. CARE

Routine pediatric care, including growth monitoring, is enough unless new symptoms develop. Infants with other persistent or recurrent symptoms, including disorders other than IHPS, require further evaluation. During postoperative abdominal ultrasonography, caution should be exercised in interpretation as the thickened muscle and enlarged diameter take eight months and one year respectively to resolve back to normal values.

10. COMPLICATIONS

Postoperative complications are rare. Nutritional errors or infections should be sought in vomiting that continues after surgery. Rarely, a second surgery may be required in the presence of incomplete pyloromyotomy.

Mucosal perforations occur in less than 1% of patients, 1 to 3 percent of patients who are treated with a laparoscopic approach by an experienced surgeon, and even rarer in those undergone an open procedure (Hall, Eaton, Seims, 2014).

IHPS and gastroesophageal reflux often coexist. Hematemesis, seen in 25% of patients with pyloric stenosis, is also a consequence of reflux-related esophagitis.

Pyloromyotomy is curative in the vast majority of infants. When diagnosed early, the prognosis is excellent. The mortality rate is extremely low.

12. MULTIDISCIPLINARY APPROACH

Rapid diagnosis and treatment of infants with pyloric stenosis requires a multidisciplinary approach. Pediatricians should first evaluate the baby and, if necessary, initiate intravenous fluid therapy. Abdominal ultrasonography should be performed by an experienced radiologist and early diagnosis should be made. The diagnosed patient is referred to a pediatric surgeon. This multidisciplinary approach results in better patient outcomes.

REFERENCES

Abdellatif, M., Ghozy, S., Kamel, MG., Elawady, SS., Ghorab, MME., Attia, AW., Le, Huyen, TT., Duy, DTV., Hirayama, K., Huy, NT. (2019). Association between exposure to macrolides and the development of infantile hypertrophic pyloric stenosis: a systematic review and meta-analysis. *Eur J Pediatr,* 178(3), 301-314.

Bašković, M., Župančić, B., Lesjak, N., Vukasović, I. (2016). Hypertrophic Pyloric Stenosis - Five-Year Retrospective Analysis. *Acta Med Croatica,* 70(2):103-6.

Cook, Sather, SD., Liacouras, CA., Previte, JP., et al. (1997). Gastric fluid measurement by blind aspiration in paediatric patients: a gastroscopic evaluation. *Can J Anaesth*, 44:168.

Danko, ME., Evans, PT., Upperman, JS. (2022). Current management of pyloric stenosis. *Semin Pediatr Surg,* 31:151145.

Galea, R., Said, E. (2018). Infantile Hypertrophic Pyloric Stenosis: An Epidemiological Review. *Neonatal Netw,* 37(4), 197-204.

Goldman, Yassen, AE., Gross, J., Novak, I., Poletto, E., Kim, JS., Son, JK., Levin, TL. (2019). Identification of clinical parameters to increase the diagnostic yield of the non-emergent upper gastrointestinal series in pediatric outpatients. *Pediatr Radiol,* 49(2):162-167.

Hall, NJ., Eaton, S., Seims, A., et al. (2014). Risk of incomplete pyloromyotomy and mucosal perforation in open and laparoscopic pyloromyotomy. *J Pediatr Surg* 2014; 49:1083.

Kamata, M., Cartabuke, RS., Tobias, JD. (2015). Perioperative care of infants with pyloric stenosis. *Paediatr Anaesth,* 25:1193.

Kim, SS., Lau, ST., Lee, SL., et al. (2005). Pyloromyotomy: a comparison of laparoscopic, circumumbilical, and right upper quadrant operative techniques. *J Am Coll Surg*, 201:66.

Koyuncu, E,. Malas, MA., Albay, S., Cankara, N., Karahan, N. (2009). The development of fetal pylorus during the fetal period. *Surg Radiol Anat,* 31(5), 335-41.

Kumar, A., Wilson, GA., Engelhardt, TE. (2014). Ultrasound guided rectus sheath blockade compared to peri-operative local anesthetic infiltration in infants undergoing supraumbilical pyloromyotomy. *Saudi J Anaesth*, 8:229.

Ogawa, Y., Higashimoto, Y., Nishijima, E., et al. (1996). Successful endoscopic balloon dilatation for hypertrophic pyloric stenosis. *J Pediatr Surg*, 31:1712.

Paramalingam, S., Hallows, R., Sheth, J., Lazner, M., Chadwick C. (2019). Initial management of pyloric stenosis, *the Medicines Governance Group*

Park, RS., Rattana, Arpa, S., Peyton, JM., et al. (2021). Risk of Hypoxemia by Induction Technique Among Infants and Neonates Undergoing Pyloromyotomy. *Anesth Analg*, 132:367.

Rosenthal, YS., Chodick, G., Grossman, Z., Shalev, V., Koren, G. (2019). The incidence of infantile hypertrophic pyloric stenosis and its association with folic acid supplementation during pregnancy: A nested case-control study. *J Pediatr Surg*, 54(4), 701-706.

Squillaro, AI., Ourshalimian, S., McLaughlin, CM., et al. (2020). Postoperative Opioid Analgesia Impacts Resource Utilization in Infants Undergoing Pyloromyotomy. *J Surg Res*, 255:594.

Staerkle, RF., Lunger, F., Fink, L., et al. (2021). Open versus laparoscopic pyloromyotomy for pyloric stenosis. *Cochrane Database Syst Rev*, 3:CD012827.

Yamashiro, Y., Mayama, H., Yamamoto, K., et al. (1981). Conservative management of infantile pyloric stenosis by nasoduodenal feeding. *Eur J Pediatr*, 136:187.

CHAPTER 3

EFFECTS OF ANTIBIOTIC ALTERNATIVES ON THE INTESTINAL MICROBIOTA AND PERFORMANCE OF BROILERS

Lec. Dr. Seyyide SARIÇAM İNCE[1]

[1] Ankara University, Faculty of Veterinary Medicine, Department of Microbiology, Ankara, Turkey. E-mail: ssaricam@ankara.edu.tr ORCID ID: 0000-0002-2386-6857.

INTRODUCTION

The poultry industry is a large food sector worldwide as poultry accounts for over 70% of total consumed meat (Ayalew et al., 2022). The industry has used antibiotics widely for over 50 years (Chowdhury et al., 2009), particularly for subtherapeutic aims, such as improve to performance, efficiency, and productivity (Mehdi et al., 2018). However, as a result of misuses or overuses, the emergence and spread of antimicrobial resistance had become a serious problem in recent years. Therefore, WHO has called for greater control of their use and the development of new antibiotics (Tacconelli et al., 2018). The use of antibiotics causes to reduce beneficial bacteria in the gut microbiota. This negative effect is evaluated together with increased intestinal pathogens in several studies (Mehdi et al., 2018). In addition, the vertical and horizontal transmission of antibiotic resistance genes accelerates the spread of resistance while antibiotic-resistant bacteria can also be transmitted from animals to humans (Abd El-Hack et al., 2022; Chowdhury et al., 2009; Ruan et al., 2021). Widespread antibiotic use also results in antibiotic residues of the products (meat and eggs), which are polluting and pose risks for both human and animal health in terms of allergenicity, carcinogenicity, and antimicrobial resistance. For example, various studies have indicated a possible correlation between antibiotic residues and the severity of bacterial infections in humans (Abd El-Hack et al., 2022; Arsene et al., 2021). Therefore, the subtherapeutic use of antibiotics has been criticized in recent years while in 2006 the European Union and various countries banned this practice (Ayalew et al., 2022). Consequently, poultry producers now prefer potential and effective alternatives to antibiotics. Nowadays, several feed additives are used in the modern broiler production as growth promoters that help improve growth performance and encourage beneficial bacteria in the gut microbiota. Thus, the exclusion of risks is possible in poultry, such as antibiotic resistance and antibiotic residue. In recent years, the most common used feed additives are bacteriocins, enzymes, essential oils, organic acids, probiotics, and prebiotics (Mehdi et al., 2018). These compounds also non-toxic and non-carcinogenic, whether for humans or animals.

They are generally both stable and metabolizable structures (Abd El-Hack et al., 2022). The following sections outline the characteristic of these alternative feed additives.

1. BACTERIOCINS

Bacteriocins are extracellular proteinaceous compounds (proteins, glycoproteins, and lipoproteins) that are stable, metabolizable and non-harmful. They are synthesized by both Gram-positive bacteria (e.g., *Bacillus* spp., *Bifidobacterium* spp., *Enterococcus* spp., *Lactobacillus* spp., *Staphylococcus* spp., and *Streptococcus* spp.) and Gram-negative bacteria (e.g., *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Pasteurella* spp., *Proteus* spp., and *Salmonella* spp.). Like antibiotics, they exhibit bacteriostatic or bactericidal activity, specifically through cell membrane dissociation, biomolecule (DNA, RNA, and proteins) synthesis inhibition, or cell lysis (Jozefiak & Sip, 2013).

Bacteriocins are widely used as feed additivities thanks to these characteristics, particular after the EU banned the use of antibiotics as growth promotors in poultry. In broiler production, the three most commonly used are pediocin, salinomycin and nisin, which help inhibit gut pathogens, specifically through antibacterial effects on *Listeria monocytogenes* or *Staphylococcus aureus* (Kieronczyk et al., 2016). Pediocin A (produced from *Pediococcus pentosaceus*) improves growth performance and feed conversion ratio (FCR) while nisin supplementation improves gut microbiota by reducing jejunal and cecal pathogens (Grilli et al., 2009). As a result, the researchers suggested using nisin as a dietary supplement (Kieronczyk et al., 2020). Additionally, the combination of nisin and salinomycin appears to regulate the intestinal microbiota and decrease total pathogen counts (Kieronczyk et al., 2016). Regarding other bacteriocins used in broilers, divercin and reuterin have antibacterial effects against fecal *Listeria innocua* and *Campylobacter jejuni* (Nazef et al., 2008) while divercin AS7 (produced from *Carnobacterium divergens*) is an effective growth promotor and gut microbiota regulator in broilers (Jozefiak & Sip, 2013).

2. ENZYMES

Enzymes, synthesized from proteins, lipids, minerals, and vitamins, are biocatalyzers of biochemical reactions within organisms. Enzymes have long been used as dietary supplements in broilers since the 1990s, including amylases, cellulases, galactosidases, lipases, mannanases, pectinases, phytases, proteases, and xylanases. Generally multi-enyzme complexes are added to the diet (Ayalew et al., 2022; Park & Carey, 2019). Many studies have demonstrated their contributions to immune response, nutritional digestibility, intestinal histology, and growth performance (Gadde et al., 2017; Mathlouthi et al., 2002).

The most widely used enzymes in broiler production, xylanase, protease, and phytase, are also produced in commercial forms. Xylanase is used to degrade xylan chains in corn and wheat while protease helps to digest corn and soybean proteins. These digestive processes help increase weight gain and gut morphology in broilers. Xylanase improves growth performance (weight gain and feed intake (FI)) while reducing intestinal viscosity and intestinal pathogen counts (*E. coli* and anaerobic bacteria) (Mathlouthi et al., 2002). It reduces cecal *Salmonella* spp. contamination, by reducing utilizable nutrients for intestinal pathogens and creating nutrient competition (Amerah et al., 2012).

Two other frequently used enzymes in broilers are protease and phytase. Protease has positive effects on FI and growth (Farrell et al., 1993; Yang et al., 2009). Kalmendal and Tauson (2012) examined the effects of protease on nutrient digestibility in broilers. Broilers in the protease-added group exhibited better starch and fat digestion than the control group (Kalmendal & Tauson, 2012). Exogenous phytase, which catalyzes the hydrolysis of phosphate residues, is often used with broilers to improve growth performance, energy utilization, nutrient digestion, and gut microbiota composition (Khan et al., 2013). Phytase supplementation is associated with higher feed conversion ratio (FCR) and body weight gain (BWG) in broilers as well as improved body weight (BW) and average daily gain (ADG) (Akter et al., 2017; Song et al., 2021). Phytase also has beneficial effects on the cecal microbiota,

specifically in terms of decreased cecal coliforms and increased the population of *Lactobacillus* spp. (Amiri et al., 2021).

3. ESSENTIAL OILS

Essential oils (EOs) are aromatic compounds of plant origin, obtained by various techniques, such as extraction, fermentation, and distillation. The most commonly used EOs include anethole, carvacrol, eugenol, garlic, lavender, and oregano thymol. They contain bioactive chemical groups that role improve feed conversion, assisting fermentation, regulating digestive enzymes, and stimulating antioxidant and antimicrobial activity (Abd El-Hack et al., 2022; Ruan et al., 2021). They provide many benefits to the broiler production, such as inhibiting pathogen proliferation, promoting beneficial bacteria, regulating immune reactions, and stimulating weight gain and growth (Mehdi et al., 2018).

Many studies have demonstrated that EOs improve growth performance in broiler production. More specifically, it was found that a diet supplemented with an EO mixture (oregano, fennel seed, citrus peel, laurel leaf, sage leaf and myrtle leaf oil) significantly increases growth performance of broilers (Bozkurt et al., 2012). Tiihonen et al. (2010) indicated that higher growth performance in the diet supplemented with cinnamaldehyte and thymol (Tiihonen et al., 2010). Within another report, it was reported that higher BW when diet supplemented with thymol and carvacrol EOs (Silva-Vazquez et al., 2018). Wade et al. (2018) found that broiler diet supplemented with thyme EO contributed the digestive activities of amylases and chymotrypsin. Therefore, the feed utilization was enhanced in broilers (Wade et al., 2018). Ding et al. (2022) reported that higher BW and FI in the thymol treatment group than the control group (Ding et al., 2022).

