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Acute Eosinophilic Appendicitis Immunohistochemical Study

A Thesis Submitted to the Faculty of Science and Health at Koya University as a Partial Fulfilment for the Degree of Masters of Science (MSc.) in Biology

By

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بسم الله الرحمن الرحيم

قَالُواْ سُبْحَٰنَكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآۖ إِنَّكَ أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ

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I declare that the Master Thesis entitled **"Acute Eosinophilic Appendicitis Immunohistochemical Study"** is my original work, and hereby certify that unless stated, all work contained within this thesis is my independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the next.

Dedication

Who took care of me and did not see the fruit of my efforts, my dear Brother, Allah bless him.

My dear Mother and Father who suffered so much for me.

My darling husband (Sardar) who infinitely supports me with all his love.

My lovely daughters (Waren & Allen) who opened my heart to life.

My dear brother (Hoshmand Jawad Hussein)

My Friends and all who love me.

Viva Examining Committee Approval

Supervisor's Approval

Hereby I'm Dr. Sarmad Raheem Kareem, state that this thesis as entitled (**Acute Eosinophilic Appendicitis Immunohistochemical Study**) was prepared under my supervision at Biology Department / Koya University by (Shahla Jawad Hussein) as a partial fulfillment for the degree of Master of Science (MSc.) in Biology.

I have read and reviewed this work and I confirm that it is an original work to the best of my knowledge.

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IV

Viva Examining Committee Approval

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List of Abbreviations

AA	Acute appendicitis
AEA	Acute Eosinophilic Appendicitis
AIR	Appendicitis inflammatory response
CBC	Completed blood count
CRP	C-reactive protein
CT	Computed tomography
EC	Eosinophilic colitis
ECP	Eosinophilic cationic protein
EDN	Eosinophil derived neurotoxin
EDTA	Ethylenediaminetetraacetic acid
EE	Eosinophilic enteritis
EG	Eosinophilic gastritis
EGIDs	Eosinophilic Gastrointestinal diseases
EOE	Eosinophilic esophagitis
EPO	Eosinophil peroxidase
GI tract	Gastrointestinal tract
GM-CSF	Granulocyte macrophage colony stimulating factor
H&E	Hematoxylin and eosin
HES	Hyper eosinophilic syndrome
HPF	High power field
IHC	Immunohistochemistry
IL-4	Interleukin-4
IL-5	Interleukin-5
LGIH	Lower gastrointestinal hemorrhage

MBP	Major basic protein
MBPmAb	Major basic protein monoclonal antibody
MGG	May Grunwald Giemsa
MPV	Mean platelet volume
MRI	Magnetic resonance imaging
NLR	Neutrophil -to- lymphocyte ratio
PAS	Pediatric appendicitis score
PDW	Platelet distribution width
PLT	Platelet
RDW	Red cell distribution width
RIF	Right iliac fossa
US	Ultrasound
X	Magnification power
WB	Washing buffer
WBC	White blood cell

Abstract

Acute appendicitis (AA) is the most common abdominal surgical emergency in the world and most cases develop as a result of luminal obstruction. The incidence of AA is strongly age dependent reaching a peak incidence in the teenagers and early 20s. Mainly clinically diagnosed based on history, physical examination and imaging with adjuvant laboratory test, but a histopathological study remains a gold standard for diagnosis of AA. Histopathologic finding of AA is the presence of neutrophils in the muscularis propria. However, an unusual variant of appendicitis which is called acute eosinophilic appendicitis (AEA) has eosinophilic infiltration of the muscularis propria and edema separating the muscle fibers without the presence of neutrophilic infiltration as its pathologic hallmark and type I hypersensitivity is responsible for its pathogenesis. So, this research was aimed to find simple and rapid way for detection of unusual form of AA that may require postoperative special treatment.

This retrospective study was carried out at Shahid Dr. Khalid teaching Hospital in (Koya city/ Kurdistan region/ Iraq), which included a randomized collection of 50 appendectomy samples during the period of six months from 1st Nov. 2021 to 1st May. 2022. The patients were admitted to emergency department suspected to have AA on the basis of history, physical examination, investigation and abdominal ultrasound. Preoperative blood samples were taken from all these patients for demonstrations of complete blood count (CBC) parameters, C. reactive protein (CRP) and peripheral blood eosinophils in blood film. After appendectomy, all samples were grossly examined (weight, dimensions & photographed) immediately fixed in 10% neutral buffered formalin for overnight then processed, paraffin embedded and stained by routine Hematoxylin & Eosin (H&E), May Grunwald Giemsa (MGG), Congo red and Immunohistochemistry (IHC) stains.

In present study 28 patients were male (56%) and 22 were females (44%). The age of the patients was ranging from (4 to 43 years), with mean (20.98) years old, and the majority of cases (60%) fall in the age group (≤20) years. Tissue sections stained by IHC (14.73 ± 1.05) and Congo red (3.50 ± 0.9540) revealed that tissue eosinophil count was highly significantly ($P < 0.0001$) increased when compared with H&E (4.082 ± 0.3769) and MGG (5.136 ± 0.3602).while the IHC significantly ($P < 0.05$) yielded a higher eosinophil counting than Congo red. There was a significant gradual increase in weight of appendix with age groups, but the dimensions except the height were significantly decreased. The actual means of CBC parameters were significantly

different from theoretical means, but there was non-significant sex difference in CBC parameters. Although a negative correlation between eosinophil infiltration and white blood cell (WBC) counts found which was statistically highly significant ($P<0.0019$), However a positive correlation between tissue and peripheral blood eosinophils was seen which was statistically significant with ($P < 0.01$).

In conclusion, this study has determined that the tissue eosinophils were demonstrated very effectively by IHC and Congo red staining as compared to H&E and MGG staining while IHC staining was significantly higher than Congo red staining and there was a positive correlation between tissue and peripheral blood eosinophils in patients with AA.

CHAPTER ONE

INTRODUCTION

1. Introduction

The vermiform appendix is a small, finger-like blind-ended tube connected to the cecum (Faisal *et al*., 2022). The appendix is arise from posteromedial wall of caecum about 2cm below the ileocecal junction (Alraddadi, 2021). Base of appendix was linked to the caecum but the location of the tip was changeable (Gali *et al*., 2022). It may be retrocecal, pelvic, subcecal, preileal, or in the correct paracolic position and this anatomical feature has important clinical implications in the setting of AA (Williams *et al*., 2008). The length of a normal appendix varies between 6 to 9 cm (Ekici *et al*., 2018) as well as it might range between 2 to 20 cm (Deshmukh *et al*., 2014a). Histologically, the appendiceal wall consist of four layers and have several unique features and specialized cells (Iurii *et al*., 2018). The innermost layer called mucosa which surrounded by submucosa with an externally located muscularis externa and serosa (Bandyopadhyay *et al*., 2022). The presence of masses of lymphoid tissue in the mucosa and submucosa of the appendix is the distinguishing feature of this anatomical structure (Salih *et al*., 2020).

The acute inflammation of the appendix is referred to as AA and it is the most common abdominal surgical emergency in the world (Teng *et al*., 2021). The severity of AA may range from mild AA to fecal peritonitis (Mekakas *et al*., 2022). The incidence of AA is highly age dependent reaching a peak incidence in the teenagers and early 20s. Males have an 8.6% lifetime chance of developing AA, while women have 6.7% (Snyder *et al*., 2018). The AA developed as a result of a luminal obstruction, which causes distention and an increase in pressure inside the lumen. This increased intraluminal pressure then caused the appendix's ischemia and mucosal hypoxia to develop, as well as ulceration, a breach in the mucous barrier, and the onset of necrosis (Sazhin *et al*., 2021). Fecalith, lymphoid hyperplasia, foreign bodies and tumors are the most typical causes of obstruction. Appendicitis is also brought on by infectious pathogens such viral, bacterial, and parasite infections that induce inflammation of the local lymphatic tissues (Shahmoradi *et al*., 2021). Clinical diagnosis is mainly based on a patient's medical history, physical findings, lab tests and hospital imaging, but histopathological analysis is still the gold standard for confirmation of diagnosis (Téoule *et al*., 2020). The clinical diagnosis of appendicitis is confirmed by the presence of multiple inflammatory cells within appendectomy specimens. The presence of neutrophils, mature lymphocytes and eosinophils within the appendix's various layers confirms a final diagnosis of appendicitis (Kafle *et al*., 2020).

Eosinophils are terminally differentiated cytotoxic effector cells that have a role in parasitic infections and allergy by releasing their granule-derived cytotoxic proteins. However, an increasing number of recent observations indicate that eosinophils are not only associated with the pathogenesis of a wide range of diseases, but also contribute to the maintenance of homeostatic responses in previously underappreciated diverse tissues, such as the GI tract and adipose tissue (Kim and Jung, 2020). The presence of eosinophils in tissues can be detected by H&E staining. However, it may become difficult. Thus, other special stains can be used for eosinophils demonstration in tissue (Ikeda *et al*., 2022). Eosinophils are usual appendix components that are found in the submucosa and lamina propria of the appendix, but not in the muscularis propria. The presence of a pure eosinophil infiltration in the muscle layer, together with inflammatory edema, raises the possibility that an allergic reaction is the main cause of the acute onset illness (Ahn and Lee, 2021). A rare variation of appendicular inflammation that may be related to allergy or parasitic infection is known AEA. It is described as the absence of neutrophils and the presence of eosinophils in the muscular layer of the appendix as observed in acute suppurative appendicitis. The presence of edema separating the muscle fibers and eosinophilic infiltration of the muscularis propria on histopathology is the gold standard for the diagnosis (Aggelidou *et al*., 2019). Compared to normal appendices, AEA had elevated eosinophil levels in all layers (Mowla, 2021). In addition, eosinophils were more sensitive than neutrophils to early acute clinical symptoms of appendicitis, indicating that increased muscularis propria eosinophils may be a marker for early symptomatic appendicitis (Zhang and Qu, 2022).

The aim of the study:

1. Find Simple & rapid way for the diagnosis of unusual form of acute appendicitis that may require postoperative special treatment.

2. Comparison of four staining method, both routine and special stain for demonstration of eosinophils in patients with acute appendicitis.

3. Find the correlation between tissue and peripheral blood eosinophils in patients with acute appendicitis.

CHAPTER TWO

LITERATURE REVIEW

2. Literature Review

2.1 Histology of Gastrointestinal (GI) Tract

The digestive system often known as the gastrointestinal (GI) tract, is made up of a series of muscular tubes. with an epithelial lining that runs from the mouth cavity to the anus. The pancreas, liver, and gall bladder work in conjunction with the mouth cavity, esophagus, stomach, small intestine, and colon to carry out the life-sustaining processes of digestion and absorption. From the oesophagus to the large intestine, the tract wall's histology is generally consistent and includes layers of mucosa, submucosa, muscularis, and serosa (McQuilken, 2021). The esophagus has a thick layer of stratified squamous epithelium. In contrast, the epithelia that are present in the stomach, the small intestine, and the colon are single-layered (Gehart and Clevers, 2019). The epithelium that makes up the innermost mucosal layer is supported by the lamina propria and muscularis mucosae. The submucosa is under the mucosa and is composed of adipose tissue, major blood vessels, and loose collagenous tissue derived from mesoderm. The muscularis externa is a thick layer of mesoderm-derived muscle that contains additional enteric ganglia. It wraps around the mucosal and submucosal layers of the intestine and generates the peristaltic forces that move food through the intestine. The muscularis externa is mostly made up of smooth muscle; however, the esophagus and the anal sphincter both include skeletal muscle as well. Finally the GI system is covered by a serosa or adventitia that develops from mesoderm (Thompson *et al*., 2018).