Many studies have demonstrated the positive effects of EOs on the intestinal microbiota in broilers to control pathogen levels. More specifically, feed supplemented with cinnamon, oregano, and thymol enhances gut microbiota while EO supplementation with thymol and cinnamaldehyde reduces intestinal pathogens (Tiihonen et al., 2010;

Ulfah, 2006). Similarly, a diet supplemented with oregano reduces the jejunal population of intestinal pathogens, such as *Staphylococcus* spp., *Klebsiella* spp., and *Proteus* spp. (Bauer et al., 2019). Oregano EO improves BW and FCR (Betancourt et al., 2019). Likewise, feed supplemented with oregano EO improves nutrient metabolism, growth performance, and intestinal microbiota (Ruan et al., 2021). Similarly, lavender EO supplementation has positive effects on both BWG and FCR, and modulates the gut microbiota by increasing probiotic bacteria populations in the ileum while reducing *E. coli* and coliform colonization (Adaszynska-Skwirzynska & Szczerbinska, 2019). Carvacrol, cinnamon, and thymol all have beneficial effects on intestinal microbiota. For example, a diet supplemented with carvacrol and thymol reduces *Campylobacter* spp. colonization while cinnamaldehyte supplementation decreases transmission of *Salmonella* Enteritidis (Abd El-Hack et al., 2021). Diets supplemented with thymol reduce the populations and biofilm formation of *E. coli* and *Salmonella* Pullorum (Ding et al., 2022).

In recent years, EO encapsulation has provided a new, more efficient approach, which enables control of EO solubility and stability during the digestive process (Abd El-Hack et al., 2022). By protecting EOs from digestion in the upper parts of intestinal system, encapsulation enables them to easily pass to lower parts in order to benefit the gut microbiota of broilers (Grilli et al., 2009).

4. ORGANIC ACIDS

Organic acids (OAs) are commonly used as feed additives in broiler production, particularly acetic, butyric, citric, formic, lactic, malic, tartaric, and propionic acids. Especially, OAs have been used increasingly frequently since the ban on dietary use of antibiotics in broilers (Ayalew et al., 2022). The EU has approved the use of OAs in broilers, whether in drinking water or feed, as a replacement for antibiotics (Saleem et al., 2020).

OAs can generally improve growth performance and nutrient utilization in broilers. More specifically, certain OAs (butyric acid, etc.) improve weight gain, nutrient absorption, and fermentation of

biomolecules (proteins and carbohydrates). When they were used individually or in combinations, OAs have positive effects on broiler growth performance (Yang et al., 2018). For example, adding acetic acid, citric acid, and lactic acid to broiler diets improves BW (Abdel-Fattah et al., 2008). Similarly, citric supplemented diet improves FI, feed efficiency, mineral utilization, and immunity while sorbic acid and fumaric acid improve digestive enzyme activities and growth (Chowdhury et al., 2021; Yang et al., 2018). Finally, a diet supplemented with formic acid and propionic acid significantly increases body weight and feed conversion (Saleem et al., 2020).

OAs also positively affect microbiota regulation in broilers. In particular, they inhibit bacteria invasion by infusing into the bacterial membrane and impairing bacterial enzymatic reactions. This increases beneficial bacteria populations while reducing bacterial invasion and intestinal pathogen populations (Ayalew et al., 2022). For example, dietary OAs (formic and propionic acids) promote population of *Lactobacillus* spp. in the broiler gut microbiota while reducing intestinal pathogens, such as *Campylobacter* spp., *Clostridium perfringens*, *Escherichia coli*, and *Salmonella* spp. (Mehdi et al., 2018). This improves the host's nutrient conversion efficiency by decreasing nutrient competition within the gut. Similarly, formic and propionic acids supplementation can improve both the immune response, specifically humoral immunity, and populations *of Lactobacillus* spp. (Emami et al., 2013). A citric acid supplanted diet decreases *Listeria monocytogenes* counts with antibacterial effect (Galli et al., 2021; Gonzalez-Fandos et al., 2020). Similarly, formic and propionic acids have antibacterial effects on *Salmonella* Typhimurium, *Campylobacter coli*, and *E. coli* (Khan et al., 2022).

In recent years, OAs and EOs have frequently been used together in broiler production to take advantage of their complementary effects. That is, combined products have a greater positive effect on growth performance than when used separately (Bozkurt et al., 2012). More specifically, a combination of sorbic acid, fumaric acid, and thymol improves growth performance in broilers (Yang et al., 2018) while an OAs-EOs mixture (thymol-benzoic acid or cinnamaldehyde-

caproic acid) has an antibacterial effect on *Salmonella* Enteritidis (Zhang et al., 2019). Similarly, an OAs and EOs supplemented diet (thyme, carvacrol, hexanoic acid, benzoic acid, and butyric acid) has positive effects on the gut microbiota by reducing intestinal population of *C. perfringens* and increasing population of *Lactobacillus* spp. (Pham et al., 2020). It was reported that a mixture of OAs and EOs (citric acid, eugenol, fumaric acid, malic acid, sorbic acid, thymol and vanillin) increased the beneficial bacteria *Firmicutes* and *Ruminococcus* while decreased harmful *Caprobacillus* in gut microbiota of broilers (Adewole et al., 2021). Finally, it is known that a combination of OAs and EOs (citric acid, eugenol, fumaric acid, malic acid, sorbic acid, thymol and vanillin) reduces cecal *E. coli* and *C. jejuni* counts (Chowdhury et al., 2021).

5. PROBIOTICS

Probiotics are defined as "adequate amounts of live microorganism that are beneficial on health". Probiotics are mostly used to improve immune reactions, intestinal health, mineral utilization, and growth performance. Their use in broilers improves the quality of the meat, specifically protein and fatty acid composition. The most commonly used probiotic microorganisms are *Bacillus subtilis (B. subtilis), Bacillus longum (B. longum), Bacillus amyloliquefaciens (B. amyloliquefaciens), Bacillus licheniformis (B. licheniformis), Bacillus coagulans (B. coagulans), Bacillus animalis (B. animalis), Bacillus thermophilum (B. thermophilum), Enterococcus faecalis (E. faecalis), Enterococcus faecium (E. faecium), Lactobacillus lactis (L. lactis), Lactobacillus plantarum (L. plantarum), Lactobacillus fermentum (L. fermentum),* and *Saccharomyces cerevisiae (S. cerevisiae)* (Arsene et al., 2021; Mehdi et al., 2018; Wang et al., 2020).

Many studies have demonstrated the antibacterial effects of probiotic supplements in broilers. For example, *B. subtilis* has an antibacterial effect on *Salmonella* spp. and enhances productivity of broilers while *B. longum* has an antibacterial effect on *Campylobacter* spp. (Park & Kim, 2014; Santini et al., 2010). *Bacillus* probiotics, including *B. amyloliquefaciens*, decrease intestinal populations of *E.*

coli (Ahmed et al., 2014). B. coagulans improves BW, ADG, and gut health in broilers while *B. animalis, B. thermophilum,* and *B. longum* have antimicrobial effects against *E. coli, Eimeria tenella, Salmonella* spp., and *Listeria* spp. (Arsene et al., 2021; Zhang et al., 2021).

The use of probiotic *E. faecium* as a dietary supplement enhances ileal colonization of lactic acid bacteria and improves growth performance (BW and FCR) of broilers (Samli et al., 2007). In addition, *E. faecium* improves mineral utilization and populations of beneficial bacteria *Ruminococcaceae,* which is associated with improved intestinal phosphorus absorption. It reduces populations of gut pathogens like *E.coli* and *Shigella* spp. (Wang et al., 2020). Similarly, addition of *E. faecium* to drinking water and *L. fermentum* to feed both increase BW (Capcarova et al., 2010).

Another widely used probiotic is *Lactobacillus* spp., which is applied as feed alternative to improve performance and quality in poultry. It also reduces *Salmonella* spp. contamination in chickens. For example, *L. fermentum* helps decrease intestinal populations of *E. coli* and *L. acidophilus* and reduce *Salmonella* spp. contamination in poultry (Huang et al., 2004). The use of 11 different *Lactobacillus* strains (*L. reuteri, L. gallinarum, L. brevis,* and *L. salivarius*) significantly decreases cecal *E. coli* populations in broilers while increasing counts of cecal *Bifidobacterium* spp. and FI (Mookiah et al., 2014). Finally, it is known that *S. cerevisiae* supplementation reduces *E. coli* counts in broiler microbiota while significantly increasing BW and FI (Koc et al., 2010).

6. PREBIOTICS

Prebiotics were first defined in 1995 by Gibson and Roberfroid as "nondigestible food ingredient that beneficially affect the growth and host health" (Gibson & Roberfroid, 1995). They also defined prebiotics as "a selectively fermented ingredient that cause specific changes on intestinal microbiota and host health" in 2010 (Roberfroid et al., 2010). More recently, prebiotics have been defined as "nondigestible compounds that modulate the gut microbiota and have beneficial effect on host health" (Bindels et al., 2015). An essential characteristic of

prebiotics is that they are nondigestible and nonabsorbable compounds (Patterson & Burkholder, 2003). Commonly used prebiotics in broiler production include fructose-oligosaccharides (FOS), galacto-oligosaccharides (GOS), mannan-oligosaccharides (MOS), xylo-oligosaccharides (XOS), and β-glukan are (Ayalew et al., 2022; Pourabedin & Zhao, 2015).

Prebiotics are similar to probiotics in terms of regulating gut microbiota and having antioxidative and antibacterial effects on host health (Ayalew et al., 2022; Teng & Kim, 2018). Several studies have demonstrated how prebiotics as a dietary supplement modulate intestinal microbiota, specifically by increasing counts of beneficial bacteria like *Lactobacillus* spp. and *Bifidobacterium* spp. while reducing counts of and invasion by intestinal pathogens, such as coliforms, *Campylobacter* spp., and *Salmonella* spp. (Patterson & Burkholder, 2003; Pourabedin & Zhao, 2015; Ricke, 2021). Diets supplemented with prebiotics increase levels of *Lactobacillus* spp. and *Bifidobacterium* spp. in the microbiota (Gaggia et al., 2010).

More specifically, a FOS-supplemented diet has a beneficial effect on ileum microbiota of broilers and helps modulate the intestinal microbiota composition by increasing beneficial bacteria like *Lactobacillus* spp. and *Bifidobacterium* spp. (Geier et al., 2009; Rebole et al., 2010). An FOS diet also increases *Lactobacillus* spp. counts while inhibiting intestinal pathogens as *E. coli* and *C. perfringens* in the broiler ileum. Another commonly used prebiotic in broilers is MOS (Kim et al., 2011). Like FOS, MOS reduces *E.coli* and *C. perfringens* counts while increasing *Lactobacillus* spp. counts (Kim et al., 2011). MOS also reduces cecal *Salmonella* spp. and *E. coli* colonization (Shanmugasundaram et al., 2013). A MOS supplemented diet reduces *E.coli* in broiler ceca and increases *Lactobacillus* spp. counts (Koc et al., 2010). MOS supplementation alters the intestinal microbiota by increasing *Lactobacillus* spp. and *Bifidobacterium* spp. and decreasing *Campylobacter* spp., *Clostridium perfringens, E. coli*, and *Salmonella* spp. MOS is frequently preferred as a supplement for broilers due to both its own anti-pathogenicity and the anti-pathogenic properties of increasing *Lactobacillus* spp. populations (Teng & Kim, 2018). Finally,

both GOS and XOS supplementation improves broiler microbiota by reducing intestinal pathogens and increasing beneficial bacteria populations (Pourabedin & Zhao, 2015; Rebole et al., 2010). For example, a GOS supplemented diet raises *Lactobacillus intestinalis* and *Faecalibacterium prausnitzii* counts in broiler ceca (Park et al., 2017).

CONCLUSION

The overuse and misuse of antibiotics in organisms has created significant problems worldwide. The spread of antibiotic resistance and residue and the killing of beneficial bacteria in the microbiota are lead the these problems. Therefore, the European Union banned the subtherapeutic use of antibiotics in 2006. As a result of this procedure, poultry producers has tended to various dietary additives to improve growth performance and gut microbiota. The most common used additives are bacteriocins, enzymes, essential oils, organic acids, probiotics, and prebiotics. In many studies, the benefits of these additivies on the broiler health were reported. These compounds enhance to growth performance of broilers. In addition they are also improve beneficial bacteria in gut microbiota while form the competition of nutrients for inntestinal pathogens.

REFERENCES

Abd El-Hack, M. E., El-Saadony, M. T., Saad, A. M., Salem, H. M., Ashry, N. M., Abo Ghanima, M. M., Shukry, M., Swelum, A. A., Taha, A. E., El-Tahan, A. M., AbuQamar, S. F., & El-Tarabily, K. A. (2022). Essential oils and their nanoemulsions as green alternatives to antibiotics in poultry nutrition: a comprehensive review. Poultry Science, 101(2), 1-21.

Abd El-Hack, M. E., El-Saadony, M. T., Shehata, A. M., Arif, M., Paswan, V. K., Batiha, G. E. S., Khafaga, A. F., & Elbestawy, A. R. (2021). Approaches to prevent and control Campylobacter spp. colonization in broiler chickens: a review. Environmental Science and Pollution Research, 28(5), 4989-5004.

Abdel-Fattah, S. A., El-Sanhoury, M. H., El-Mednay, N. M., & Abdel-Azeem, F. (2008). Thyroid Activity, Some Blood Constituents, Organs Morphology and Performance of Broiler Chicks Fed Supplemental Organic Acids. International Journal of Poultry Science, 7(3), 215-222.

Adaszynska-Skwirzynska, M., & Szczerbinska, D. (2019). The effect of lavender (Lavandula angustifolia) essential oil as a drinking water supplement on the production performance, blood biochemical parameters, and ileal microflora in broiler chickens. Poultry Science, 98(1), 358-365.

Adewole, D. I., Oladokun, S., & Santin, E. (2021). Effect of organic acids-essential oils blend and oat fiber combination on broiler chicken growth performance, blood parameters, and intestinal health. Anim Nutr, 7(4), 1039-1051.

Ahmed, S. T., Islam, M. M., Mun, H. S., Sim, H. J., Kim, Y. J., & Yang, C. J. (2014). Effects of Bacillus amyloliquefaciens as a probiotic strain on growth performance, cecal microflora, and fecal noxious gas emissions of broiler chickens. Poultry Science, 93(8), 1963-1971.

Akter, M., Iij, P. A., & Graham, H. (2017). Increased iron level in phytase-supplemented diets reduces performance and nutrient utilisation in broiler chickens. British poultry science, 58(4), 409-417.

Amerah, A. M., Mathis, G., & Hofacre, C. L. (2012). Effect of xylanase and a blend of essential oils on performance and Salmonella colonization of broiler

chickens challenged with Salmonella Heidelberg. Poultry Science, 91(4), 943-947.

Amiri, M. Y. A., Jafari, M. A., & Irani, M. (2021). Growth performance, internal organ traits, intestinal morphology, and microbial population of broiler chickens fed quinoa seed-based diets with phytase or protease supplements and their combination. Tropical Animal Health and Production, 53(6), 1-8.

Arsene, M. M. J., Davares, A. K. L., Andreevna, S. L., Vladimirovich, E. A., Carime, B. Z., Marouf, R., & Khelifi, I. (2021). The use of probiotics in animal feeding for safe production and as potential alternatives to antibiotics. Veterinary World, 14(2), 319-328.

Ayalew, H., Zhang, H. J., Wang, J., Wu, S. G., Qiu, K., Qi, G. H., Tekeste, A., Wassie, T., & Chanie, D. (2022). Potential Feed Additives as Antibiotic Alternatives in Broiler Production. Frontiers in Veterinary Science, 9, 1-15.

Bauer, B. W., Radovanovic, A., Willson, N. L., Bajagai, Y. S., Van, T. T. H., Moore, R. J., & Stanley, D. (2019). Oregano: A potential prophylactic treatment for the intestinal microbiota. Heliyon, 5(10), 1-11.

Betancourt, L., Hume, M., Rodriguez, F., Nisbet, D., Sohail, M. U., & Afanador-Tellez, G. (2019). Effects of Colombian oregano essential oil (Lippia origanoides Kunth) and Eimeria species on broiler production and cecal microbiota. Poultry Science, 98(10), 4777-4786.

Bindels, L. B., Delzenne, N. M., Cani, P. D., & Walter, J. (2015). Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol, 12(5), 303-310.

Bozkurt, M., Kucukyilmaz, K., Catli, A. U., Cinar, M., Cabuk, M., & Alcicek, A. (2012). Effects of administering an essential oil mixture and an organic acid blend separately and combined to diets on broiler performance. Archiv Fur Geflugelkunde, 76(2), 81-87.

Capcarova, M., Weiss, J., Hrncar, C., Kolesarova, A., & Pal, G. (2010). Effect of Lactobacillus fermentum and Enterococcus faecium strains on internal milieu, antioxidant status and body weight of broiler chickens. Journal of Animal Physiology and Animal Nutrition, 94(5), 215-224.

Chowdhury, M. A. K., Dhara, A. K., Dey, S., Sarkar, A., Tactacan, G. B., & Haldar, S. (2021). Dietary supplementation of a microencapsulated essential oil-organic acid blend with and without a protease affects performance, nutrient accretion, cecal microbiota, and circulatory biomarker concentrations in male broiler chickens. Letter in Animal Biology, 1(1), 19-32.

Chowdhury, R., Islam, K. M. S., Khan, M. J., Karim, M. R., Haque, M. N., Khatun, M., & Pesti, G. M. (2009). Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. Poultry Science, 88(8), 1616-1622.

Ding, Y. A., Hu, Y., Yao, X. F., He, Y. C., Chen, J. L., Wu, J., Wu, S. L., Zhang, H. H., He, X., & Song, Z. H. (2022). Dietary essential oils improves the growth performance, antioxidant properties and intestinal permeability by inhibiting bacterial proliferation, and altering the gut microbiota of yellow-feather broilers. Poultry Science, 101(11), 1-12.

Emami, N. K., Naeini, S. Z., & Ruiz-Feria, C. A. (2013). Growth performance, digestibility, immune response and intestinal morphology of male broilers fed phosphorus deficient diets supplemented with microbial phytase and organic acids. Livestock Science, 157(2-3), 506-513.

Farrell, D. J., Martin, E., Dupreez, J. J., Bongarts, M., Betts, M., Sudaman, A., & Thomson, E. (1993). The Beneficial-Effects of a Microbial Feed Phytase in Diets of Broiler-Chickens and Ducklings. Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde, 69(5), 278-283.

Gadde, U., Kim, W. H., Oh, S. T., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. Anim Health Res Rev, 18(1), 26-45.

Gaggia, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol, 141(1), 15-28.

Galli, G. M., Aniecevski, E., Petrolli, T. G., da Rosa, G., Boiago, M. M., Simoes, C. A. D. P., Wagner, R., Copetti, P. M., Morsch, V. M., Araujo, D. N., Marcon, H., Pagnussatt, H., Santos, H. V., Mendes, R. E., Loregian, K. E., & Da Silva, A. S. (2021). Growth performance and meat quality of broilers fed

with microencapsulated organic acids. Animal Feed Science and Technology, 271, 1-8.

Geier, M. S., Torok, V. A., Allison, G. E., Ophel-Keller, K., & Hughes, R. J. (2009). Indigestible carbohydrates alter the intestinal microbiota but do not influence the performance of broiler chickens. J Appl Microbiol, 106(5), 1540-1548.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr, 125(6), 1401-1412.

Gonzalez-Fandos, E., Martinez-Laorden, A., & Perez-Arnedo, I. (2020). Combined Effect of Organic Acids and Modified Atmosphere Packaging on Listeria monocytogenes in Chicken Legs. Animals (Basel), 10(10), 1-17.

Grilli, E., Messina, M. R., Catelli, E., Morlacchini, M., & Piva, A. (2009). Pediocin A improves growth performance of broilers challenged with Clostridium perfringens. Poultry Science, 88(10), 2152-2158.

Huang, C. H., Qiao, S. Y., Li, D. F., Piao, X. S., & Ren, J. P. (2004). Effects of Lactobacilli on the performance, diarrhea incidence, VFA concentration and gastrointestinal microbial flora of weaning pigs. Asian-Australasian Journal of Animal Sciences, 17(3), 401-409.

Jozefiak, D., & Sip, A. (2013). Bacteriocins in Poultry Nutrition - a Review. Annals of Animal Science, 13(3), 449-462.

Kalmendal, R., & Tauson, R. (2012). Effects of a xylanase and protease, individually or in combination, and an ionophore coccidiostat on performance, nutrient utilization, and intestinal morphology in broiler chickens fed a wheat-soybean meal-based diet. Poult Sci, 91(6), 1387-1393.

Khan, R. U., Naz, S., Raziq, F., Qudratullah, Q., Khan, N. A., Laudadio, V., Tufarelli, V., & Ragni, M. (2022). Prospects of organic acids as safe alternative to antibiotics in broiler chickens diet. Environmental Science and Pollution Research, 29(22), 32594-32604.

Khan, S. A., Chaudhry, H. R., Butt, Y. S., Jameel, T., & Ahmad, F. (2013). The Effect of Phytase Enzyme on the Performance of Broiler Flock (A-Review). Poultry Science Journal, 1(2), 117-125.

Kieronczyk, B., Pruszynska-Oszmalek, E., Swiatkiewicz, S., Rawski, M., Dlugosz, J., Engberg, R. M., & Jozefiak, D. (2016). The nisin improves broiler chicken

growth performance and interacts with salinomycin in terms of gastrointestinal tract microbiota composition. Journal of Animal and Feed Sciences, 25(4), 309-316.

Kieronczyk, B., Rawski, M., Mikolajczak, Z., Swiatkiewicz, S., & Jozefiak, D. (2020). Nisin as a Novel Feed Additive: The Effects on Gut Microbial Modulation and Activity, Histological Parameters, and Growth Performance of Broiler Chickens. Animals, 10(1), 1-15.

Kim, G. B., Seo, Y. M., Kim, C. H., & Paik, I. K. (2011). Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poult Sci, 90(1), 75-82.

Koc, F., Samlı, H., Okur, A., Ozduven, M., Akyurek, H., & Senkoylu, N. (2010). Effects of Sacchromyces Cerevisiae and/or Mannanoligosacchride on performance, blood parameters and intestinal microbiota of broiler chicks. Bulgarian Journal of Agricultural Science, 16, 643-650.

Mathlouthi, N., Mallet, S., Saulnier, L., Quemener, B., & Larbier, M. (2002). Effects of xylanase and beta-glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal microflora of broiler chickens fed a wheat and barley-based diet. Animal Research, 51(5), 395-406.

Mehdi, Y., Letourneau-Montminy, M. P., Gaucher, M. L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S. K., Cote, C., Ramirez, A. A., & Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. Animal Nutrition, 4(2), 170-178.

Mookiah, S., Sieo, C. C., Ramasamy, K., Abdullah, N., & Ho, Y. W. (2014). Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. Journal of the Science of Food and Agriculture, 94(2), 341-348.

Nazef, L., Belguesmia, Y., Tani, A., Prevost, H., & Drider, D. (2008). Identification of lactic acid bacteria from poultry feces: Evidence on anti-Campylobacter and anti-Listeria activities. Poultry Science, 87(2), 329-334.

Park, J., & Carey, J. B. (2019). Dietary Enzyme Supplementation in Duck Nutrition: A review. Journal of Applied Poultry Research, 28(3), 587-597.

Park, J. H., & Kim, I. H. (2014). Supplemental effect of probiotic Bacillus subtilis B2A on productivity, organ weight, intestinal Salmonella microflora, and breast meat quality of growing broiler chicks. Poult Sci, 93(8), 2054-2059.

Park, S. H., Perrotta, A., Hanning, I., Diaz-Sanchez, S., Pendleton, S., Alm, E., & Ricke, S. C. (2017). Pasture flock chicken cecal microbiome responses to prebiotics and plum fiber feed amendments. Poult Sci, 96(6), 1820-1830.

Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. Poult Sci, 82(4), 627-631.

Pham, V. H., Kan, L. G., Huang, J. Y., Geng, Y. Q., Zhen, W. R., Guo, Y. M., Abbas, W., & Wang, Z. (2020). Dietary encapsulated essential oils and organic acids mixture improves gut health in broiler chickens challenged with necrotic enteritis. Journal of Animal Science and Biotechnology, 11(1), 1-18.

Pourabedin, M., & Zhao, X. (2015). Prebiotics and gut microbiota in chickens. FEMS Microbiol Lett, 362(15), 1-8.

Rebole, A., Ortiz, L. T., Rodriguez, M. L., Alzueta, C., Trevino, J., & Velasco, S. (2010). Effects of inulin and enzyme complex, individually or in combination, on growth performance, intestinal microflora, cecal fermentation characteristics, and jejunal histomorphology in broiler chickens fed a wheat- and barley-based diet. Poult Sci, 89(2), 276-286.

Ricke, S. C. (2021). Prebiotics and alternative poultry production. Poult Sci, 100(7), 1-12.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., Wolvers, D., Watzl, B., Szajewska, H., Stahl, B., Guarner, F., Respondek, F., Whelan, K., Coxam, V., Davicco, M. J., Leotoing, L., Wittrant, Y., Delzenne, N. M., Cani, P. D., Neyrink, A. M., Meheust, A. (2010). Prebiotic effects: metabolic and health benefits. Br J Nutr, 104(2), 1-63.

Ruan, D., Fan, Q. L., Fouad, A. M., Sun, Y. Y., Huang, S. S., Wu, A. J., Lin, C. X., Kuang, Z. X., Zhang, C., & Jiang, S. Q. (2021). Effects of dietary oregano essential oil supplementation on growth performance, intestinal antioxidative capacity, immunity, and intestinal microbiota in yellow-feathered chickens. Journal of Animal Science, 99(2), 1-11.

Saleem, K., Saima, Rahman, A., Pasha, T. N., Mahmud, A., & Hayat, Z. (2020). Effects of dietary organic acids on performance, cecal microbiota, and gut morphology in broilers. Tropical Animal Health and Production, 52(6), 3589-3596.

Samli, H. E., Senkoylu, N., Koc, F., Kanter, M., & Agma, A. (2007). Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Archives of Animal Nutrition, 61(1), 42-49.

Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D., & Biavati, B. (2010). Characterization of probiotic strains: An application as feed additives in poultry against Campylobacter jejuni. International Journal of Food Microbiology, 141, 98-S108.

Shanmugasundaram, R., Sifri, M., & Selvaraj, R. K. (2013). Effect of yeast cell product supplementation on broiler cecal microflora species and immune responses during an experimental coccidial infection. Poult Sci, 92(5), 1195-1201.

Silva-Vazquez, R., Duran-Melendez, L. A., Hernandez-Martinez, C. A., Gutierrez-Soto, J. G., Hume, M. E., & Mendez-Zamora, G. (2018). Effects of two sources of Mexican oregano oil on performance, blood profile, carcass variables, and meat of broilers. Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science, 47, 1-10.

Song, T. T., Yu, C. Y., Zhao, X., Chen, F. L., Liu, Y. J., Yang, C. W., & Yang, Z. B. (2021). Effects of thermostable phytase supplemented in diets on growth performance and nutrient utilization of broilers. Animal Science Journal, 92(1), 1-8.

Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D. L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., Outterson, K., Patel, J., Cavaleri, M., Cox, E. M., Houchens, C. R., Grayson, M. L., Hansen, P., Singh, N., Theuretzbacher, U., Magrini, N., Working group, W. P. P. L. (2018). Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infectious Diseases, 18(3), 318-327.

Teng, P. Y., & Kim, W. K. (2018). Review: Roles of Prebiotics in Intestinal Ecosystem of Broilers. Frontiers in Veterinary Science, 5, 1-18.

Tiihonen, K., Kettunen, H., Bento, M. H. L., Saarinen, M., Lahtinen, S., Ouwehand, A. C., Schulze, H., & Rautonen, N. (2010). The effect of feeding essential oils on broiler performance and gut microbiota. British poultry science, 51(3), 381-392.