2.2 Large Intestine

The large intestine is around 1.5m (or five feet) long and has a greater diameter than the small intestine. The large intestine is composed of the caecum, colon (which includes the ascending, transverse, descending, and sigmoid colons), rectum, anal canal and anus (Figure 2.1). It begins at the point where the ileum is connected to the large intestine, which is known as the ileocecal junction, and it terminates at the anus (Azzouz and Sharma, 2018).

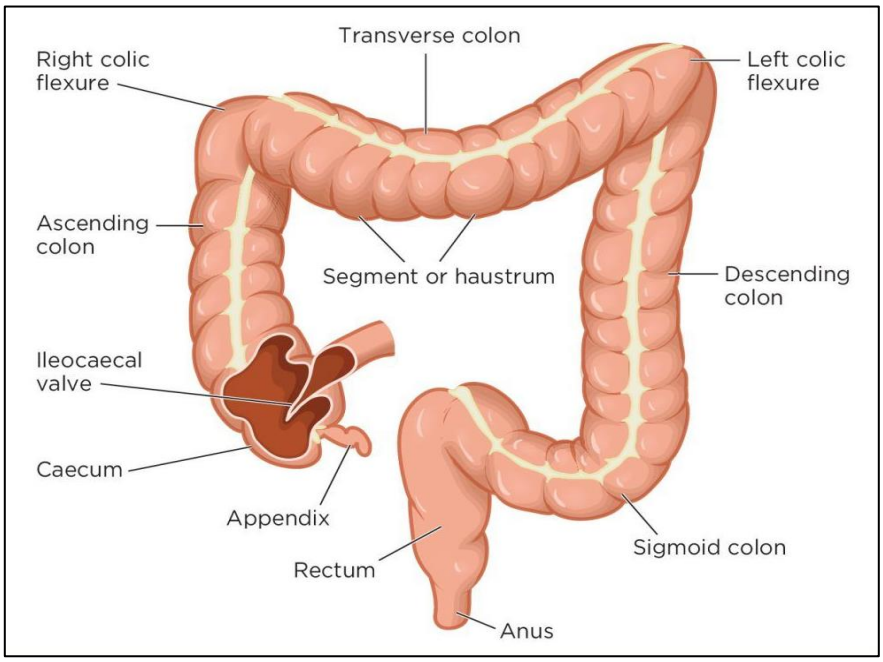


Figure 2.1: Large intestinal parts (Standring, 2019)

Although the wall of the large intestine is histologically comparable to that of other parts of the digestive system, there are important distinctions between the two. The mucosa does not have villi but has a significant number of goblet cells. Although it may be seen the longitudinal muscle layer is only partially developed. The whole of the length of the colon is covered by three distinct bands of muscle that are referred to as taeniae coli. Taeniae coli contraction presses on the colon's wall and forms a series of pouches known as haustra. Pieces of connective tissue loaded with fat called epiploic appendages are linked to the outside of the colon (Bass and Wershil, 2016). The large intestine doesn't produce any digesting enzymes, in contrast to the small intestine. Before the chyme reaches the large intestine, the small intestine finishes chemical digestion. Along with removing waste, the large intestine also absorbs water and electrolytes (Ogobuiro *et al*., 2021).

2.3 Appendix

Berengario da Carpi was the first person to document the existence of the human vermiform appendix in 1521. Furthermore, there has long been attention in the pathology of this organ. At St. George's Hospital in London, Claudius Amyand conducted the first appendectomy in 1735. But, it wasn't until 1880 when Lawson Tait achieved the distinction of becoming the first surgeon to surgically remove an inflamed appendix (Williams and Myers, 1994).

2.3.1 Appendix Morphology and Anatomy

The appendix also called (the vermiform appendix) is a tube that is linked to the cecum and has a blind end. It is shaped like a finger. Vermiform is a Latin word that meaning "worm-shaped" (Faisal *et al*., 2022). Appendix is a true diverticulum of the cecum unlike acquired diverticular disease which is identified by a protuberance of a section of the intestinal wall layers. the development of abnormal pouches in the intestinal wall is known as diverticulosis, inflammation or infection of these abnormal pouches is known as diverticulitis (Iurii *et al*., 2018). The large intestine's posteromedial wall of the caecum is where the appendix develops. About two centimeters below the ileocecal valve in the right lower abdomen is where this little, blind-end-like tube extension from the caecum is found (Iqbal *et al*., 2018). A two-layered peritoneum fold mesoappendix, suspends it from the terminal ileum, which provides support. The normal length of appendix is (6-9 cm) with average length (2-20 cm) and much longer in male compared to females (Deshmukh et al., 2014b). Since the appendix is the most mobile abdominal structure and has no permanent place, it is regarded as the most changeable abdominal organ (Mwachaka *et al*., 2014). As contrast to the base of the appendix, which is often situated in the posteromedial aspect of the caecum (about 2 centimeters under the ileocecal valve, at the confluence of three taenias), the tip of the appendix may be found in a number of different places. Differences in the vermiform appendix's location are often caused by the caecum's complex and variable embryonic development or the appendix's varying length (Nayak *et al*., 2013).

There is a wide range of variability in both the position of the appendix and its length in various populations, but there are currently few studies that link these variations to acute appendicitis. Gladstone and Wakeley conducted the first main appendix location research. According to this research, 69.2% of the participants were postcecal, 27.5% were pelvic, 0.9% were preileal, 0.5% were post ileal, and 18.6% were subcecal (Iqbal *et al*., 2012). Since the vermiform appendix may vary in location and create symptoms that resemble those of other surgical and non-surgical acute abdominal disorders, it is crucial to be aware of these changes. Acute appendicitis may be correlated with vermiform appendix anatomical abnormalities (Vidya and Kuberappa, 2016).

2.3.2 Appendix Blood Supply

According to embryology, the midgut gives rise to the most proximal portion of the large intestine while the hind gut provides the distal half. The distal half of the large intestine receives its blood supply from the inferior mesenteric artery, whereas the proximal half receives its blood supply from the superior mesenteric artery. Both of these arteries belong to the midgut and the hindgut, respectively (Lee *et al*., 2014). The whole length of the appendicular tube is covered by a triangular-shaped peritoneal fold known as the mesoappendix. It has a free border through which the ileocolic artery's branch known as the appendicular artery, which supplies the vermiform appendix with blood (Jagdish and Ashoka, 2018). The appendicular vein drains venous blood, which joins with the ceacal vein to form the ileocolic vein (Kuzan *et al*., 2019).

2.3.3 Histology of Appendix

Histologically the wall of the appendix is quite similar to the intestinal wall of the colon in that it consists of four different tissue layer which include mucosa, submucosa, muscularis externa, and serosa (Figure 2.2). The characteristic that distinguishes the appendix from other anatomical structures is the presence of lymphoid tissue masses in the mucosa and submucosa of the appendix (Bharti *et al*., 2016). The mucosa is made up of the lamina propria, muscularis mucosae and columnar epithelium that contains enterocytes and goblet cells. In along with macrophages, the lamina propria also contains a sizeable population of plasma cells, which are responsible for the production of immunoglobulin IgA or IgG Similar to the existence of intraepithelial lymphocytes (IELs) in the epithelium of the colon, intraepithelial lymphocytes (IELs) in the appendix are mostly made up of very small CD8+ regulatory T (Treg) cells (Vitetta *et al*., 2019). Additionally, compared to the colon, the appendix is home to Lieberkuhn crypts. Paneth cells, which are typically found in the small intestine and have the main function of producing antimicrobial peptides, may be found near the bottom of those crypts (Junqueira and Carneiro, 2005).

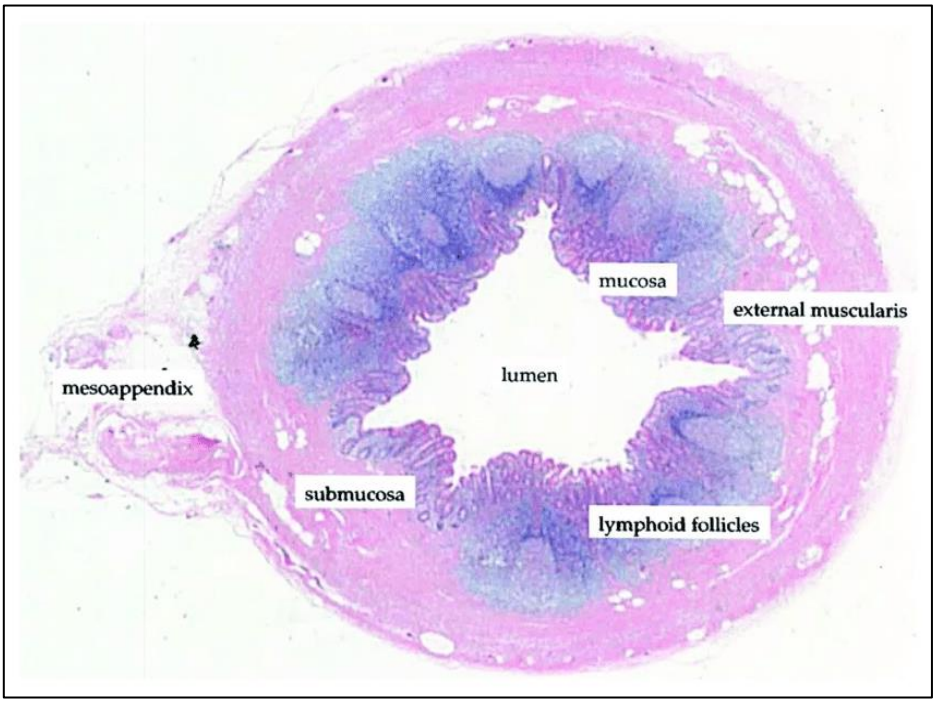


Figure 2.2: Histological transverse section of the vermiform appendix (Kooij *et al.*, 2016)

The submucosa is made up of connective tissue, and it may be identified by the presence of a significant number of lymphoid follicles that extend from the submucosa into the lamina propria. These lymphoid follicles are what give the submucosa its distinctive appearance (Figure 2.3). They are identical to Peyer's patches, which are seen in the small intestine, despite the fact that their presence or compared structure, is not detectable in a healthy colon. In this lymphoid tissue's mantle zone, which is located mostly closest to the lumen there are many closely packed B lymphocytes but just a few T cells. The unique germinal center's dark area is located the furthest from the lumen. It is composed of macrophages, centroblasts, and proliferating B lymphocytes, all of which are responsible for the majority of the follicle's creation via monoclonal development (Treuting *et al.*, 2018). These centroblasts are responsible for the development of the subsequent cell type known as the centrocyte. Centrocytes and follicular dendritic cells (FDCs) both originate in the light zone. Between the dome epithelium and the lymphoid follicles is an area of the immune system known as the mixed cell zone. This region of the immune system is distinguished by the presence of lymphocytes including (B and T cell) subsets and macrophages. There are T cell regions at the base of the lymphoid follicles that contain T cells and macrophages, with eight times as many CD4+ T cells as CD8+ T cells (Chandan, 2019) .