Ulfah, M. (2006). Essential oils as multi-function feed additive (MFA) to improve broilers performance, metabolism, dung consistency and efficiency of production. Journal of Agriculture and Rural Development in the Tropics and Subtropics, 88, 50-55.

Wade, M. R., Manwar, S. J., Kuralkar, S. V., Waghmare, S. P., Ingle, V. C., & Hajare, S. W. (2018). Effect of thyme essential oil on performance of broiler chicken. Journal of Entomology and Zoology Studies, 6(3), 25-28.

Wang, W., Cai, H., Zhang, A., Chen, Z., Chang, W., Liu, G., Deng, X., Bryden, W. L., & Zheng, A. (2020). Enterococcus faecium Modulates the Gut Microbiota of Broilers and Enhances Phosphorus Absorption and Utilization. Animals (Basel), 10(7), 1-13.

Yang, X., Xin, H., Yang, C., & Yang, X. (2018). Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens. Anim Nutr, 4(4), 388-393.

Yang, Z. B., Huang, Z. Y., Zhou, J. P., Yang, W. R., Jiang, S. Z., & Zhang, G. G. (2009). Effects of a new recombinant phytase on performance and mineral utilization of laying ducks fed phosphorus-deficient diets. Journal of Applied Poultry Research, 18(2), 284-291.

Zhang, B., Zhang, H. R., Yu, Y., Zhang, R. Q., Wu, Y. P., Yue, M., & Yang, C. M. (2021). Effects of Bacillus Coagulans on growth performance, antioxidant capacity, immunity function, and gut health in broilers. Poultry Science, 100(6), 1-9.

Zhang, S., Shen, Y. R., Wu, S., Xiao, Y. Q., He, Q., & Shi, S. R. (2019). The dietary combination of essential oils and organic acids reduces Salmonella enteritidis in challenged chicks. Poultry Science, 98(12), 6349-6355.

CHAPTER 4

OCULAR DELIVERY SYSTEMS AND APPLICATIONS DEVELOPED BY QbD PERSPECTIVE: FROM THE PAST TO THE FUTURE

Dr. Burcu UNER[1,2], Dr. Pankaj DWIVEDI[3],
Dr. Juste BARANAUSKAITE[4]

[1] Institute of Graduate Studies in Health Sciences, Istanbul, Turkey,
[2] University of Health Science and Pharmacy in St. Louis, St. Louis, USA,
Burcu.uner@uhsp.edu, ORCID ID: 0000-0003-4691-0432
[3] University of Health Science and Pharmacy in St. Louis, St. Louis, USA,
Pankaj.dwivedi@uhsp.edu, ORCID ID: 0000-0002-3548-3984
[4] Yeditepe University, Faculty of Pharmacy, Istanbul, Turkey
juste.ortasoz@yeditepe.edu.tr, ORCID ID: 0000-0002-0358-5759

1. INTRODUCTION

Eye is one of the most complex organs that converts the light reflected from objects into electrical impulses and transmits them to the brain via the optic nerve. The eye might be investigated in two different perspectives which are anatomical and functional. In context with anatomical. The eye composes two different parts; anterior and posterior segments. While the anterior segment consists of the cornea, anterior chamber, iris, posterior chamber, ciliary body and lens, posterior segment consists of the vitreous, retina, retinal pigment epithelium, and choroid **(Figure 1).**

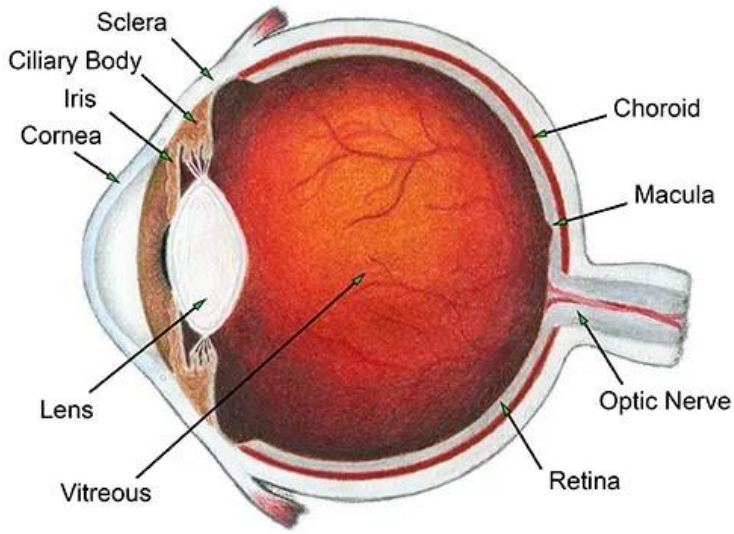


Figure 1. Anatomical investigation of eye division (Pautler, 2021)

The major duty of the components in the anterior segment is permeating the light stimuli to the posterior segment as well as that focus on the macula. The posterior segment, which forms the posterior 2/3 of the eye, includes the vitreous humor, retina, macula, and optic nerves (Gaudana, Jwala, Boddu, & Mitra, 2009; Wadhwa, Paliwal, Paliwal, & Vyas, 2009). In addition, the eyeball can be functionally examined in three layers. The outermost protective layer consists of the

sclera and cornea. The cornea is a transparent structure with a diameter of 11.7 mm and a thickness of 0.5 - 0.7 mm, which protects the eye against infections, physical damage, and transmits light to the lens and retina by refracting it (Yasukawa et al., 2004). The sclera protects the eye from internal and external forces and ensures that the shape of the eye remains intact is the structure of the uttermost layer of the eye. The middle layer includes the ciliary body, choroid, and iris. The choroid is a highly vascularized structure located among the retina and the sclera. The iris is the structure that plays a role in regulating the amount of light reaching the retina by controlling the pupil opening (DelMonte & Kim, 2011). The retina is the layer that forms the inner layer and contains the basic nerve cells and structures for the sense of sight. And last structure is the ciliary body controls the shape of the lens (Müller, Pels, & Vrensen, 2001).

1.1 Ocular Barriers

The ocular drug delivery process is difficult due to the anatomical and physiological barriers in the eye. In the treatment of superficial diseases of the eye, systemic drug administration is not a preferable option as so the obstacle components such as the blood-aqueous barrier and the blood-retina barrier block desired level of the drug (Figure 2). Hence, for overcoming of this problem, high systemic drug dosage is being applied; unfortunately, this approach causes side effects in patients. For this reason, topical drug administration is frequently preferred in the treatment of superficial diseases of the eye, as well as eye drops constitute more than half of ophthalmic preparations (Singh, Ahmad, & Heming, 2011).

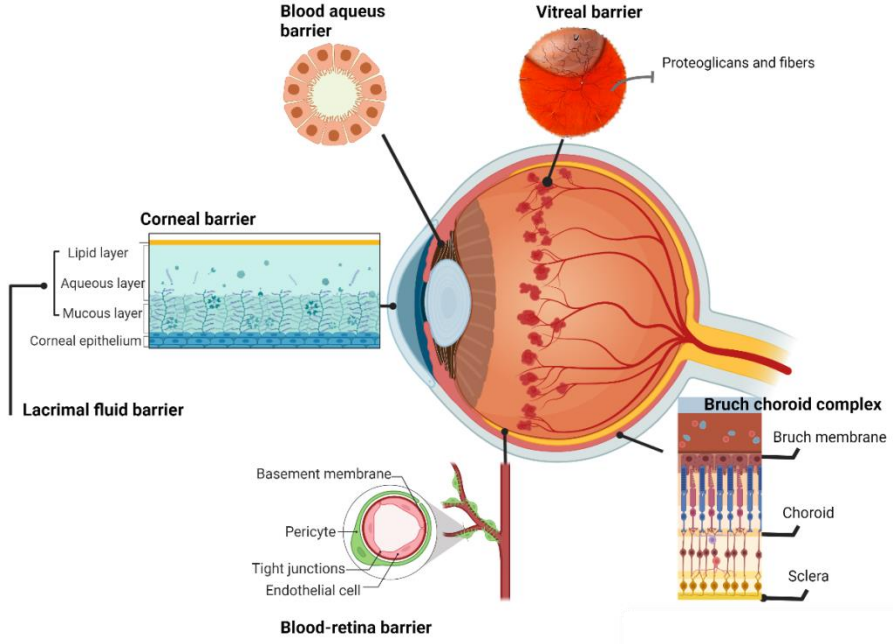


Figure 2 Encountering the barriers on ophthalmic administration

However, low ocular bioavailability (< 5%) is observed with topical administration of drugs to the eye. The reasons for this can be summarized as follows (Kompella, Kadam, & Lee, 2010):

-After the drug is applied to the eye, the drug solution is diluted with lacrimal fluid.

- The volume of lacrimal fluid in the eye is approximately 7 to 10 μL, as well as the volume of each drop applied to the eye is approximately 50 μL. Therefore, approximately 80% of the amount of drug administered immediately protrudes from the surface of the eye or is removed from the conjunctival tract.
- Topically applied drug continues to dilute with increasing tear, normal tear cycle as well.
- Consequently, regarding other barriers in the eye, the penetration of the drug into the back of the eye is restricted.

Solutions are one of the traditional ophthalmic drug forms that are used ubiquitously forms for the topical application of drugs to the eye. The crucial points regarding the solution's formulation are solubility, ocular toxicity, pKa, the effect of pH, tonicity, buffer capacity, viscosity, compatibility with other ingredients in the formulation, preservatives to be used, comfort in eye application and can be listed as convenience (Gericke et al., 2019; Winter, Anderson, & Braun, 2010).

In order to boost the ocular bioavailability by decreasing the loss of the drug, dozens of strategies (such as increasing viscosity, developing into the ointment, adding a cationic agent, prodrug development, and penetration enhancer addition) had been utilized by the researchers (Gaudana, Ananthula, Parenky, & Mitra, 2010). Unfortunately, these strategies were not very effective at improving bioavailability.

1.2 Novel ocular drug delivery systems

New drug delivery systems are being developed since traditional strategies are not adequately efficient in terms of increasing the accommodation in the precorneal area, preventing it from moving away from this area, and increasing drug penetration through the cornea. These systems can be listed as polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, nanosuspensions, liposomes, micelles, dendrimers, hydrophilic gels, inserts, and ocular minitablets (Hornof, Toropainen, & Urtti, 2005; Irimia et al., 2018; Urtti, 2006)

The pharmaceutical industry is encountered many problems for instance insufficient bioavailability and low water solubility of newly developed drug molecules. Hereby, the requirement is available to occur the action taken promptly for improving the novel drug delivery system that overcomes these disadvantages(Urtti, 2006). These delivery systems should not have acute and chronic toxicity, as well as they should have adequate drug loading capacity, drug targeting, and controlled release properties. Meanwhile, the chemical and physical stability of the drug must be ensured. Size reduction is not one of the best methods only, it is also increasing the solubility of poorly water-

soluble agents of biopharmaceutical classification system (BCS) classes II and IV(C. Freitas & Müller, 1999; Fresta, Guccione, Beccari, Furneri, & Puglisi, 2002).

Colloidal systems are drug delivery systems in micron and nanometer sizes, in solid or semi-solid form and show all the advantages of controlled release systems (Yalpani, 1997). In addition, they increase solubility and bioavailability of inferior drugs by acting as preservatives for sensitive drug compounds (Fialho & da Silva-Cunha, 2004). Parameters that need to be controlled to select the most suitable colloidal system are indicated in the **Table 1** (Chrysantha Freitas & Müller, 1998; Müller et al., 2001; Winter et al., 2010).

Table 1: Specifications that affecting on the colloidal systems (Chrysantha Freitas & Müller, 1998; Müller et al., 2001; Winter et al., 2010)

Parameters of colloidal particles				
Electrostatic	**Morphological**	**Solution**	**Optical**	**Quantitative**
Surface charge	Size	Turbidity	Refractive index	Loading capacity
Zeta Potential	Molecular weight	viscosity	Coefficient absorption	Encapsulation efficiency
		stability		

Moreover, regarding the carrier systems: *in-vivo* degradation time, toxicity, biocompatibility with ocular tissues, and excretion time from the body are also important except for the specified parameters in the Table 1.

2. QUALITY BY DESIGN (QBD) – OCULAR APPLICATIONS

Quality by Design (QbD) is a design concept that aims to cover the entire lifecycle of a product, from the design stage to the marketed stage, as well as an initiative initiated by the United States Food and Drug Administration (FDA). QbD is a new paradigm that emphasizes understanding the product and process within the product development process and throughout the product lifecycle. As can be seen from the studies in the literature, the basis of QbD studies is the scientific

understanding of the product and the process, hereby statistical experimental designs (Design of Experiments, DoE).

In the literature, reducing the number of experiments and obtaining more useful data. Numerous experimental designs have been described that can provide. If the purpose of an experimental design is to classify many variables as "dependent" and "independent", first-order designs such as Plackett-Burman or Taguchi may be preferred.

However, if the aim is to predict a response variable mathematically (estimation) or to optimize a process, quadratic models such as Box-Behnken, Central Composite, or Optimal designs should be preferred. As seen in the literature examples, the use of appropriate software in the creation is a crucial part of the process as much as selecting the suitable type of the DoE model, and the quality of the data to be obtained from the study as well. By looking at the characteristics given in **Table 1** (dependent), the roadmap used in the selection of the appropriate formulation can be determined. Determining of the levels of independent variables in two-level experimental designs is a critical point. If the gap between the low and high levels is too narrow, a significant factor may be considered meaningless, or on the contrary, if the gap between the two levels is too wide, a nonsignificant factor may be considered significant under normal conditions and may mask the effects of other factors (Ahuja, Ferreira, & Moreira, 2004; Tekade & Chougule, 2013).

Khan et al., investigated the main effects of various adjuvants and process parameters using a seven-factor, twelve-experiment Plackett-Burman design and used X-Stat® software. For this purpose, cumulative drug release was determined as the independent variable, and the lag time and the time when 50% of the total drug was released were considered by constraints. In the quantitative analysis, the most significant factors were determined, and the significance of these factors was tested by analysis of variance (Khan et al., 2000).

Kiss et al. determined the changing active substance (w/w, %), lipid (w/w, %), and surfactant (w/w, %) ratios; investigated their effects on particle size (nm), zeta potential (mV), loading capacity (%EE) and PDI using two-factor, eight-experimental full factorial design.

Consequently, it was observed that the surfactant ratio had a significant effect on both size and EE% ($p<0.05$), it was determined that the lipid ratio did not have a significant effect ($p>0.05$) (Kiss et al., 2020)

The effect of formulation components on the release of besifloxacin- loaded nanostructured lipid carrier was studied using Design-Expert® software (Stat-Ease Inc., USA) with the aid of a Box-Behnken Design by using amount of drug (X_1), lipid:polymer ratio (w/w, %) (X_2) and surfactant concentration (X_3) are independent variants, as well as the particle size (Y_1) and %EE (Y_2) are dependent variants. ANOVA was used to validate the optimization design and correlation of response of 3D plots was generated. As a result of the analysis of variance, it was seen that the factors had significant effects on the response variables ($p<0.05$) (Ferreira et al., 2007).

Fangueiro et al. used Softisan® 100 (w/w, %), Lipoid S75 (soybean phosphatidylcholine) (w/w, %), and Poloxamer 188 (w/w, %) to had a lipid nanoparticle with a water/oil/water structure containing positive potential. In the Taguchi factorial design model performed with CTAB, it has been shown that the lecithin concentration in the formulation (Lipoid S75) is the key factor affecting the nanoparticle size and PDI ($p < 0.05$). The optimized formulation containing CTAB (0.5% of the lipid phase) appeared to be biocompatible in the human retinoblastoma cell line (Fangueiro et al., 2014).

Recently, formulations can be created by using 2 different design models at the same time in order to obtain precise results. Paclitaxel nanoparticles were prepared by Yerlikaya et al. using both Plackett-Burman and Box-Behnken designs. After determining paclitaxel amount (w/w,%), PLGA amount (w/w,%) , PLGA molecular weight, PLGA terminal group, surfactant concentration (w/w,%), surfactant type, homogenization rate (rpm) and homogenization time parameters (min) as potential risk factors, these risk factors effects were specified on the critical quality properties of nanoparticles, which were determined using the Plackett-Burman experimental design. As a result, it was seen that the amount of PLGA (w/w,%), surfactant concentration (w/w,%), and homogenization rate (rpm) of the emulsion affected the particle size, zeta potential and encapsulation efficiency properties of

paclitaxel nanoparticles, therefore these factors were critical formulation and process parameters. The formulation and process parameters were investigated in Box-Behnken experimental design after determining critical factors as a result of the Plackett-Burman experimental design.

Fifteen formulations in the Box-Behnken test matrix were prepared and analyzed for particle size, zeta potential, and %EE. When the results obtained were evaluated by regression and analysis of variance, it was seen that the mathematical models established could explain the effect of factors on particle size and encapsulation efficiency ($p<0.05$), but the model established for zeta potential was not statistically significant ($p>0.05$) (Yerlikaya et al., 2013).

4. FUTURE SIGHT

Process validation approaches consisting of different stages adopted in drug production for the last 10 years have brought significant advantages in the production of a targeted quality drug product. Although QbD-based product development, design, and continuous process verification phases require detailed planning and investment, it has been the most beneficial approach in terms of stakeholders (patient, industry, official authority) that concern patient health in general.

For the design and development of desired robust ocular products in terms of quality target product profile (QTTP) is mandatory given the biopharmaceutical properties of the active substance. These properties are called critical quality properties (CQAs) and include physical (particle size distribution/morphology, polymorphism, water solubility), chemical (pKa, chemical, photolytic, and oxidative stability), biological/microbiological property (partition coefficient, membrane permeability, bioavailability, microbial limits) or the property of an output material, including the finished drug product, that must be within an appropriate limit to achieve the desired product quality.

The criticality of an attribute is decided by the degree of damage done to it. The ocular product design defines whether the product meets

the patient's requirements and maintains its performance throughout the shelf life specified both by clinical and stability studies. It is obvious that it provides benefits both on the basis of the patient and company while reducing the possible errors in production to the lowest levels and ensuring the delivery of quality products to the patient. However, since the application of current approaches in process validation requires a significant culture change; regulators, industry, and academics, all together with a multidisciplinary approach needs to work.

5. CONCLUSION

DoE approach provides the most appropriate result by changing many variables at once, instead of changing one variable at a time, which is the traditional method. In this way, many factors are evaluated both simultaneously and independently of each other. Thus, the interactions between the variables are more detailed, interpretable, and clear in a short time. Based on these studies, it is clear that the designs of nanoparticles have been prepared successfully with the QbD perspective. In this way, in the future, it will be possible to use drugs that are clinically critical and costly more effectively and to improve new drug carrier systems in a less complicated and time-consuming manner.

REFERENCES

Ahuja, S. K., Ferreira, G. M., & Moreira, A. R. (2004). Application of Plackett-Burman design and response surface methodology to achieve exponential growth for aggregated shipworm bacterium. *Biotechnology and Bioengineering, 85*(6), 666-675. doi:https://doi.org/10.1002/bit.10880

DelMonte, D. W., & Kim, T. (2011). Anatomy and physiology of the cornea. *J Cataract Refract Surg, 37*(3), 588-598. doi:10.1016/j.jcrs.2010.12.037

Fangueiro, J. F., Andreani, T., Egea, M. A., Garcia, M. L., Souto, S. B., Silva, A. M., & Souto, E. B. (2014). Design of cationic lipid nanoparticles for ocular delivery: Development, characterization and cytotoxicity. *International journal of pharmaceutics, 461*(1-2), 64-73.

Ferreira, S. C., Bruns, R., Ferreira, H. S., Matos, G. D., David, J., Brandão, G., . . . Souza, A. (2007). Box-Behnken design: an alternative for the optimization of analytical methods. *Analytica chimica acta, 597*(2), 179-186.

Fialho, S. L., & da Silva-Cunha, A. (2004). New vehicle based on a microemulsion for topical ocular administration of dexamethasone. *Clin Exp Ophthalmol, 32*(6), 626-632. doi:10.1111/j.1442-9071.2004.00914.x

Freitas, C., & Müller, R. H. (1998). Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLN™) dispersions. *International journal of pharmaceutics, 168*(2), 221-229.

Freitas, C., & Müller, R. H. (1999). Stability determination of solid lipid nanoparticles (SLN) in aqueous dispersion after addition of electrolyte. *J Microencapsul, 16*(1), 59-71. doi:10.1080/026520499289310

Fresta, M., Guccione, S., Beccari, A. R., Furneri, P. M., & Puglisi, G. (2002). Combining molecular modeling with experimental methodologies: mechanism of membrane permeation and accumulation of ofloxacin. *Bioorg Med Chem, 10*(12), 3871-3889. doi:10.1016/s0968-0896(02)00350-4

Gaudana, R., Ananthula, H. K., Parenky, A., & Mitra, A. K. (2010). Ocular drug delivery. *Aaps j, 12*(3), 348-360. doi:10.1208/s12248-010-9183-3

Gaudana, R., Jwala, J., Boddu, S. H., & Mitra, A. K. (2009). Recent perspectives in ocular drug delivery. *Pharm Res, 26*(5), 1197-1216. doi:10.1007/s11095-008-9694-0

Gericke, A., Wang, X., Ackermann, M., Neufurth, M., Wiens, M., Schröder, H. C., . . . Müller, W. E. G. (2019). Utilization of metabolic energy in treatment of ocular surface disorders: polyphosphate as an energy source for corneal epithelial cell proliferation. *RSC Adv, 9*(39), 22531-22539. doi:10.1039/c9ra04409d

Hornof, M., Toropainen, E., & Urtti, A. (2005). Cell culture models of the ocular barriers. *Eur J Pharm Biopharm, 60*(2), 207-225. doi:10.1016/j.ejpb.2005.01.009

Irimia, T., Ghica, M. V., Popa, L., Anuţa, V., Arsene, A. L., & Dinu-Pîrvu, C. E. (2018). Strategies for Improving Ocular Drug Bioavailability and Corneal Wound Healing with Chitosan-Based Delivery Systems. *Polymers (Basel), 10*(11). doi:10.3390/polym10111221

Khan, M. A., Sastry, S. V., Vaithiyalingam, S. R., Agarwal, V., Nazzal, S., & Reddy, I. K. (2000). Captopril gastrointestinal therapeutic system coated with cellulose acetate pseudolatex: evaluation of main effects of several formulation variables. *Int J Pharm, 193*(2), 147-156. doi:10.1016/s0378-5173(99)00324-5

Kiss, E. L., Berkó, S., Gácsi, A., Kovács, A., Katona, G., Soós, J., . . . Budai-Szűcs, M. (2020). Development and Characterization of Potential Ocular Mucoadhesive Nano Lipid Carriers Using Full Factorial Design. *Pharmaceutics, 12*(7). doi:10.3390/pharmaceutics12070682

Kompella, U. B., Kadam, R. S., & Lee, V. H. (2010). Recent advances in ophthalmic drug delivery. *Therapeutic delivery, 1*(3), 435-456.

Müller, L. J., Pels, E., & Vrensen, G. F. (2001). The specific architecture of the anterior stroma accounts for maintenance of corneal curvature. *Br J Ophthalmol, 85*(4), 437-443. doi:10.1136/bjo.85.4.437

Pautler, S. (2021). Anatomy of the Eye. Retrieved from https://www.scottpautlermd.com/anatomy-of-the-eye/

Singh, V. P., Ahmad, R., & Heming, T. A. (2011). *THE CHALLENGES OF OPHTHALMIC DRUG DELIVERY: A REVIEW*.

Tekade, R. K., & Chougule, M. B. (2013). Formulation Development and Evaluation of Hybrid Nanocarrier for Cancer Therapy: Taguchi Orthogonal Array Based Design. *BioMed Research International, 2013*, 712678. doi:10.1155/2013/712678

Urtti, A. (2006). Challenges and obstacles of ocular pharmacokinetics and drug delivery. *Adv Drug Deliv Rev, 58*(11), 1131-1135. doi:10.1016/j.addr.2006.07.027

Wadhwa, S., Paliwal, R., Paliwal, S. R., & Vyas, S. P. (2009). Nanocarriers in ocular drug delivery: an update review. *Curr Pharm Des, 15*(23), 2724-2750. doi:10.2174/138161209788923886

Winter, K. N., Anderson, D. M., & Braun, R. J. (2010). A model for wetting and evaporation of a post-blink precorneal tear film. *Math Med Biol, 27*(3), 211-225. doi:10.1093/imammb/dqp019

Yalpani, M. (1997). Polymeric Biomaterials, Edited by Severian Dumitriu, Marcel Dekker, New York, ISBN 0-8247-8969-5, x + 845 pp, 1994, $195.00. *Journal of Carbohydrate Chemistry, 16*(2), 249-250. doi:10.1080/07328309708006526

Yasukawa, T., Ogura, Y., Tabata, Y., Kimura, H., Wiedemann, P., & Honda, Y. (2004). Drug delivery systems for vitreoretinal diseases. *Prog Retin Eye Res, 23*(3), 253-281. doi:10.1016/j.preteyeres.2004.02.003

Yerlikaya, F., Ozgen, A., Vural, I., Guven, O., Karaagaoglu, E., Khan, M. A., & Capan, Y. (2013). Development and evaluation of paclitaxel nanoparticles using a quality-by-design approach. *Journal of pharmaceutical sciences, 102*(10), 3748-3761.

CHAPTER 5

STEM CELLS AND THEIR USAGE IN THE TREATMENT OF DISEASES

Assist. Prof. Dr. Fatih TAŞ[1]

[1] Siirt University, Faculty of Medicine, Department of Histology and Embryology, Siirt, Türkiye, ftas85@yahoo.com, Orcid ID: 0000-0001-9817-4241

INTRODUCTION

Stem cells are the main cells responsible for the development of all tissues and organs in our body. These cells can renew and differentiate themselves according to appropriate signals. In this respect, it can be said that a living organism starts from a single stem cell. Stem cells, which play an active role in our body, have important duties both in renewing our body and in healing damaged/diseased areas. Therefore, stem cells are involved as an alternative treatment strategy for the repair of tissues and organs. In this section, current information about stem cell therapy, which has an important place in the treatment of various diseases, will be discussed.

1. GENERAL CHARACTERISTICS OF STEM CELLS

Undifferentiated cells in the living body, which can regenerate themselves and have the ability to differentiate into many cells according to the needs of the body, are called "Stem Cells". Stem cells can form the basic structure of most tissues, according to the signal they receive through genes. The telomere chain of stem cells is quite long; accordingly, telomerase enzyme activity is high. Therefore, cells have an unlimited ability to divide, thanks to the telomerase enzyme activity of the cells. Thus, stem cells can reproduce for a very long time by self-replication. Stem cells play an important role in the production and repair events in the organism with their differentiation and unlimited division potential (Cerci and Erdost 2019).

Stem cells, according to their potential to differentiate into other cell types, can be called Totipotent, Pluripotent, and Multipotent. Of these, totipotent cells are defined as stem cell types that can form a complete individual, and they have unlimited differentiation capability. These cells provide the formation of all embryonic and post-embryo tissue structures. Pluripotent cells, on the other hand, originate from the three germ layers of embryonic development and can differentiate into about 200 different cells. They can be a source for the formation of many tissues and organs, but they do not have the potential to form a new individual. On the other hand, Multipotent cells transform in a single direction and into a much more limited number of cell types than

pluripotent cells. Therefore, while pluripotent stem cells appear in early developmental stages; Multipotent cells are more common in childhood and adulthood. Blood stem cells in the bone marrow can be given as an example of multipotent stem cells (Sağsöz and Ketani 2008).