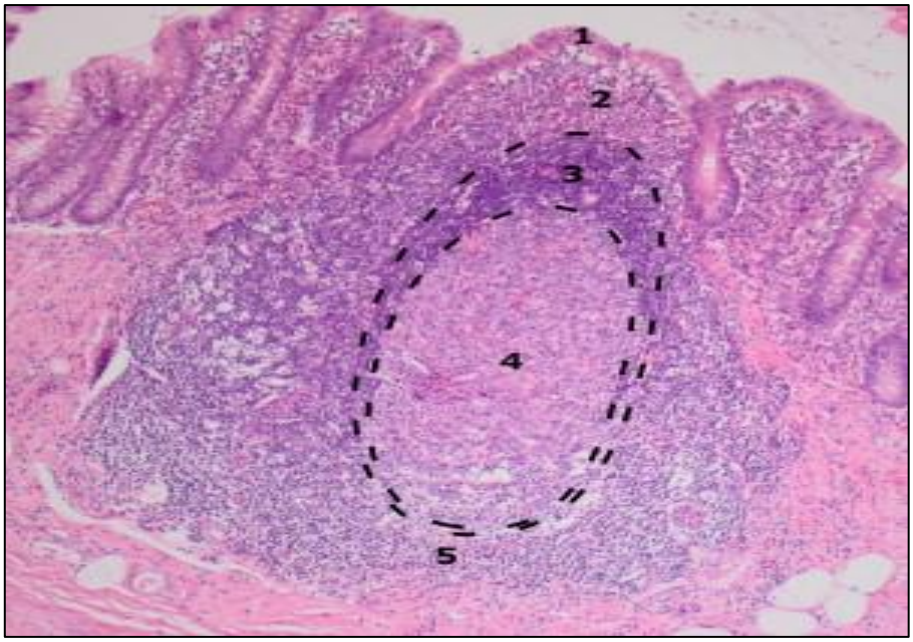


Figure 2.3: Appendiceal lymphoid follicle (de Costa, 2022)

The muscularis externa that surrounds the appendix consists of an inner circular muscle layer in addition to a thin layer of exterior longitudinal muscle. Its structure is analogous to the colon. A serosa outside of the muscle layers contains loose connective tissue, capillaries, and nerves. A thin layer of mesothelial cells makes up the peritoneum's outermost layer (Kooij *et al*., 2016).

2.3.4 Function of Appendix

For a very long time the appendix of a human being has been regarded as a vestigial organ, which refers to an organ that has lost its function as a result of evolutionary processes. In contrast, during the last several years, various findings have developed that relate the appendix to a variety of immunological activities in humans (Lee and Bae, 2018). The appendix has been shown to play an important role in maintaining intestinal health. This theory suggests that the appendix might function as a reservoir or "safe home" for the microbial gut flora allowing them to rapidly repopulate after being eliminated from the colon (Sahami *et al*., 2016). Research studies have also shown the presence of a biofilm, or thin coating of microorganisms, mucus, and immune system components, on the lining of the stomach. These biofilms seem to be the most apparent in the appendix. It is believed that the lumen of the appendix is protected from diarrhoeal elimination due to its location in the most proximal region of the colon, due to its

location and slender (worm-like) lumen, its interaction with feces is relatively low. Hence, it is believed that the biofilm in the appendix functions as a "safe home" for commensal bacteria and makes it easier for them to inoculate the intestine after a digestive illness (Guinane *et al*., 2013). The vermiform appendix is often known as the tonsils of the abdomen because to the large number of lymphoid cells it contains. Lymphocytes enter the vermiform appendix's mucous membrane and submucosa, where they create lymph nodes and interstitial clusters. Hence the vermiform appendix has an immunological purpose (Radenković *et al*., 2021). Within this critical formation, B-cell subpopulations in especially are the primary sites for the growth, proliferation, and differentiation of immunocompetent cells (Kisera *et al*., 2019).

2.4 Appendicitis

The inflammation of the appendix's inner lining is known as appendicitis that may extend to other parts of the organ and the surrounding tissue. Typically, AA develops aggressively within 24 hours after the start and requires immediate medical attention or surgery. But, chronic appendicitis is a more chronic and uncommon form of the illness. Traditionally, the initial symptom of appendicitis is periumbilical or widespread abdominal discomfort followed by pain in the right lower quadrant (Jones *et al*., 2021). Appendicitis may present itself as simple or uncomplicated appendicitis which is defined as a phlegmonous inflamed appendix without signs of necrosis or perforation. Alternatively, it may present itself as complicated appendicitis with inflammation having resulted in gangrene or perforation with or without the development of an abscess. 13–20% of individuals with AA present with perforation (Di Saverio *et al*., 2020). Necrosis is the distinguishing characteristic between uncomplicated and complicated appendicitis. The presence of necrosis characterizes complicated appendicitis (Bhangu *et al*., 2015). The histopathological analyses were retrospectively examined in order to categorize cases of appendicitis. Three distinct categories of appendicitis were identified: phlegmonous appendicitis, gangrenous appendicitis, and perforated appendicitis. Phlegmonous appendicitis is characterized by the presence of transmural infiltration of neutrophils in the appendix, without the occurrence of gangrene or perforation. Gangrenous appendicitis is distinguished by the occurrence of ischemic regions accompanied by transmural myonecrosis, whereas perforation is identified by the presence of a transmural defect. In clinical settings, the histological observation of phlegmonous appendicitis is closely associated with uncomplicated courses, on the other hand,

gangrenous appendicitis and perforation are classified as instances of acute complicated appendicitis (Rawolle *et al*., 2019).

2.4.1 Acute Appendicitis (AA)

The AA is the most common cause for emergency surgery all around the world and its symptoms may range from mild AA to fecal peritonitis. It is associated with blockage, decreased blood flow, ischemia damage to the mucosa and bacterial infection. The appendix may be influenced by other abdominal organ inflammation (Mandeville *et al*., 2015). The AA is a condition that can effect individuals across various age groups. However, it is most common among teens and young adults with an incidence rate of 1.17 per 1000. In terms of lifetime risk, males have an 8.6% probability of developing AA, while females have a slightly lower risk at 6.7% (Snyder *et al*., 2018). The AA diagnosis is still difficult especially in young women who have a wider variety of differential diagnoses than male (Ashbrook *et al*., 2022). The negative appendectomy rate may be reduced with the proper diagnosis. Symptoms often overlap with those of other diseases and are non-specific (Shogilev *et al*., 2014). If the appendicitis is complex treatment may be challenging. The postoperative mortality rate following an appendectomy is relatively low, with estimates ranging from (0.07 - 0.7) % in patients without perforation, and from (0.5 - 2.4) % in patients with perforation. Furthermore, in the case of uncomplicated AA, the postoperative complication rates exhibited a range of 10% to 19%, while in patients with complicated AA, these rates increased to 30% (Sartelli *et al*., 2018).

2.4.2 Histological Types of Acute Appendicitis (AA)

Based on its pathological appearance the AA has been classified in various studies as acute localized or (catarrhal) appendicitis characterized by acute mucosal and submucosal inflammation, lymphoid hyperplasia, acute suppurative appendicitis, normal appendix without any pathological changes, gangrenous appendicitis and perforative appendicitis (Kareem and Karim, 2014). The histological classification of AA is determined by the degree to which inflammation spreads across the various layers of the appendix. In catarrhal appendicitis, the inflammation consisting of neutrophils and edema is in the initial stage and is extends to the mucosal or submucosal area. In the early stages of catarrhal appendicitis, neutrophil- and edema-rich inflammation is localized to the mucosal or submucosal region. A significant amount of substantial edema with or without pus characterizes the inflammation in suppurative appendicitis.

All of the layer's exhibit inflammation. In gangrenous appendicitis necrosis is present in the appendix wall as well as dilated, congested blood vessels in the serosa and inflammation in all of the appendix's layers. Perforated appendicitis leads to the perforation of both the muscularis propria and serosa due to the inflammatory process. The serosa is the site from which the inflammation originates (Tayfur and Balci, 2019).

2.4.3 Epidemiology

Appendicitis is a global problem but there is a wide variance in incidence between countries and an increase in the number of cases being recorded in newly industrialised countries. The AA forms an important emergency in the day-to-day surgical practice. It affects human beings irrespective of age, nationality and religion. Global incidence tends to be lower in winter as opposed to summer, for unknown reasons (Ferris *et al.*, 2017). One of the most striking epidemiologic highlights of an inflamed appendix is the wide variation in occurrence by age and sex. The incidence of appendicitis is highest in older children and young adults, but can theoretically present at any age, with a male to female ratio of 1.4:1. Young children tend to have a wider, funnel-shaped appendix, which reduces the likelihood of occlusion and therefore of developing appendicitis. In older people the lumen is often obliterated, with similar effect. In this study, the most elevated occurrence of an inflamed appendix was seen in individuals between 4-20 years, both in males and females. This perception changes with the results in different studies where they have observed them. In Sulaimani AA is the most common cause of acute non-traumatic surgical abdomen and its diagnosis remains a challenge (Sarla, 2019).

It has been hypothesised that a low dietary fiber intake predisposes to appendicitis which may explain the higher incidence in Western countries. Appendicitis is the most common cause of the acute abdomen in the UK and about 10% of the population will develop AA. It occurs in about 7% of the US population. It is suspected to be lower in incidence in African and Asian countries due to the higher fiber content in the diet. Higher fiber decreases the formation of faecoliths and hence reduces risk of obstruction. It can occur at any age but tends to be highest in the 10 to 20 age group (Chandrasekaran and Johnson, 2014). Mortality from acute appendicitis in developed countries is low, at 0.3%, but rises significantly to 1.7% after perforation, demonstrating the importance of early diagnosis and treatment. Children and the elderly often present later and consequently have more advanced disease and higher chance of perforation already on presentation (Chhabra and Kenny, 2018).