Stem cells are generally divided into two groups as Embryonic Stem Cells and Adult Stem Cells.

Embryonic stem cells contain the fertilized oocyte formed after conception, that is, the dividing cells of the zygote called blastomeres. They are totipotent cells capable of forming an entire organism. 4-5 days after fertilization, these totipotent cells become more specialized and turn into pluripotent cells obtained from the inner cell layer of the blastocyst. The differentiation potential of these cells is higher than that of adult stem cells. Diseases in which embryonic stem cells are commonly used include diabetes, central nervous system (CNS) diseases, liver diseases and transplantation, and heart diseases and transplantation (Cannazik and Polat 2014).

Adult stem cells are a group of undifferentiated cells found in tissues that develop in humans or animals after birth. When damage occurs in the tissues and organs that make up the organism, it provides the regeneration of damaged structures. They are cells with multipotent characters. These cells can also transform into other cell types other than the specialized cell type of the tissue from which they originate. Obtaining muscle cells from adult blood stem cells can be given as an example. Since adult stem cells cannot be obtained from every tissue, some tissues such as skin, muscle, and bone marrow are used for this procedure (Cannazik and Polat 2014).

2. STEM CELL THERAPY

It has been known for many years that tissues have their own stem cells, albeit with limited differentiation features, and enter clinical applications. Stem cells are being investigated especially for their potential roles in curative-regenerative medical applications (Şahin et al. 2005). The reproduction of adult and mesenchymal stem cells in vitro and their use in tissue engineering are important for reconstructing different types of tissues (Özmen et al. 2006). The fact that tissues such

as neurons and heart muscle, which are sensitive and do not have the ability to regenerate under normal conditions, can be repaired with stem cell applications is among the promising treatment applications in the future (Şahin et al. 2005). Stem cell transplantation has been successfully used in the treatment of many oncological, hematological, immunological, and metabolic diseases.

2.1. Stem cell therapy in neurological diseases

In previous years, it was thought that central nervous system cells did not have the ability to regenerate. Today, it is known that these cells can also regenerate in case of need. Limited neurogenesis in the central nervous system complicates the treatment of neurological diseases. Stem cells provide trophic healing and stimulate endogenous neurons, astrocytes and vessels. In addition, it provides multi-faceted healing thanks to the secretion of growth factors and its anti-inflammatory effects (Deda 2008).

The important point in stem cell therapy is to correctly decide which cell to use for which type of neurological disease. Neuronal progenitor cells are used in Parkinson's disease. It is recommended in mixed pools of progenitor cells, spinal cord injuries and motor neuropathies. Glial and oligodendrocytic progenitor cells are used in the treatment of myelin diseases. Neural stem cells are recommended in lysosomal storage diseases. The important point here is that these cells are used in a way that does not harm the patient (Goldman and Windrem 2006).

Stem cell and regenerative approaches are gaining importance in neurological diseases. Diseases in which these applications are used or tried; strokes, epilepsy, traumatic brain or spinal cord injuries, neurodegenerative diseases (ALS, Parkinson's, Alzheimer's and Huntington's disease), Multiple Sclerosis, and genetic diseases can be given as examples (Deda 2008). Today, traditional methods applied in the treatment of these diseases cannot provide a definitive solution. Therefore, stem cell applications can be an alternative treatment method in the treatment of these diseases. The important thing here is

to develop and implement common protocols about which stem cells will be used in which diseases and in what way.

2.2. Stem cell therapy applications in dentistry

In dental practice, damaged tooth tissue is restored with various synthetic materials. Recently, studies on a new treatment concept have been carried out in this field with dental pulp stem cell studies and tissue engineering techniques. Dental pulp stem cells have recently become an important research topic, as they are less invasive than other stem cell acquisition methods and do not have ethical problems. A full understanding of the potentials and properties of dental pulp stem cells will be beneficial in terms of developing clinical treatment models and creating a new treatment alternative for dental diseases. Dental pulp stem cell studies are used especially in the creation of tissues such as dentin, pulp, cementum, and periodontal ligament, and thus in the repair of damaged tissue.

In addition to the advantages of being easily obtained, dental pulp stem cells have the potential to easily reach a sufficient number of cells for cellular treatment by showing a very high population increase. In this way, dental pulp stem cells have become an important source of stem cells for autologous stem cell transplantation (Atalayın et al. 2012). Although the regeneration capacity of dental pulp stem cells in dentin/pulp and bone tissue has been demonstrated in vivo, extensive studies are needed to examine the persistence of stem cell characteristics in vitro and optimal tissue regeneration in vivo.

2.3. Stem cell therapy in diabetes mellitus

Diabetes mellitus (DM) has two types, insulin-dependent type I and insulin-independent type II. In type II diabetes, there is a failure to release insulin from pancreatic beta cells. In type I diabetes, on the other hand, there is the destruction of pancreatic beta cells due to immunodeficiency. In both types of diabetes, insulin deficiency or absence is observed because the beta cells of Langerhans islets are damaged. Diabetic stem cell studies have gained importance in order to recover beta cells from different sources or to ensure the replication of mature cells. In order to carry out these studies, it is important to know

the molecular properties of beta cells from embryonic development and the mechanisms of destruction of these cells. If these mechanisms are well understood, they can be a solution to the permanent treatment of diabetes (Mıcılı and Özoğul 2007).

Today, methods such as regular insulin injection, β-cell, or pancreas transplantation are used for the treatment of diabetes. Studies have shown that both pluripotent and multipotent cells can be used in the treatment of diabetes. It has been shown that the expression of insulin and GLUT-2 mRNA increased and blood glucose levels decreased in diabetic mice administered amniotic stem cells. Amniotic stem cells differentiated into cells that produce insulin and glucagon, thereby increasing the levels of these hormones. It has been stated that with these effects, it can be used in the treatment of diabetes (Kaya and Tutun 2021).

2.4. Stem cell therapy in liver diseases

Advances in stem cell biology and the discovery of pluripotent stem cells have made the possibility of cell therapy and tissue regeneration a clinical reality. Cell therapies offer great potential to repair, restore, replace or regenerate affected organs and may outperform any pharmacological or mechanical application. It is stated that adult stem cells, especially bone marrow-derived stem cells, benefit liver and pancreatic islet cell regeneration (Levicar et al. 2007).

Liver failure is a life-threatening liver disease involving severe acute deterioration of liver function. Emergency liver transplant is the only curative treatment for liver failure, but is difficult to access due to shortage of organ donors. Stem cells, including embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, hematopoietic stem cells, and hepatic progenitor cells, have the capacity to proliferate and differentiate. Therefore, stem cells can be used in various liver diseases, including hereditary liver diseases, cirrhosis and liver failure. Stem cell transplant is a promising but challenging strategy. Further research is required for the safe and effective application of this new strategy to humans (Tao et al 2018).

2.5. Stem cell transplantation in heart and liver failure

Although adult stem cells have high differentiation potential, they do not have as high proliferation potential as embryonic and fetal stem cells. In addition, they may not be suitable for transplantation because they have DNA anomalies and genetic defects due to environmental factors. Studies in animal models reveal that various chronic diseases such as liver and heart failure can be successfully treated with the transplantation of pluripotent or fetal stem cells (Yılmaz and Uçar 2006).

In studies on stem cells, it is necessary to control the differentiation properties of these cells and to provide an appropriate environment for in vitro culture processes. In addition, it is important to fully reveal the use of stem cells in transplantation and, most importantly, the mechanisms of rejection of transplanted cells by the recipient as a result of immune reactions. At this point, with the help of cloning technology, the production and use of genetically identical stem cells of the same karyotype and ultimately not rejected by the recipient with immunological reactions added a different dimension to research (Yılmaz and Uçar 2006).

2.6. Stem cell therapy in inflammatory bowel disease

The main goal in the treatment of inflammatory bowel diseases (IBD) is to restore normal intestinal function by controlling inflammation and healing of the mucosa. In addition, it is necessary to ensure the continuation of this remission state by eliminating the symptoms and signs of the disease. Applications such as steroids, anti-inflammatory drugs, and surgical interventions used for this may be insufficient (Leitner and Vogelsang 2016). Today, there is a need for more effective treatment methods for such diseases. At this point, stem cell applications, which have been studied in recent years, can provide a permanent solution.

In a study on Crohn's patients, it was seen that autologous applications were more effective and safer than allogeneic applications. In one of the studies, it was stated that autologous hematopoietic stem cell transplantation was performed in four Crohn's patients who were

unresponsive to conventional therapy, and the patients remained in remission during the post-transplant one-year follow-up period. On the other hand, the mechanism of action of hematopoietic stem cell transplantation in Crohn's disease is not fully known (Craig et al. 2003). Recently, studies on mesenchymal stem cell (MSC) transplantation have been conducted in treatment-resistant Crohn's disease cases. In vivo and in vitro experimental studies have shown that MSC has immunomodulatory effects as well as providing regeneration of damaged tissues (Uccelli et al. 2008). It reveals that stem cell transplantation, which is accepted as an experimental field of application in IBD today, can take its place as a more effective, permanent, and safe treatment method for inappropriate indications.

2.7. Stem cell therapy in immunodeficiency, metabolic disorders and various blood diseases

Bone marrow transplantation is used in the treatment of many childhood diseases such as hematological malignancies, hemoglobinopathy, and bone marrow deficiencies. Bone marrow transplantation is defined as hematopoietic stem cell transplantation (HSCT) due to the use of peripheral blood and umbilical cord blood as stem cell sources. This practice is a part of the treatment protocols in some diseases and as the main treatment option in others (Yeşilipek 2014). For example, in megakaryocytic thrombocytopenia, which is a genetic disease, allogeneic hematopoietic stem cell transplantation is used as the only curative treatment method (Yeşilipek 2000).

Stem cell transplant; is performed as a curative treatment method for blood diseases, various cancers, immune deficiencies, some hereditary metabolic diseases, and autoimmune diseases. At this point, especially hematopoietic stem cell transplantation; It is successfully applied in diseases such as leukemia, lymphoma, myelodysplastic syndrome, congenital neutropenia, and aplastic anemia. In addition, it has been reported that similar treatment practices have been successfully applied in systemic lupus erythematosus, an autoimmune disease, or Hurler Syndrome, a hereditary metabolic disease (Kuşkonmaz 2016).

2.8. Other uses of stem cell therapy

After high-dose chemotherapy and radiotherapy are applied in the treatment of some cancer diseases, stem cells taken from healthy individuals are given to these patients. In this way, the remaining cancer cells in the patient are destroyed and the patient's immune system is regenerated/strengthened. In addition, stem cells are also used in the field of dermatology. An example of this is the use of stem cells and successful results in the treatment of hair loss caused by alopecia areata, an autoimmune disease. Also, stem cells; It is used for research or treatment in many fields such as orthopedics, acute/chronic kidney failure, tissue engineering, and veterinary medicine. Stem cells can be used more effectively for the treatment of these diseases by using the data to be obtained through studies on stem cell surface markers (Avcı 2022).

CONCLUSION

New studies are needed to understand the physiology of stem cells and to solve the problems that arise in stem cell therapy in humans. In particular, the approach of collecting stem cells from patients and re-implanting them in the patient may be more reliable than applications such as organ transplantation and immunosuppressive therapy. In addition, stem cells can be used as an effective method for many diseases for which there is no effective treatment today.

REFERENCES

Atalayın Ç, Ergücü Z, Tezel H. Diş hekimliğinde kök hücre ve dental pulpa kök hücreleri. Gazi Üniversitesi Diş Hekimliği Fakültesi Dergisi. 2012;29(2):115-20.

Avcı M. Kök Hücre; Tanımı ve Genel Özellikleri, Kullanım Alanları, Tarihi, Yüzey Belirteçleri. Arşiv Kaynak Tarama Dergisi. 2022;31(2):87-93.

Cannazik O, Polat B. Kök hücre ve veteriner hekimlikte uygulama alanları. Atatürk Üniversitesi Veteriner Bilimleri Dergisi. 2014;9(3).

Çerci E, Erdost H. Kök hücre. Atatürk Üniversitesi Veteriner Bilimleri Dergisi. 2019;14(2): 221-8.

Craig RM, Traynor A, Oyama Y, Burt RK. Hematopoietic stem cell transplantation for severe Crohn's disease. Bone Marrow Transplant. 2003;32(1):S57-9.

Deda H. Nörolojik Hastalıklarda Kök Hücre Tedavileri. Journal of Nervous System Surgery. 2008;1(3):142-52.

Goldman SA, Windrem MS. Cell replacement the-rapy in neurological disease. Phil Trans R Soc B. 2006;361:1463-75.

Kaya MM, Tutun H. Kök Hücre Üretimi, İzolasyonu ve Tedavide Kullanımı. Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni. 2021;12(2):55-78.

Kuşkonmaz, BB. Hematopoetik Kök Hücre Transplantasyonu ve Çocuklardaki Kazanılmış Aplastik Anemi Tedavisinde Transplantasyonun Yeri. Bone Marrow Transplant. 2016;51:778.

Leitner, GC, Vogelsang H. Pharmacological-and non-pharmacological therapeutic approaches in inflammatory bowel disease in adults. World journal of gastrointestinal pharmacology and therapeutics. 2016;7(1):5.

Levicar N, Dimarakis I, Flores C, Tracey J, Gordon MY, Habib NA. Stem cells as a treatment for chronic liver disease and diabetes. Handbook of experimental pharmacology. 2007;(180):243–62.

Mıcılı SC, Özoğul C. Diyabette kök hücreler. Dokuz Eylül Üniversitesi Tıp Fakültesi Dergisi. 2007;21(2):109-17.

Özmen S, Fındıkçıoğlu F, Siemionow M. Kök hücreler. Türk Plastik Rekonstrüktif ve Estetik Cerrahi Dergisi. 2006;14(3):187-96.

Sağsöz H, Ketani MA. Kök hücreler. Dicle Üniversitesi Veteriner Fakültesi Dergisi. 2008;2: 29-33.

Şahin F, Saydam G, Omay SB. Kök hücre plastisitesi ve klinik pratikte kök hücre tedavisi. Türk hematoloji-onkoloji dergisi. 2005;1(15):48-56.

Tao YC, Wang ML, Chen EQ, Tang H. Stem Cells Transplantation in the Treatment of Patients with Liver Failure. Current stem cell research & therapy. 2018;13(3):193-201.

Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat. Rev. Immunol. 2008;8(9):726-36.

Yesilipek MA, Hazar V, Kupesiz A, Yegin O. Peripheral stem cell transplantation in a child with amegakaryocytic thrombocytopenia. Bone Marrow Transplant. 2000;26:571-2.

Yeşilipek MA. Çocuklarda hematopoetik kök hücre nakli. Turk Pediatri Ars. 2014;49:91-8.

Yılmaz O, Uçar M. Kök Hücre Çalışmaları Ve Terapötik Klonlama. Hayvancılık Araştırma Dergisi. 2006;16(1):26-31.

CHAPTER 6

PNEUMOTHORAX

Dr. Ali Zeki BEDIR[1] , Assoc. Prof. Dr. Safak KARACAY[2]

[1] Yeditepe University, Faculty of Medicine, Department of Pediatrics, Istanbul, Türkiye. zekibedir@gmail.com, Orcid ID: 0000-0003-2530-0109.
[2] Yeditepe University, Faculty of Medicine, Department of Pediatric Surgery, Istanbul, Türkiye. safakkaracay@gmail.com, Orcid ID: 0000-0001-8615-7727.

INTRODUCTION

Pneumothorax is a thoracic emergency caused by the collection of free air between the parietal and visceral pleural sheets. It is commonly observed after rupture of sub-pleural bullae in the lung. Although it was previously thought to be observed as the result of complications of infectious diseases such as tuberculosis, it has been realized that pneumothorax can also be observed in healthy individuals. It is observed more frequently in men. Etiologically, it is classified as spontaneous and acquired (*Light R. 2001 - Baumann MH, Noppen M. 2004*) Acquired pneumothorax may develop due to trauma or may be iatrogenic. (*Bozkurt AK. 2002*) Pneumothorax is classified in three groups as traumatic, iatrogenic and spontaneous.

Traumatic pneumothorax is observed after blunt or penetrating trauma of the thorax. After the impact, there is a rapid air flow into the pleural cavity. This airflow may be caused by a defect in the thoracic wall in penetrating traumas or by a defect in the tracheobronchial system or costal dislocation as observed in blunt traumas. There are three types of traumatic pneumothorax according to the mechanism: simple, open and tension pneumothorax.

Simple pneumothorax is frequently observed due to rib fractures as a result of blunt trauma. Simple pneumothorax may not be recognized by chest radiography and may not even cause clinical symptoms. Large pneumothoraxes with clinical symptoms are quickly treated with tube thoracostomy. The most important determinant of treatment is the patient's general condition and clinical follow-up.

Open pneumothorax is observed as a result of penetrating trauma. It is observed as a result of air entry through the defect caused by trauma. An opening is observed in the thoracic wall associated with the pleural cavity. There is a continuous inflow of air from the atmosphere into the pleural cavity, the negative pressure is first equalized with the air pressure in the lung and then begins to increase. Subsequently, clinical deterioration is observed. The passage should be closed quickly with a sterile gauze. Before tube thoracostomy application, it should be ensured that there is no air entry from the open

defect which is very important to prevent tension pneumothorax. (*Öztürk CA. 2013*)

Tension pneumothorax occurs with unilateral air leakage inside the pleural space. As this air passage from the tracheobronchial tree or lung parenchyma into the intrapleural space increases, mediastinal shift develops. As pleural pressure increases, ventilation tends to deteriorate rapidly, while at the same time the heart is compressed due to the push on the mediastinum. As venous return to the heart will decrease, cardiac output will also decrease and respiratory distress will deepen. The most important condition for treatment is to ensure rapid oxygenation of the patient and tube thoracostomy and closed underwater drainage.

Iatrogenic pneumothorax is observed especially in neonatal groups which is usually observed after central catheter placement and biopsies. In children, iatrogenic pneumothorax is more common than traumatic pneumothorax as the age decreases (*Harrison TR. 2015*) The most common causes other than central catheter placement are thoracentesis and bronchoscopy for diagnostic and therapeutic purposes. It can also be observed due to difficult intubation and high pressure mechanical ventilation. (*Dubus T, Uzman O, Demiryontar D, Kiraz R, Vatansever 2012*) These cases often improve with oxygen supplementation and aspiration of air with a needle. However, if no improvement is observed during follow-up, tube thoracostomy and closed drainage may be necessary.

Spontaneous pneumothorax is generally classified into two groups as primary and secondary. If there is no underlying pathology related to the respiratory system and the lung is structurally normal, it is called primary pneumothorax; if there is another underlying lung disease, it is called secondary pneumothorax. (*Shields TW. The pleura. In Shields TW, LoCiceroIII J, Reed CE, Feins RH, 2009*)

Primary Spontaneous Pneumothorax

Primary spontaneous pneumothorax is the accumulation of air between the pleural sheets in the absence of clinically or radiologically proven lung disease or history of trauma. This event is thought to be

caused by rupture of the alveoli in the blebs. (*Baumann MH. 2003*) Blebs are localized air accumulations smaller than 1 cm while bullae are larger parenchymal air accumulations. Inflammation caused by smoking is generally the responsible etiological agent (*Sahn SA, Heffner JE. 2000*) It is generally thought to pass through genetic transmission.

Secondary Spontaneous Pneumothorax

Secondary spontaneous pneumothorax is observed in individuals with underlying lung disease. (*Henry M, Arnold T and Harvey J. 2003*) The most common underlying cause is chronic obstructive pulmonary disease, as it is frequently seen in the adult patient group. In adults, necrosis is observed in the peripheral parts of the lung due to progressive inflammation. As a result of this necrosis, air fills between the ruptured alveoli and pleural layers. The best example of this is the necrotizing alveolitis observed in the lungs after acquired immune deficiency syndrome in the adult population.

1. PHYSIOLOGY

The pleura is a serous layer of mesothelial cells lining the inner surface of the lung and thoracic cavity. It consists of two sheets, parietal and visceral. The pleural space is between these two layers and contains pleural fluid. The visceral pleura penetrates the lung fissures and covers the entire area. Above, the parietal pleura covers the interior of the mediastinum, the thoracic wall and the diaphragm.

The lung parenchyma tends to collapse due to its elastic structure and the thoracic wall tends to expand. This creates negative pressure between the pleural layers. This negative pressure is important for lung movement. Lung movements occur in two ways. The first of these is by shrinking and expanding the thoracic cavity due to the diaphragmatic movements. The second is by regulating the antero-posterior diameter of the chest with the help of intercostal muscles. Physiologic breathing is caused by diaphragm movements, whereas in forced breathing the intercostal muscles are involved.

1.1 Fetal lung development

Lung development starts in the fetal period and continues until 8 years of age and is analyzed in five different periods (*Henry Gray. 2016*)

The embryonic period covers the first 4 weeks. Organogenesis is observed in this period. Alveolarization and microvascular formations are observed. Primitive trachea formation and basic right and left bronchi develop. Various malformations can be observed as a result of errors during organogenesis in this period. The most important of these are congenital anomalies such as tracheoesophageal fistula, esophageal atresia and diaphragmatic hernia.

The pseudoglandular period covers the prenatal period between 5-16 weeks. During this period, there is a simple respiratory tree and it is filled with fluid. It does not adequately support gas exchange. Development up to the terminal bronchiole stage is observed during this period. (*Warburton D, El-Hashash A, Carraro G, Tiozzo C, Sala F, Rogers O, et al. 2010*)

The canalicular period occurs between the prenatal weeks 16-25. During this period, the cranial part of the lung develops faster than the caudal part. Vascularization and angiogenesis are more prominent. The canaliculi that will develop into alveolar ducts are formed and airway epithelial differentiation is observed. Completion of this stage is very important for preterm babies. Bronchopulmonary dysplasia is frequently observed in babies born at the end of this period because lung maturation is not complete. (*Schittny JC. 2017*) In this disease, in which prematurity is the most important risk factor, acute damage causes many structural and physiologic changes.

The saccular period is between the prenatal 25-36th weeks. This is the period when bronchial branching is completed. The air-blood barrier and the surfactant production is mainly observed at this stage. Preterm infants have to provide gas exchange during these stages, and the main reason for administering surfactant to the infant during these stages is to mimic this physiology.

The alveolarization stage is the last stage of lung development and continues until the age of 8 years. The formation of air spaces is dominated by fully functional alveoli in which basic air exchange takes place. Surfactant is in sufficient quantity and reduces surface tension. This prevents collapse of the lungs (*Warburton D, El-Hashash A, Carraro G, Tiozzo C, Sala F, Rogers O, et al. 2010*).

1.2 Lung Ventilation

The main purpose of respiration is to provide tissues with oxygen and to remove accumulated carbon dioxide. The first of the four main steps to ensure this physiology is ventilation. The other steps are diffusion, perfusion and regulation (*Guyton A, Hall J. 2006)*

Owing to the flexible structure of the lung, there is no problem in air inlet and outlet as long as there is no obstructive pathology. Since the lungs do not have a complete connection with other tissues, they are thought to float in pleural fluid. This fluid is calculated to be approximately 0.1 ml/kg and is clear in nature. This is the area where effusions are observed. Excess fluid drains into the lymphatics and is the main cause of the negative pressure between the two pleural sheets.

In physiologic respiration, energy is expended mainly during inspiration, because muscle contractions are observed. Expiration is passive and does not consume as much energy as inspiration. Only 3-5% of the daily energy requirement is used for respiration (*Guyton A, Hall J. 2006*)

1.3 Respiratory Physiology of the Newborn

The baby should inhale and exhale as soon as the labor finishes. To do this, the baby must start active breathing. If there are problems during this transition, hypoxic ischemic encephalopathy may occur because the brain will be deprived of oxygen. The alveoli open with the first respiration after birth. A time period and effort is required for the alveoli to open completely. In this process, the newborn may have respiratory problems. The most common one is transient tachypnea of the newborn. Babies with is transient tachypnea and similar respiratory problems may need to be closely monitored. These babies may need supportive oxygen therapy. (*Guyton A, Hall J. 2006)* Delivery of

oxygen in a step wise fashion from the noninvasive to the invasive methods is preferred. In this period, complications such as pneumothorax may be occur.

1.4 Pathophysiology of pneumothorax

The pressure in the pleural space, which is considered to be between the visceral and parietal leaves of the pleura, is negative compared to atmospheric pressure. Because of this negative pressure, the thoracic wall tends to expand. Since the lungs are elastic, they cause the tissues to collapse inward. If a tear develops in this interstitial space, air fills the intrapleural space. Air continues to pass until the pressure stabilizes. This results in total or partial lung collapse. This event is called pneumothorax. In the following process, ventilation is impaired first, and then it starts to affect perfusion and vital signs.

2. ETIOLOGY

Subpleural blebs and bullae, especially in the lung apex, are most commonly responsible for the etiology. Anatomical abnormalities are also frequently demonstrated even in the absence of an underlying disease. The most important risk factor is smoking. In a study conducted in a group of patients newly diagnosed with primary spontaneous pneumothorax, the risk of developing pneumothorax was 12% in healthy men and smokers, while this rate was 0.1% in non-smokers. (*Bense L, Eklund G, Wiman L-G. 1987*)

The most common etiologies of secondary spontaneous pneumothorax are chronic obstructive pulmonary disease, bullous emphysema, malignancies, Marfan syndrome. There is a risk of recurrence depending on the underlying disease. Infections also play an important role in the etiology, especially in the pediatric population. They are encountered both in the acute and in the recovery period. Although tuberculosis is the most common infection, viral infections and parasitic infections are also involved in the etiology. Covid-19 infection should be considered in the foreground in terms of pneumothorax in a complaint such as chest pain. Previous Covid-19 infection should not be ignored in terms of predisposition to secondary bacterial and fungal infections. SARS-CoV-2 virus may present with a

clinical presentation such as acute chest pain or respiratory distress such as pneumothorax after the disease, although there are limited cases reported. One of such cases presented to our clinic.

A 15-year-old male patient was admitted to our emergency department with sudden chest pain that started 2 hours ago. He had no additional complaints other than chest pain and did not describe respiratory distress. It was learned that 1 month ago, the patient was quarantined due to the detection of a Covid-19 case in the family and that he spent this period with subfebrile fevers and had no additional complaints during this period. The SARS-CoV-2 PCR test obtained from him during this period was positive and he was followed up with symptomatic treatments at home. After quarantine, everyone in the family tested negative. When further investigations were performed, pneumothorax was diagnosed and no risk factor could be found in the etiology of spontaneous pneumothorax in detailed examinations. As a result of the evaluations, SARS CoV 2 was considered as the cause of pneumothorax.

In traumatic pneumothoraxes, cardiac catheterization used to be most frequently blamed for the etiology, but it has been replaced by mechanical ventilation, resuscitation, and central venous catheterization. (*Antoni R, Ponka J. 2000*).

3. EPIDEMIOLOGY

Pneumothorax is seen in all periods including newborn, infant, school child, adolescent, adult and old age. Approximately 20.000 cases of spontaneous pneumothorax are detected annually in the USA. Among these cases, primary and secondary pneumothorax cases are very close in frequency. It is observed 6 times more frequently in males than in females. (*Sadikot RT, Greene T, Meadows K, Arnold AG. 1997)* However, this rate has been decreasing over the years.