2.4.4 Etiology

For those with AA, an appendectomy one of the most often used surgical treatments (Sartelli *et al*., 2018). Despite improvements and the fact that appendectomy is one of the more popular surgical operations the etiology of appendicitis is poorly understood and paraclinical, clinical and imaging modalities cannot reliably determine the disease's severity (Maghsoudi *et al*., 2021). The most frequent causes of AA are infection and luminal obstruction. Ficalith, lymphoid follicle hyperplasia, foreign body obstruction and malignancies such carcinoid, adenocarcinoma, lymphoma and serous cystadenoma are the most frequent causes of luminal obstruction. The AA can also arise from the inflammation of nearby lymphatic tissues in response to various infectious pathogens, including viral, bacterial and parasitic infections (Shahmoradi *et al*., 2021). The most frequent causes of AA are feces and viral infections. Whereas tumors, inflammatory bowel conditions and parasites are much less likely to cause this illness (Özturk *et al*., 2017). *Enterobius vermicularis* is the parasite that is often found after appendectomy. Furthermore, there is evidence suggesting a correlation between appendicitis and certain parasites including *Entamoeba histolytica*, *Schistosoma* sp, *Taenia* sp, *Ascaris lumbricoides* and although rarely *Balantidium coli* (Çalli *et al*., 2014). The development of the disease often arises due to an elevation in pressure within the lumen caused by the obstruction resulting from fecaloid debris. Subsequently, infection ensues as a result of bacterial translocation. The blockage causes inflammation, ischemia, distention and bacterial proliferation. Necrosis, gangrene and perforation happen without treatment. If the omentum covers the perforation an appendiceal abscess develops (Maghsoudi *et al*., 2021).

2.4.5 Pathogenesis

While the specific pathogenesis of AA is yet unknown it is multifactorial. However, it is clear that luminal obstruction is typically present. It was formerly thought that a primary lumen blockage and a subsequent bacterial infection were the causes of appendicitis (Kabir *et al*., 2017). The most commonly identified aerobic pathogens associated with AA include Escherichia coli, Klebsiella pneumoniae, peptostreptococcus, pseudomonas species and Bacteroides fragilis. Fecalith, lymphoid hyperplasia, foreign bodies, parasites, various bacteria, viruses, benign or malignant tumors and others are only a few of the reasons of luminal blockage (Ceresoli *et al*., 2016). The obstruction observed in preschoolers is commonly attributed to lymphoid hyperplasia, a condition characterized by the excessive growth of lymphoid tissue in the submucosa of the

appendix. This growth in size and number of lymphoid tissue is known to increase with age, reaching its peak during adolescence. Therefore, the risk of developing AA is higher during this period. Additionally, although less frequently, obstruction in preschoolers can also be caused by the presence of fecalith (Rothrock and Pagane, 2000). Lymphoid hyperplasia has been found to be associated with various inflammatory and infectious diseases, which are includes gastroenteritis, amebiasis, respiratory infections, measles, and infectious mononucleosis. Faecoliths are formed through the accumulation of fecal debris and calcium salts on inspissated feces within the lumen of the vermiform appendix. The appendix experiences luminal obstruction as a result of the ongoing production and deposition of fluids and mucus by epithelial cells. This obstruction leads to an increase in intraluminal pressure, subsequently causing distension of the appendix (Di Saverio *et al*., 2020). The proliferation of intestinal bacteria within the appendix results in their multiplication, subsequently causing bacterial invasion of the edematous wall. Furthermore, the impaired blood flow, reduced venous drainage, and consequent formation of blood clots within the appendicular artery and vein serve to increase the inflammatory cascade resulting in ischemia, necrosis, gangrene and perforation (Figure 2.4). Appendix perforation may cause a localized appendicular abscess or generalized peritonitis. It has been observed that younger children have a greater chance to develop diffuse peritonitis due to the relatively less developed omentum, whereas older children benefit from a more develop omentum which provides better protection against this condition (Jaffe and Berger, 2005, Levin, 2019).

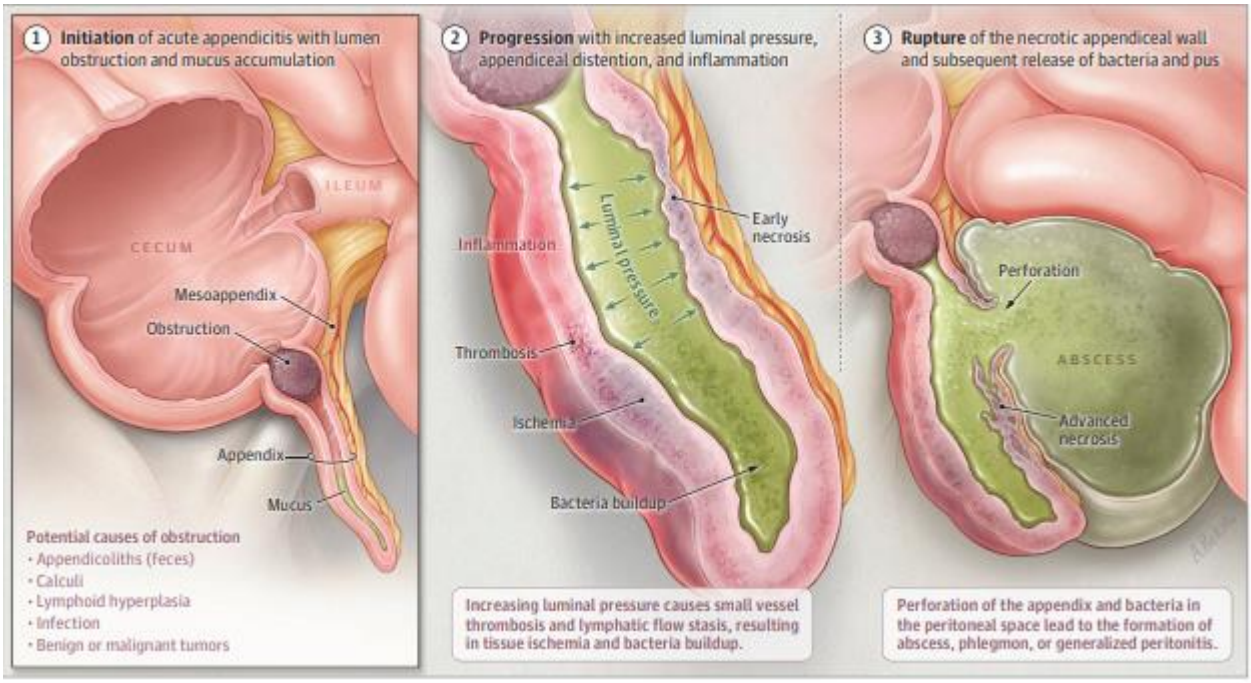


Figure 2.4: Pathophysiology of acute appendicitis (Moris *et al*., 2021)

2.4.6 Clinical Features

The symptoms of AA usually correlate with those of other diseases and are non-specific. The classic clinical signs of AA are present in about half of cases (Shogilev *et al*., 2014). The main initial symptom of AA is a continual pain in the upper or middle portion of the abdomen. The presence of inflammation in the appendix leads to the stimulation of the parietal peritoneum located in the anterior abdominal wall. Consequently, pain is transmitted to the lower right quadrant, where it displays as typical sensitivity and muscular spasm upon palpation. This symptom may not always be present in appendix positions that are abnormal. Patients with AA have pain that is distressing, nausea, vomiting, and/or lack of appetite. The anatomical position of the appendix may vary or the patient's age may prevent this traditional presentation from occurring, with uncommon presentations often occurring in newborns and the elder (Mostbeck *et al*., 2016). Elevated body temperature which does not exceed (38.5℃) is one of the symptoms of AA. However, if it is higher, it must be considered gangrene, perforation or some other cause of diseases. If the inflammation worsens, the so-called lucid period without pain will arise when the swollen appendix ruptures. Acute abdominal pain, which is a potentially lethal disease is soon followed by severe pain over the whole abdomen and established indicators of widespread peritoneal hyperarousal (Di Saverio *et al*., 2016).

2.4.7 Diagnosis

The diagnoses of AA are done on the basis of patient's medical history, doing a physical examination, obtaining laboratory results and obtaining imaging at the hospital. Despite this, the clinical diagnosis remains difficult in both the pediatric and adult populations because the symptoms and signs that manifest themselves are often unusual. Some of the historical signs include pain that moves from the periumbilical region to the right iliac fossa (RIF), nausea and vomiting, fever, abdominal rigidity and McBurney's soreness (Humes and Simpson, 2006). Several simple and understandable scoring techniques have been utilized as a systematic algorithm to determine the risk of AA. However, none of these methods have achieved broad consensus or widespread acceptance (Gregory *et al*., 2016). The Alvarado score, Pediatric Appendicitis Score (PAS) and Appendicitis Inflammatory Response score (AIR) involve common clinical and laboratory indicators to categorize individuals into low, moderate, or high-risk groups, possibly helping with a quick and specific diagnosis (Snyder *et al*., 2018). Another critical challenge is the use of imaging techniques for diagnostic purposes. The techniques involved in this category are ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI) (Sippola *et al*., 2020b).

2.4.7.1 Laboratory Testing

The CBC is frequently employed as a diagnostic laboratory test for the diagnosis of AA. Numerous studies have been conducted to investigate the diagnostic significance of various hematological parameters, such as WBC count, neutrophil-to-lymphocyte ratio (NLR), platelet distribution width (PDW), mean platelet volume (MPV), red cell distribution width (RDW), platelet count (PLT), lymphocyte count (L), neutrophil count (N), CRP level and lymphocyte-C-reactive protein ratio (LCR) in the diagnosis of AA (Sippola *et al*., 2020b). Increased levels of WBC, CRP, granulocytes or the percentage of polymorphonuclear cells all increase the possibility of appendicitis. The diagnosis of AA cannot be made with sufficient precision using the WBC count or inflammatory biomarkers alone. However, the utilization of laboratory tests can be beneficial when utilized together with clinical decision-making guidelines, performing into consideration symptoms and signs, or when combined with imaging investigations as a component of a systematic examination (Rud *et al*., 2019). The primary laboratory test necessary for the first diagnosis of AA is a CBC to check for a shift to the left, or increased segmented neutrophils (more than 75%), since they are high in the early stages of the inflammatory process,

or stabs or bands (more than 5%). However, a few hours later the total number of leucocytes increases and you will notice a leukocytosis of more than 10.000/ml. A urinalysis is helpful to check for acetone, which suggests a fasting condition associated with anorexia. It may also reveal a few red blood cells owing to an inflammatory activity surrounding the appendix. Further testing should be done if the urine has an abnormally high number of red blood cells, which might indicate ureteral calculi. The CRP test is a non-specific test that just identifies the presence of an inflammatory condition and it does not diagnose any specific disease. In addition, it would not be required since the function that is performed served by leukocytosis and the shift to the left is the same (Alvarado, 2016).

2.4.7.2 Imaging

Imaging modalities that include US, CT and MRI are the three most often used imaging essential for determining a diagnosis AA. Individuals with a clinical diagnosis of appendicitis have the probability of negative appendectomy that is reduced by about 15% when accurate imaging is performed (Shogilev *et al*., 2014). It is highly recommended to use imaging techniques like US and CT to provide a more certain diagnosis of appendicitis (Kotagal *et al.*, 2015). Imaging methods have complications and limitations; however, they may increase diagnostic accuracy. Concerns about radiation exposure through CT have grown, particularly for pregnant individuals. In contrast, US, which is a non-invasive diagnostic procedure that also exposes patients to less radiation can only provide images with a limited field of view and a poor degree of quality. Furthermore, US is limited in its ability to produce high-resolution images of organs that contain air. However, in certain clinical contexts, these imaging modalities could not be generally available, requiring the use of more broadly available pathology testing (Fedko *et al*., 2014). By improved diagnostic accuracy, US and CT have the potential to enhance clinical outcomes. While radiation-free, ultrasound has the relative disadvantage of operator dependence and a narrow range of sensitivity. US also has the significant benefit of being radiation-free. With reported accuracy rates of 93-98%, CT demonstrates increased sensitivity in the diagnosis of AA. Also, it can identify complications and their severity and in unfavorable circumstances it could be able to identify other diagnoses. It is also a quick procedure that is economical. Nevertheless, it may be difficult to diagnose appendicitis on a CT scan since symptoms can range from mild findings linked to early appendiceal inflammation to significant abnormalities when complications arise (Viswanthan *et al*., 2017).

2.4.8 Management of Acute Appendicitis

Appendectomy is widely regarded as the sole efficacious treatment for AA, as it involves a full removal of the vermiform appendix (Michalinos *et al.*, 2014). Deciding to perform an appendectomy based only on clinical characteristics might result in the removal of healthy appendices (negative appendectomy). Yet, delaying needed surgery for individuals with suspected appendicitis might result in serious complecations (Elsherbiny *et al.*, 2020). Less than 1% of people who get an uncomplicated appendicitis die, however, this risk might rise to 5% or more in older patients and children. As the clinical symptoms of AA in these latter age groups are often unclear and have a higher risk of complications, the diagnosis is frequently delayed (Mohamed and Bhat, 2010). The AA if left untreated may result in severe sometimes fatal consequences include appendiceal perforation, abscess development and peritonitis (Charfi *et al.*, 2014). Laparoscopic appendectomy progressively replaced open appendectomy as the most common surgical procedure during the last 40 years. The utilization of laparoscopic appendectomy has been associated with a decrease in postoperative pain and a faster recovery process, resulting in shorter hospital stays and a quicker return to a state of optimal health compared to the open surgical approach (Meinzer *et al.*, 2020). Recent clinical studies have provided evidence supporting the possibility and effectiveness of utilizing antibiotics as the sole form of treatment for uncomplicated appendicitis, thereby avoiding the need for surgical intervention (Sippola *et al.*, 2020a).

2.5 Eosinophils

Eosinophils are a kind of terminally differentiated leukocyte that found in the blood and connective tissues. These cells account for around (1-5) % of the total number of leukocytes in the peripheral blood of healthy persons (Jung, 2015). Eosinophils stain with acidophilic dyes a feature noted in 1879 by Paul Ehrlich, who first described eosinophils and appreciated their increased presence in patients with asthma and helminth infections, among other conditions (McBrien and Menzies-Gow, 2017).

2.5.1 Eosinophil Cell Structure

Eosinophils are granulocytes usually measuring (10–16) μm in diameter, with a segmented bilobed nucleus. Due to the acidic dye eosin's avidity for the essentially charged intracellular granules located only inside the cytoplasm, this bilobed cell displays the relatively high staining

that distinguishes it from other similar cells (Abdala-Valencia *et al*., 2018). Characteristic of this cell is the presence of a large number of molecules with pleiotropic functions, such as cationic granule proteins, chemokines, cytokines, growth factors, immunomodulatory molecules and lipid mediators, mainly accumulated within the intracellular compartment (Figure 2.5).

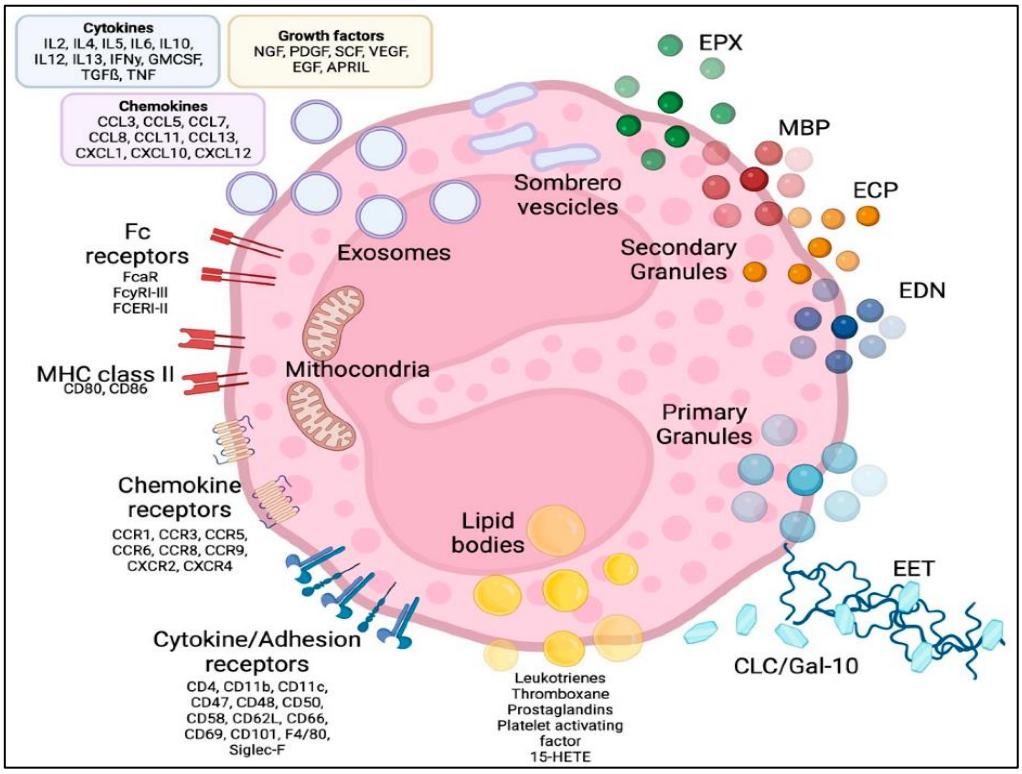


Figure 2.5: Cell structure of eosinophil (McBrien and Menzies-Gow, 2017)

Eosinophils also have a large array of transmembrane proteins (integrins) and surface receptors which mediate the interaction with the micro-environment. Thy also allow the response to multiple stimuli or integrate with the innate and adaptive branches of the immune system involved in inflammatory responses and homeostasis (Sastre *et al*., 2018). Eosinophil surface receptors are (cytokine receptors, adhesion receptors, chemoattractant receptors, Fc receptors, major histocompatibility complex class II (MHC-II), pattern recognition receptors (PRRs), Lipid Mediator Receptors, inhibitory receptors and Siglec-8) (McBrien and Menzies-Gow, 2017). The eosinophil also has many intracellular receptors that regulate its function (such as some toll-like receptors and the glucocorticoid receptor) (Rodrigo-Muñoz *et al*., 2021). The presence of approximately 200 large specific granules within each individual cell which is also known as secondary granules, is a characteristic feature that distinguishes eosinophils from other

granulocytes like neutrophils and basophils (Ueki *et al*., 2017). Specific granules consist of a dense crystalline core and a matrix, surrounded by a membrane. They contain a large number of mediators capable of inducing inflammation and/or tissue damage, including basic proteins, cytokines, chemokines, growth factors, and enzymes. The predominant substances are the proteins which include major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN). MBP is located in the core of the eosinophilic granule while ECP, EPO and EDN are stored in the surrounding matrix (Lacy, 2017).

Interestingly, each of these proteins is cytotoxic to a variety of targets, including host tissue, and ECP and EPX are members of the pancreatic ribonuclease family so these proteins are significant contributors to eosinophil-mediated inflammation. There are three further varieties of eosinophil granules mostly seen in immature eosinophil promyelocytes, Primary granules are smaller than specific granules. They are the principal location of Charcot–Leyden crystal protein (galactin-10) hexagonal bipyramidal crystals, which exhibit lysophospholipase activity and have been identified in tissues subject to eosinophilic inflammation. Eosinophils also have lipid bodies which come in different numbers. Activated eosinophils and eosinophils from persons with eosinophilia had more of these lipid bodies (Melo and Weller, 2018).

2.5.2 Eosinophils Origin and Distribution

Eosinophils are derived from hematopoietic CD34+ stem cells in the bone marrow. primarily in response to a specific combination of transcription factors and cytokines (Kim and Jung, 2020). The process of lineage specification is established through the interaction of various transcription factors, including GATA-binding protein 1 (GATA-1), PU.1 from the E26 transformation-specific family, IFN consensus sequence binding protein, and members belonging to the CCAAT-enhancer-binding protein family (Figure 2.6). GATA-1 is the transcription factor that plays an essential role in the development of eosinophils. Differentiation and proliferation of eosinophils are further regulated by IL-5, IL-3 and GM-CSF, which share a common β-chain receptor. In addition to their primary functions, these cytokines also play a role in regulating the differentiation and proliferation of eosinophils. The IL-5 exhibits a distinct characteristic among cytokines as it plays an essential role in promoting the specific differentiation and mobilization of eosinophils from the bone marrow during inflammation as well as for the eosinophil homing into various tissues in the steady state. This unique function of IL-5 remains important, despite the

ability of IL-3 and GM-CSF to induce the development of multiple lineages of hematopoietic cells (Singh *et al*., 2020).