4. CLINICAL FINDINGS

Typical symptoms in a patient with pneumothorax are chest pain and respiratory distress. Symptoms are related to the patient's age group, the size of the pneumothorax and the underlying clinical features of the patient. Most patients usually have acute onset of unilateral chest

pain. The type of pain may be sharp or superficial. Dyspnea is related to the severity of the case. Chest pain is predominant in primary spontaneous pneumothorax, while increased respiratory effort is more common in secondary pneumothorax. Acute onset of pain begins to decrease over time even if the pneumothorax is not treated and is replaced by respiratory distress, cough and dyspnea. (*Sadikot RT, Grene T, Meadows K, Arnold AG. 1997*)

As with the symptoms, the size of the pneumothorax area determines the findings on physical examination. Physical examination is often normal in cases with a pneumothorax size of less than 15% (*Light RW. 1995*)

If the patient's lung collapse is evident, inspection reveals decreased participation of the hemithorax on that side in respiration. Respiratory sounds are decreased, vibration thoracic decreases on palpation, and hyper sonority is obtained during percussion. Tachycardia is the most common clinical finding..

5. DIAGNOSIS

A careful physical examination alone is not sufficient for the diagnosis of pneumothorax. Supportive radiologic evaluation is often needed because the symptoms will become more prominent in the later stages. The first method to be used for the diagnosis is posteroanterior chest radiography. It is the most valuable diagnostic method and a high rate of diagnosis can be made. (*Glazer H, Anderson D, Wilson B, Molina P, Sagel S. 1989*) The sensitivity is 83% and the finding is the appearance of a visceral pleural line with no evidence of vascular or lung parenchyma in the radiolucent area between this line and the chest wall. (*Seow A, Kazerooni EA, Pernicano PG, Neary M. 1996*) In half of the cases, an air-fluid level is seen in the costophrenic angle. This may be the only abnormality in some cases. In addition, lateral and lateral decubitus radiographs are useful in the diagnosis as minimal pneumothorax may not be visible on radiographs.

Lateral radiographs may be performed when a pneumothorax is suspected and standard posteroanterior chest radiographs do not provide adequate confirmation. It is not routinely used in daily clinical

practice. Lateral decubitus radiographs are more commonly used in trauma patients with restricted mobility; however, the sensitivity is lower than posteroanterior radiographs. (*Beres R, Goodman L. 1993*) Expiratory radiography is not recommended today.

Ultrasonography is very valuable in the diagnosis of pneumothorax and is nowadays much more preferred because it is radiation free. It is preferred more frequently in pregnant women and children. It is mainly preferred in trauma patients in the supine position.

Computed tomography is the gold standard for definitive diagnosis of pneumothorax (*Kelly A-M, Weldon D, Tsang AY, Graham CA. 2006*) It provides a serious advantage in estimating the size and valuable in detecting pathologies that cannot be detected on posteroanterior radiographs. It is also very effective in emphysematous diseases, detection of lung anatomical abnormalities and differential diagnosis of pneumothorax. It is not used as a primary diagnostic tool due to high radiation exposure, difficulty of application and accessibility.

6. TREATMENT

Although the rationale for the treatment of all types of pneumothorax is basically the same, the order of approach varies in some cases. The first aim is to evacuate the air in the pleural space and restore the lung expansion. Subsequently, the secondary goal should be to prevent the recurrence of this condition. The most important factors in deciding the treatment method are the patient's clinical condition and the size of the pneumothorax. There are 5 basic approaches. These are observation, needle aspiration, percutaneous drainage catheter, tube thoracostomy and surgery.

Invasive procedures are not preferred in patients with good physical condition and without dyspnea (usually in asymptomatic cases) and especially in patients with a pneumothorax area less than 15-20%. These patients are followed up with posteroanterior chest radiographs. The absorption rate of pneumothorax in room air is 1.25% per day. This rate can be accelerated 3-4 times with oxygen administration (*Bigger IA. 1937*) Oxygen supplementation during the

observation, accelerates the absorption of intrapleural air and also corrects momentary hypoxia. The biggest risk for the patient under observation is sudden complications. Therefore, this method carries certain risks, observation should last at least 24-48 hours and close follow-up examinations are valuable until complete resorption occurs. If the patient experiences shortness of breath or there are paradoxic changes in vital signs, observation is immediately abandoned and more aggressive approaches are initiated. While the recurrence rate is lower with the observation method used especially in the neonatal period, in studies conducted in the adult group, the recurrence rate in pneumothoraxes that resolved by observation was found to be significantly higher than in patients treated with tube thoracostomy. (*Donahue DM, Wright CD, Viale G, Mathisen DJ, 1993*)

When the pneumothorax area exceeds 15-20%, observation is not sufficient in treatment, needle aspiration is necessary. The fact that it is easy to perform and less invasive are the advantages in its application. In the past, the second midclavicular line was preferred, but now the entry point on anteroaxillary line through the fourth or fifth intercostal space is recommended. In children, there are authors that still recommend the second midclavicular intercostal space entry.16-18 After the intervention is performed with a catheter cannula, aspiration is performed until the air suction stops. If no resorption is observed on follow-up radiographs, the tube thoracostomy method should be performed. Needle aspiration is recommended as the first step treatment for tension pneumothorax.

Percutaneous drainage catheter is used in small and moderate pneumothorax. In spontaneous pneumothorax, the success rate with a special catheter system called pleurocan is 59%, while the success rate with Heimlich valve has been shown to be higher. The use of these valves facilitates mobilization and patient care. Disadvantages include catheter kinking, occlusion and bleeding. Rapid evacuation of air may cause pulmonary edema. However, high suction power may also cause persistent air leakage. The catheter is clamped and monitored; if no complications are observed, the catheter is removed. (*So S, Yu D, 1982*)

Chest tube is used with a closed drainage system and provides unidirectional drainage from the pleural space. It is vital for tension pneumothorax. After tube thoracostomy, the drain is usually placed in a closed underwater drain. Tube thoracostomy can be performed under sedation in agitated patients, but local anesthesia is usually sufficient. Pneumothorax, hemothorax, chylothorax, empyema, pleural effusion are indications for tube thoracostomy. It is the treatment of choice for moderate to large pneumothorax. It is preferred if the patient is symptomatic or if complications develop in the observation period, in tension pneumothorax or if adequate re-expansion cannot be achieved.

When performing tube thoracostomy, the patient is given detailed information about the procedure. The area is cleaned with antiseptic solution. Local anesthesia is instilled after determining the zone where the tube thoracostomy will be applied. The pleural cavity is entered with the needle tip. Soft tissues are dissected after a 1-2 cm skin incision. The pleura is opened by blunt dissection from the upper edge of the costa and the thorax is entered. After crossing the pleura, the drain is pushed towards the apex through the thoracic cavity using forceps. The drain is fixed to the skin and the distal end is connected to a closed underwater system. The dressing around the drain is applied and a control chest x-ray is obtained.

Lung expansion with tube thoracostomy has been reported to be 90% in the first pneumothorax, 52% after the first recurrence and 15% after the second recurrence. (*Sahn SA, Heffner JE. Spontaneous pneumothorax. N Engl J Med 2000; 342(12):868-74.*) While the procedure is technically easy to perform, its' complications may be serious. The most common complication is the malposition of the tube. Other complications include bleeding, nerve damage, infection of the site, chylothorax, pain and reexpansion edema. The tube thoracostomy procedure can be terminated 24 hours after the end of air leak, after full expansion is observed on chest radiography and after the total daily drainage decreases below 100-200 ml. (*Roman M, Mercado D, 2006*)

Surgery is usually considered in patients when previous treatment modalities are inadequate. The main indications are recurrent pneumothorax, persistent air leakage lasting more than seven days,

bilateral pneumothorax, pneumothorax in a patient with pneumonectomy pneumothorax of special groups of people such as divers and pilots. (*Beauchamp G, Ouellette D, 2002*) Surgical procedures include video-assisted thoracic surgery (VATS), thoracotomy and pleurodesis. The main goal of surgical treatment is to treat the parenchymal lesion and prevent recurrence.

VATS is the first choice of surgical method considered for the treatment of spontaneous pneumothorax having advantages of reduced hospitalization and less postoperative pain. VATS is performed with double lumen endobronchial tubes under general anesthesia. Thoracotomy is usually performed as an axillary thoracotomy with anterior, posterolateral muscle sparing. There has been a marked decrease in the use of thoracotomy due to VATS. It is mainly preferred in cases of recurrence of primary spontaneous pneumothorax. Pleurodesis is used in cases of frequent recurrence when the risk of open surgery is high. A chemical fluid is introduced into the pleural cavity through a tube. However, pleurodesis performed by VATS is considered to be more effective than that performed through a thoracic tube. (*Hwong TM, Ng CS, Lee TW, et al. 2004*)

REFERENCES

Light, R. Transudative pleural effusions. (2001). Pleural diseases 4th ed Baltimore: Williams and Wilkins, 96-107.

Baumann, MH., Noppen, M. Pneumothorax. (2004). Respirology, 9(2),157-164.

Bozkurt, AK. Pneumothorax. (2002). Respiration, 4(supplement 1),206-9.

Öztürk, CA. Thoracic Trauma and Treatment. (2013). In: Yucel O, editor. Thoracic Trauma and Treatment. 1st ed. Ankara: Derman Tıbbi Yayıncılık. p.133-8.

Harrison, TR. Disorders of the Pleura. (2015) In: Dennis L. Kasper, Stephen L. Hauser, J. Larry Jameson, Anthony S. Fauci, Dan L. Longo JL, editor. Harrison's principle of internal medicine. 19th Ed. New York: McGraw Hill Medical Books; p. 1716-9.

Dubus, T., Uzman, O., Demiryontar, D., Kiraz, R., Vatansever, S. Iatrogenic Pneumothoraces: Analysis of 27 Cases. (2012). Istanbul Med J, 13(3):133-6.

Shields, TW. The pleura. (2009). In Shields TW, LoCiceroIII J, Reed CE, Feins RH, ed. General Thoracic Surgery, vol 1, 7th ed. Philadelphia: Lippincott Williams and Wilkins, 740-61.

Baumann, MH. Management of primary spontaneous pneumothorax? Pro: blebs do cause primary spontaneous pneumothorax. (2003). J Bronchol, 9:313-8.

Sahn, SA., Heffner, JE. Spontaneous pneumothorax. (2000) N Engl J Med, 342(12):868-74.

Henry, M., Arnold, T., and Harvey J. BTS guidelines for the management of spontaneous. (2003). Thorax pneumothorax, 58(2):39-52.

Henry, Gray. Thorax (2010) In: Jonathan D Spratt, editor. Gray's Anatomy. 41st ed. London: Elsevier Limited; 2016. p. 898-969

Warburton D, El-Hashash A, Carraro G, Tiozzo C, Sala F, Rogers O, et al. Lung Organogenesis. In: Current Topics in Developmental Biology. p. 73-158.

Schittny, JC. Development of the lung. (2017). Cell Tissue Res, 367(3):427-44.

Guyton, A., Hall, J. respiratory physiology. (2006). In: John E, editor. Textbook of medical physiology. 11th ed. Philadelphia: Saunders Elsevier p. 471-501.

Guyton, A., Hall, J. Physiology of the fetus and newborn. (2006). In: John E, editor. Textbook of medical physiology. 11th ed. Philadelphia: Saunders Elsevier, p. 1044-52.

Bense, L., Eklund, G., Wiman, L-G. Smoking and the increased risk of contracting spontaneous pneumothorax. (1987). Chest, 92(6):1009-1012.

Antoni, R., Ponka, J. The hazard of iatrogenic pneumothorax in certain diagnostic and therapeutic procedures. (1961). Surgery, gynecology & obstetrics. 113:24-32.

Weissberg, D., Refaely. Y. Pneumothorax: experience with 1,199 patients. (2000). Chest, 117(5):1279-1285.

Sadikot, RT., Greene, T., Meadows, K., Arnold, AG. Recurrence of spontaneous pneumothorax. (1997). Thorax 52:805.

Sadikot, RT, Grene, T., Meadows, K., Arnold, AG. Recurrence of primary pneumothorax. (1997). Thorax, 52(9):805-9.

Light, RW. Pleural diseases. (1995). 3rd ed. Baltimore: Williams and Wilkins, 7-13, 242-77.

Glazer, H., Anderson, D., Wilson, B., Molina, P., Sagel, S. Pneumothorax: appearance on lateral chest radiographs. (1989). Radiology, 173(3):707-711.

Seow, A., Kazerooni, EA., Pernicano, PG., Neary, M. Comparison of upright inspiratory and expiratory chest radiographs for detecting pneumothoraces. (1996). AJRAmJRoentgenol, 166:313.

Beres, R., Goodman, L. Pneumothorax: detection with upright versus decubitus radiography, (1993) Radiology. 186(1):19-22.

Kelly, A-M., Weldon, D., Tsang, AY., Graham, CA. Comparison between two methods for estimating pneumothorax size from chest X-rays. (2006). Respiratory medicine, 100(8):1356-1359.

Bigger, IA. Operative Surgery. (1937). Vol 2. St. Louis: C.V. Mosby Company, 605

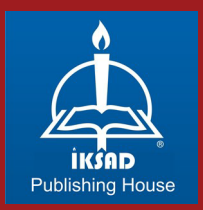
Donahue, DM., Wright, CD., Viale, G., Mathisen DJ. Resection of pulmonary blebs and pleurodesis for spontaneous pneumothorax, (1993). Chest. 104(6):1767-1769.

So, S., Yu, D. Catheter drainage of spontaneous pneumothorax: suction or no suction, early or late removal? (1982). Thorax, 37(1):46-48.

Roman, M., Mercado, D. Review of chest tube use. (2006) Medsurg Nurs, 15(1):41-3.

Beauchamp, G., Ouellette, D. Spontaneous pneumothorax and pneumomediastinum. (2002). I: Pearson FG, ed. Thoracic surgery Philadelphia: Churchill Livingstone, 1195-1213.

Hwong, TM., Ng, CS., Lee, TW., et al. Video-assisted thoracic surgery for primary spontaneous hemopneumothorax. (2004). European journal of cardio-thoracic surgery, 26(5):893-896.



ISBN: 978-625-6380-50-9