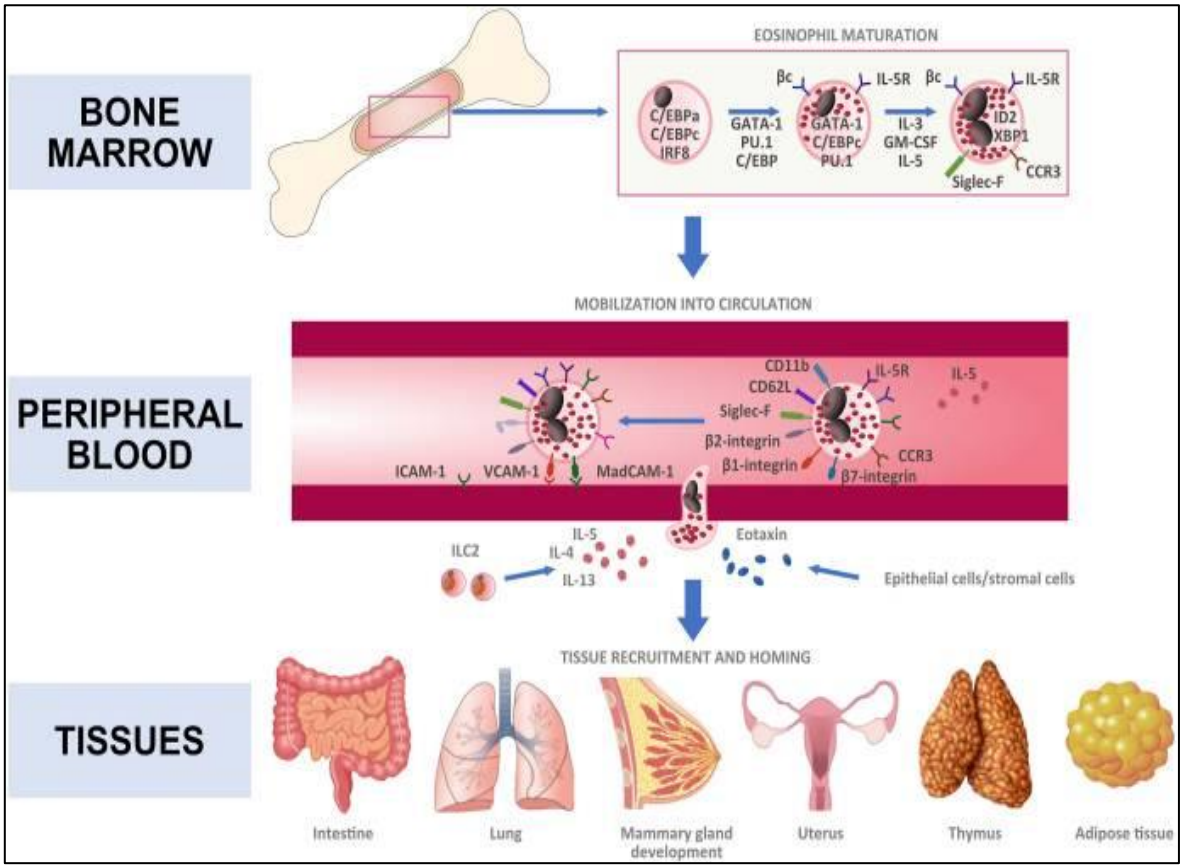


Figure 2.6: Development of eosinophils expressing various types of functional cell surface molecules: from bone marrow to tissues (Lombardi *et al*., 2022)

So IL-5 is a cytokine that plays a crucial role in the processes of eosinophil proliferation, maturation, activation and recruitment (McBrien and Menzies-Gow, 2017). It has been more than a century since the initial identification of eosinophils in both the bloodstream and certain tissues (Klion *et al*., 2020b). Eosinophils are released from the bone marrow and migrate into the bloodstream. Subsequently, they are quickly recruited to peripheral tissues through a regulated mechanism that involves the arranged interaction between various networks. They migrate to the peripheral tissues under homeostatic conditions, or to inflammatory sites in response to recruitment signals, primarily IL-5 and eotaxin-1 (CCL11) which play a crucial role in preserving the structural integrity of organs and enhancing the immune function of B and T cells (Marichal *et al*., 2017). This process involves the participation of eotaxin-1, integrins (α4β1, α4β7) and integrin receptors located on the endothelium, including MAdCAM-1, VCAM-1 and ICAM-1.

Under homeostatic conditions, eosinophils are primarily recruited within the digestive system, adipose tissue, lung, mammary gland, thymus and uterus. The mucosal lining of the digestive system contains the majority of eosinophils (Jackson *et al.*, 2022).

Eosinophil counts in human blood typically vary from 0-500 per μL (1-5% of circulating leucocytes), although under some situations they may rise by 20 times or more. Their lifespan in humans is around 24 hours once in the circulation under normal, the duration of conditions is nearly two times longer than the lifespan of neutrophils (Farahi *et al*., 2012). Although certain eosinophils have been observed to migrate to the liver and spleen, the primary site of eosinophil accumulation and residency within the body during homeostasis is the mucosa surface of the gastrointestinal tract, spanning from the stomach to the intestine. The migration of eosinophils from the circulation into intestinal tissues, is a multi-step route involving several interactions between activated adhesion molecules on endothelial cells (e.g. vascular cell adhesion molecule (VCAM)) and counter-ligands on the surface of the eosinophils (Schnyder *et al*., 1996). Eosinophils are typically rare or not present in the upper or lower airways, as well as in the esophagus (Bochner, 2018). It is widely believed that the lifespan of eosinophils in tissues is limited to a few days. However, in cases of prolonged inflammation, where tissue-resident cells and potentially eosinophils themselves are producing GM-CSF or other cytokines, it is probable that eosinophils may survive for extended periods, possibly lasting weeks(Weller and Spencer, 2017).

2.5.3 Eosinophils in Health

During homeostasis eosinophils are predominantly located in various tissues including the thymus, uterus, adipose tissue and lamina propria of the gastrointestinal (GI) tract. This distribution highlights the diverse physiological roles performed by eosinophils (Weller and Spencer, 2017). Eosinophils are increasingly being considered to be multifunctional leukocytes due to the fact that they are found in a broad range of tissues and produce a number of different immune mediators and contribute to homeostatic immune responses in the body (Long *et al*., 2016). Eosinophils are terminally differentiated cytotoxic effector cells that have a role in parasitic infections and allergy by releasing their granule-derived cytotoxic proteins. However, an increasing number of recent observations indicate that eosinophils are not only associated with the pathogenesis of a wide range of diseases, but also contribute to the maintenance of homeostatic responses in previously underappreciated diverse tissues, such as the gastrointestinal

(GI) tract and adipose tissue (Kim and Jung, 2020).The intrinsic roles of eosinophils are much more complex, including the maintenance of homeostasis, host defense against infectious agents, innate immunity activities, immune regulation through Th1/Th2 balance, anti-inflammatory, and anti-tumorigenic effects (Kanda *et al*., 2021).

Moreover, eosinophils have a main role in tissue damage through eosinophil-derived cytotoxic mediators that are involved in eosinophilic inflammation, as documented in Th2-high asthma and other eosinophilic-associated diseases (Choi *et al*., 2020). The number of eosinophils in the thymus declines with age (Tulic *et al*., 2009). Eosinophils may have a role in T cell selection. In a mouse model of MHC I-restricted acute negative selection, eosinophil recruitment to the corticomedullary region of the thymus and association with apoptotic bodies has been demonstrate (Throsby *et al*., 2000). Eosinophils also enhance the ability of macrophages to phagocytose apoptotic thymic cell (Kim *et al*., 2010). Additionally, eosinophils are involved in allergic reactions where they release cytotoxic proteins from their granules. Eosinophils possess Fc receptors that facilitate their interaction with the adaptive immune system, in addition to pattern recognition receptors that enable the detection of pathogens in innate immune responses. These receptors facilitate the involvement of eosinophils in both inflammatory responses and homeostasis (Loktionov, 2019). Eosinophils play a crucial role in the maintenance of immunological homeostasis due to their function as effector immune cells involved in host defense, as well as their ability to modulate both innate and adaptive immune responses. Additionally, it is likely that they play a role in tissue healing and the regulation of functional homeostasis in various tissues. To assure host defense against parasite, fungi, bacterial, and viral infections, inflammatory stimuli activate a complex, eosinophil-centered signaling network made up of T2 lymphocytes, B cells and mast cells as well as circulating platelets and cells resident at sites of inflammation (Ramirez *et al*., 2018). There might exist more vital functions for eosinophils and the substances they secrete. these functions include glucose and lipid homeostasis, tissue growth and remodeling, liver and muscle repair, neuronal control, epithelial regulation, and microbiome regulation. Additionally, it involves immunoregulation specifically focusing on the maintenance of immunologic fitness during the aging process (Brigger *et al*., 2020). Eosinophils migrate to the GI tract during embryonic development. Prior to the development of any viable gut flora (Mishra *et al*., 1999). In health, they are present throughout the GI tract with the notable exception of the esophagus. Eosinophils contribute to the immune

defense against gut microorganisms, due to multiple antimicrobial properties. Other potential homeostatic roles for eosinophils within the gut are not currently well defined but may relate to their ability to interact with the enteric neuronal system and increase smooth muscle reactivity (via release of MBP) (Jacoby *et al*., 1993). Eosinophils have also been implicated in the regeneration of liver tissue (Goh *et al*., 2013) and skeletal muscle. The increased presence of eosinophils in preovulatory ovarian follicles and in endometrium has prompted speculation that they may have a role in tissue remodeling related to ovulation and menstruation (Heredia *et al*., 2013).

Eosinophils appear to infiltrate primary and secondary lymphoid organs, including the thymus, lymph nodes, spleen and Peyer's patches in the gut. This infiltration is believed to potentially contribute to the maturation and migration of various other immune cells. Throughout the bone marrow and the stomach, eosinophils support plasma cell survival (Beller *et al*., 2014) and maintain a physiological equilibrium between the lung and gut's T-helper and T-regulatory responses (Mesnil *et al*., 2016, Sugawara *et al*., 2016). Moreover, they may influence the immune response's properties by carrying out antigen presentation. In addition to their roles in immunomodulation, eosinophils are necessary for the normal development of the mammary gland as well as the structural integrity of nonlymphoid tissues such as adipose tissue where they are responsible for regulating glucose tolerance and preventing obesity. While it is less obvious what their supposed homeostatic function is in the normal uterus eosinophils are also identified there (Marichal *et al.*, 2017). Lastly, eosinophils have the ability to create a variety of growth factors which may aid in tissue healing (Lee *et al*., 2010).

2.5.4 Eosinophils in Inflammatory Processes

Eosinophils can regulate local immune and inflammatory responses, and their accumulation in the blood and tissue is associated with several allergic, rheumatologic, infectious, neoplastic and rare idiopathic disorders. Although eosinophils can contribute to tissue homeostasis in steady state conditions, many studies have trended toward focusing on the contribution of eosinophils in the pathogenesis of eosinophil-associated diseases. Indeed, eosinophils may exert their biological effects via cytotoxic mediators such as type 2 cytokines (IL-4, IL-5, IL-9, IL-13, and IL-25), type 1 cytokines (IL-12, IFN-γ), acute proinflammatory cytokines (TNF-a, IL-1b, IL-6, and IL-8), chemokines, and lipid mediators (PAF and LTC4) (Kanda *et al*., 2021). Activation of eosinophils and release of proinflammatory lipid mediators, cytokines, free oxygen radicals, highly charged

cationic proteins contribute to the onset and maintenance of tissue inflammation. Furthermore, eosinophil accumulation in blood and tissues has been related to a defect in their apoptotic death (Shen and Malter, 2015).

Eosinophils may also play a role in tissue repair and regeneration; for example, muscle damage promotes rapid recruitment of eosinophils in the inflammatory foci and eosinophils play a key role in muscle regeneration during muscle injury as a major source of IL-4. The IL-4 produced by eosinophils activates muscle resident fibrocyte-adipocyte progenitors (FAPs), which induce regeneration of injured muscles (Aoki *et al*., 2021). Eosinophils can induce angiogenesis by the production of pre-formed pro-angiogenic mediators, among others the vascular endothelial growth factor (VEGF). Eosinophil-derived IL-4 is also required for liver regeneration. Eosinophil-derived IL-4 induces the proliferation of quiescent hepatocytes and regulates the regeneration of the liver (Li and Hua, 2017). Furthermore, eosinophils confer protection following myocardial infarction (Lavine, 2020). Thus, eosinophils play a profound role in tissue repair and regeneration in various organs. The study of various types of eosinophilic inflammation may increase our understanding of the biological responses of eosinophil leukocytes to different inflammatory stimuli and recent research suggests that eosinophils may have additional roles in these settings that are related to control and resolution of inflammation (Strandmark *et al*., 2016).

2.5.5 Eosinophils in Disease

Clinical diseases defined by systemic eosinophilia and eosinophilic infiltration of target organs have a pathologic function for eosinophils (Chusid, 2018). Since eosinophils have been associated to a variety of pathological conditions, having a raised blood count should prompt further testing for eosinophilic disease (Klion *et al*., 2020a). Eosinophils are thought to be terminal cells that are engaged in the processes of hypersensitivity as well as host defense against parasite infection. However, more researchs have revealed that this multifunctional cells possesses the capability to produce immunoregulatory cytokines and soluble mediators, participate in maintaining tissue homeostasis, and regulate both innate and adaptive immune reactions (Liao *et al*., 2016). Elevated eosinophil counts in blood and tissue are a feature of many pathological processes. Eosinophils can migrate and accumulate in a wide variety of tissues and, by infiltrating a target organ, can mediate the development of several inflammatory diseases (Quirce *et al*., 2023). Eosinophils play a pathological role in various diseases, including asthma,

chronic rhinosinusitis with nasal polyps (CRSwNP), eosinophilic gastrointestinal (GI) disorders and systemic hypereosinophilic diseases such as eosinophilic granulomatosis with polyangiitis (EGPA) and hypereosinophilic syndrome (HES). Eosinophils are capable of producing granule proteins and chemical mediators that have the potential to play a role in tissue remodeling, repair and the persistence of diseases (Abdala-Valencia *et al*., 2018).

2.5.6 Eosinophilic Disorders of the Gastrointestinal (GI) Tract

Various GI tract disorders characterized by eosinophilic inflammation are associated with diverse clinical presentations which are based on the specific region of the GI tract affected by eosinophilic inflammation. The eosinophilic gastrointestinal diseases (EGIDs) are a class of conditions distinguished by GI dysfunctional symptoms combining with eosinophilic inflammation which most often affects the mucosa but may also occur in the muscular or serosal layer. The IL-5 plays an essential role in promoting eosinophil production and activation which results in an increase eosinophils in response to certain immunological responses such as those to allergic illness and helminth infections (Egan and Furuta, 2018). Eosinophilic gastrointestinal illnesses (EGIDs) involve several subclasses, including eosinophilic esophagitis (EoE), eosinophilic gastritis (EG), eosinophilic enteritis (EE), eosinophilic colitis (EC) and acute eosinophilic appendicitis (AEA). These subclasses are distinguished based on the specific sites within the gastrointestinal tract that are affected. Nevertheless, there are few studies that quantify eosinophils in normal GI mucosa, making it difficult to distinguish pathologic quantities of eosinophils. Eosinophils are often present in the mucosa of all regions of the GI tract, with the exception of the esophagus (Collins *et al*., 2018).

2.5.7 Acute Eosinophilic Appendicitis (AEA)

The AEA is an uncommon form of appendiceal inflammation that may have an association with allergies. It is characterized by the infiltration of eosinophils within the muscular layer of the appendix, in contrast to the presence of neutrophils observed in cases of acute suppurative appendicitis. The gold standard for diagnosing this condition is the presence of edema that separates the muscle fibers and the infiltration of eosinophils within the muscularis propria, as determined by histopathological examination (Aggelidou *et al*., 2019). The AEA displays with symptoms which have a similarity to those observed in cases of acute suppurative appendicitis (Kim *et al*., 2019). On the opposite side, it can reveal itself as a bleeding in the lower

gastrointestinal tract (LGIH). Although appendiceal bleeding caused by AEA is exceedingly uncommon, medical professionals should take it into consideration when making a differential diagnosis (Ahn and Lee, 2021). There is a possibility that acute eosinophilic appendicitis is either an early stage of the development of appendicitis or a subtype of acute appendicitis (Yaeger *et al*., 2018).

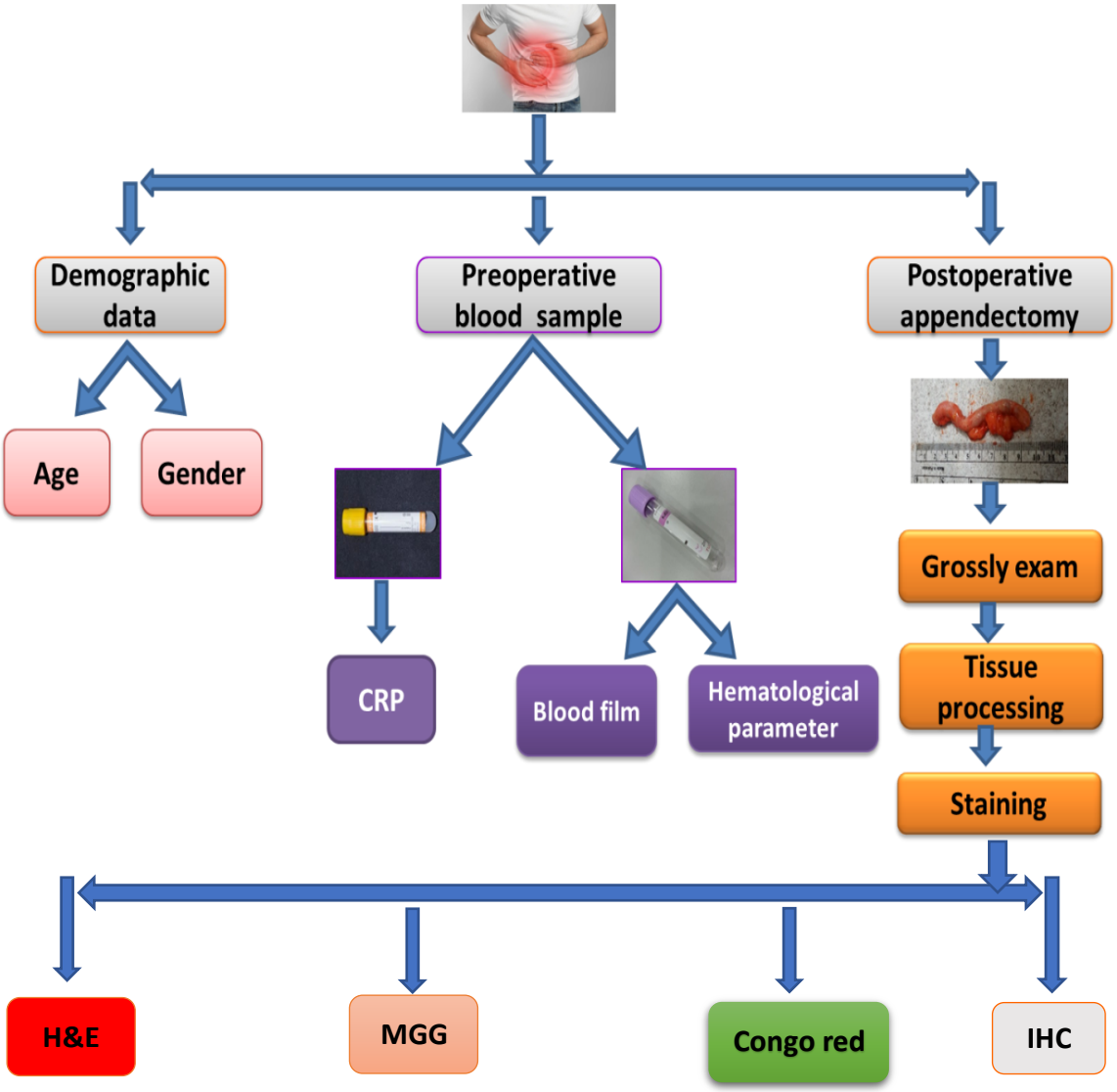
CHAPTER THREE

MARTIALS AND METHODS

3. Materials and Methods

3.1 Experimental Design

A prospective study was conducted at Shahid Dr. Khalid Teaching Hospital in Koya city/ Kurdistan Region/ Iraq, during the period of six months from 1st Nov. 2021 to 1st May. 2022. The study was approved by the ethical committee in department of biology which included a randomized collection of fifty appendectomy samples from patients admitted to emergency department suspected to have AA on the basis of history, physical examination, investigation and abdominal ultrasound as shown in (Schematic 3.1).



Schematic 3.1: Experimental design diagram

3.2 Materials

3.2.1 Instruments:

A retrospective study was included a randomized collection of fifty appendectomy samples from patients suspected to have acute appendicitis (AA) and preoperative blood samples were taken from all patients for demonstration of tissue and peripheral blood eosinophil's. In the present study the following instruments were used as shown in (Table 3.1).

Table 3.1: Instruments, supplier companies and their origin states:

No.	Instruments	Supplier companies and their origin status
1	Balance	Bio, Germany
2	Freezer machine	Ej, USA
3	Refrigerator	Daeivoo, China
4	Water bath	Thermo, USA
5	Microtome	Microm HM325, Germany
6	Oven SAKURA,	PM-400, Japan
7	Microwave Oven	Hinari Ellipse. EMX710PSL, china
8	Hot plate	Thro, china
9	Centrifuge	Hettich EBA20, Germany
10	Distillation water	GFL/ML 2001/2, Germany
11	Deionizer water	SG Co., Germany
12	Magnetic mixer	LabSco, Germany
13	Magnetic stirrer	Bibby Sterilin, Ltd., UK
14	Microscope	Motic, japan
15	Fleucare	China
16	CBC devices	Swelab, Sweden

3.2.2 Equipment:

A retrospective study was included a randomized collection of fifty appendectomy samples from patients suspected to have acute appendicitis (AA) and preoperative blood samples were taken from all patients for demonstration of tissue and peripheral blood eosinophil's. In the present study the following equipment was used as shown in (Table 3.2).

Table 3.2: Equipment and their supplier companies

No.	Item	Model and Company
1	Syringe	Nanjing Aagela Label Co., Ltd, China
2	Eppendorf tube	Citotest, China
3	Gel tube	Aman, China
4	EDTA tube	Aman, China
5	Cylinder	Isolab, France
6	Conical flask	Isolab, France
7	Beaker	Chengduchina, China
8	Coupling jar	Isolab, China
9	Washing bottle	Citotest, China
10	Ice pad	Ecomercchub, China
11	Surgical tools	Sabro, Italy
12	Container 100ml	Biozek, Netherland
13	Tissue cassette	Bio-Optika, Italy
14	Bio Mold	Bio-Optica, Italy
15	Label slides	Citoclas, China
16	Microscopic Cover slide	Citoclas, China
17	Filter paper	Citotest, China
18	Funnel	Isolab, France

3.2.3 Chemicals:

A retrospective study was included a randomized collection of fifty appendectomy samples from patients suspected to have acute appendicitis (AA) and preoperative blood samples were taken from all patients for demonstration of tissue and peripheral blood eosinophil's. In the present study the following chemicals were used as shown in (Table 3.3).

Table 3.3: Chemicals, supplier companies and their origin states:

No.	Chemicals	Company
1	Formalin	Aqua Medical, Turkey
2	Ethanol	Scharlau, Spain
3	Methanol	Scharlau, Spain
4	Xylene	Bio-Optica, Italy
5	Paraffin wax	Bio-Optica, Italy
6	DPX Mountant Standard Viscosity	Atomscientific, UK
7	Oil immersion	Scharlau, Spain
8	Hematoxylin & Eosin	Bio-Optica, Italy
9	May Grunwald Giemsa stain	Atom Scientific, UK
10	Congo red stain	Sigma Aldrich, Germany

3.3 Methods

3.3.1 Demographic and clinical data

Demographic data involved patients age and gender. The clinical data involves multiple factors related to pain, such as its duration, severity, nature, onset point and any shifting patterns. Samples of blood and urine were submitted for laboratory analysis. Preoperative assessments included the performance of both pelvic and abdominal ultrasounds for all the patients. The treatment to all patients consisted of either an open or laparoscopic appendectomy.

3.3.2 Blood collection

Preoperatively blood samples were collected in EDTA and clot activating Gel tubes from all the patients. EDTA anticoagulated blood used to measure CBC parameters and for peripheral blood eosinophils in blood film. The gel tubes for CRP test. Eosinophils in blood film were counted per 200 WBC by using high power field (1000 x).

3.3.3 Sample collection

After appendectomy all the samples were grossly examined fresh for weight, dimensions & photographed then immediately fixed in 10% neutral buffered formalin and sections from appendicular base, center, and tip were obtained. Sections were routinely processed, paraffin embedded and stained by routine H&E stain (Bancroft and Stevens,1982). All paraffin embedded samples were further stained by three stains MGG stain (MGG stain kit protocol, Atom Scientific), Congo red stain (Sigma Aldrich protocol) and IHC (DAKO Company Kit Protocol).

Eosinophils were counted under light microscope in the (mucosa and submucosa) layers of all three submitted sections in all cases. From both layers ten (10) fields (best oriented and stained areas not necessary in adjust fields) were selected from each section for eosinophils counting. The average eosinophil count for each case obtained and expressed as number of cells per high-power field (HPF) (1000 x).

3.4 Peripheral Blood Eosinophils

3.4.1 Blood Sample Collection

Preoperative EDTA blood samples for each patient used to measure CBC and an air dried smear is stained with Giemsa stain.

3.4.2 Procedure (Fraser *et al*., 2010)

1. One drop of blood sample was added on slide (8) microliter.
2. Blood sample was Smear and Air- dried.
3. Air-dried smear were fixed with methanol.
4. Smear was Air-dry until all methanol has evaporated.
5. 3-4 drops of Giemsa stain was added on end of slide for (2) min.
6. The slide was rinsed with tap water until any excess Giemsa stain is eliminated.
7. Slide was allowing to air dry, examined the samples using a microscope.

3.5 Hematoxylin and Eosin (H&E) Stain

The hematoxylin and eosin (H&E) is a routine stain used for microscopic examination of tissues that have been fixed, processed, embedded and sectioned. The H&E procedure stains the nucleus and cytoplasm contrasting colors to readily differentiate cellular components. However, staining results are dependent on proper specimen processing, which involves tissue preservation, dehydration, clearing, and paraffin infiltration (Feldman and Wolfe, 2014). Hematoxylin has a deep blue-purple color and stains nucleic acids. Eosin is pink and stains proteins nonspecifically (Fischer et al., 2008).

3.5.1 Sample Collection

All appendectomy specimens were stained by H & E according to (Bancroft and Gamble, 2008) with slight modifications.

3.5.2 Tissue Processing:

1. Ethanol (1) 50%.....2hrs
2. Ethanol (2) 50%.....2hrs
3. Ethanol (1) 70%.....2hrs
4. Ethanol (2) 70%.....2hrs
5. Ethanol (1) 90%.....2hrs
6. Ethanol (2) 90%.....2hrs
7. Ethanol (1) 100%.....4hrs
8. Ethanol (2) 100%.....6hrs
9. Xylol (1).....1hrs
10. Xylol (2).....1hrs
11. Paraffin (1),(60^{O}C) oven .....2 hrs.
12. Paraffin (2),(60^{O}C) oven .....2 hrs.
13. Blocking
14. Sectioning.

ddd

3.5.3 Staining:

1. Put slides in oven 60^{O}C .....10 min
2. Xylol (1).....10 min
3. Xylol (2).....10 min

4. Ethanol (1) 100%.....3 min
5. Ethanol (2) 100%.....3 min
6. Ethanol 90%.....3 min
7. Ethanol 70%.....3 min
8. (D.W).....1 min
9. Hematoxylin 1.5min
10. Tap water.....1.5min
11. Eosin.....30 secs.
12. Wash in tap water
13. Wash in D.W…….2min
14. Ethanol 70%.....3 min
15. Ethanol 90%.....3 min
16. Ethanol (1) 100%.....3 min
17. Ethanol (2) 100%.....3 min
18. Xylol .....10 min
19. DPX and Cover.

3.6 May Grunwald Giemsa (MGG) Stain

May Grünwald Giemsa (MGG) stain is a Romanowsky stain, which is used routinely for staining of air-dried cytological smears, blood and bone marrow smears. The MGG staining allows differentiating white blood cells and erythrocytes. This stain according to Pappenheim can also be used for tissue sections in histology. MGG in tissue sections are more variable than in blood films because (and not only due to tissue fixation) tissue sections contain more stainable components. May Grünwald is an acidic stain while Giemsa is a basic stain. May-Grünwald staining combines the effect of acidic eosin and alkaline methylene blue. Giemsa staining makes effect of azure. Acidic components, such as DNA and chromatin, stain blue or purple, while basic components, such as cytoplasm and proteins stain pink or red (Kuhlmann, 2018).

3.6.1 Reagents:

- May Grunwald stain
- Giemsa stain
- Sorensen’s buffer (200 *concentration) pH 6.8

3.6.2 Procedure: According to Manufacturer's Protocol

1.Dewax sections by xylene (20) min.

2. Hydrate sections through alcohols (%100 ethanol two changes, each change (5) min, %90 ethanol (5) min and % 70 ethanol (5) min.

3. Rinse in tap water.

4. Filter May Grunwald stain, dilute with Sorensen's buffer 1:1 and stain for 15 min.

5. Tip of stain but do not rinse in buffer.

6. Filter Giemsa stain and dilute 1part stain to 9 parts buffer and stain for 10min.

7. Rinse in Sorensens buffer pH 6.8.

8. Dehydrate (%70 (3) min, %90(3) min and %100 (1) for (3) min %100 (2) for (3) min).

9. Clearing sections with xylene (two changes, each change for five minutes)

10. DPX and cover.

3.7 Congo red Stain

Congo red is an acid-base indicator dye which is commonly used histological dye for amyloid detection and one of the major methods used to detect the amyloid structure of protein aggregates. The specificity of this staining results from Congo red's affinity for binding to fibril proteins enriched in β-sheet conformation (Kuhlmann, 2018). The Congo red staining principle is based on the formation of hydrogen bridge bonds with the carbohydrate component of the substrate. Congo red is an anionic dye and is capable of depositing itself in amyloid fibrils, which then exhibit a conspicuous dichroism under polarized light. The tissue stained with Congo red appears orange-red under the transmitted-light microscope; under polarized light, however, the amyloid deposits show up as brilliant green double-refraction images against a dark background. Other structures also stained by Congo red, e. g. collagen, however are not visualized under polarized light. Eosinophils can be dyed orange-red color clearly with Congo red in distinct contrast with other cellular components (Yakupova et al., 2019).

3.7.1 Solutions and Reagents:

- **0.5% Congo Red in 50% Alcohol;**
  1. Congo Red……..0.5g
  2. 50% Alcohol ……100ml
- **1% sodium hydroxide;**

1. Sodium Hydroxide ….1g
2. Distilled Water……100ml

- **Alkaline Alcohol Solution;**
  1. 1% Sodium Hydroxide…1ml
  2. 50% Alcohol……100ml

3.7.2 Procedure: (McAlpine and Bancroft, 1964)

1. Deparaffinization of sections by xylene…… (20) min.
2. Hydrated through graded alcohol (%100 ethanol two changes each change (5) min, %90 ethanol (5) min and % 70 ethanol (5) min).
3. Washed by distilled water for one minute.
4. Stained sections with Congo red solution for 20 minutes.
5. Washed by running water for one minute.
6. Differentiate quickly to alkaline alcohol for (5-10 dips).
7. Rinsed for one minute in tap water.
8. Counter stains with Gill hematoxylin for 30 seconds.
9. Washed for 2 minutes by running water.
10. Dehydrated by serial of alcohol (70% (3) min, 90% (3) min and 100% two changes each change for (3) minutes).
11. cleared section with xylene (two changes, each for three minutes).
12. Mounting by DPX and cover

3.8 Immunohistochemistry (IHC) Stain (DAKO Company Kit Protocol).

Immunohistochemistry (IHC) is a powerful technique that exploits the specific binding between an antibody and antigen to detect and localize specific antigens in cells and tissue, most commonly detected and examined with the light microscope (Magaki et al., 2019).The IHC is an important application of monoclonal as well as polyclonal antibodies to determine the tissue distribution of an antigen of interest in health and disease (Duraiyan et al., 2012). The IHC stains were used to evaluate the expression of major basic protein (MBP) which is markers of eosinophil degranulation. MBP serves as a marker of eosinophil's and is used to observe the distribution and degranulation of eosinophil's (Du et al., 2016).

3.8.1 Solutions and Materials Used in IHC Staining

1. My BioSource. Come: Eosinophil major basic protein (#MBS219842).
   anti-PRG2 antibody: Mouse Eosinophil major basic protein monoclonal antibody (BMK-13)
   - Product Name: mouse anti human eosinophil Eosinophil major basic protein
   - Gene Name: anti-PRG2 antibody
   - Clonality: Monoclonal
   - Isotype: IgG1
   - Clone Number: BMK-13
   - Host: Mouse
   - Perservative Stabilisers: 0.02% Sodium Azide (NaN3) 0.1% Bovine Serum Albumin
   - Buffer Solution: Phosphate buffered saline.
   - Target Species: Human

2. Dako EnVision™ FLEX detection system (Code K8000) includes

   - EnVision™ FLEX Antibody Diluent 120 mL (Code K8006) DM830, ready-to-use, Tris buffer, pH 7.2, containing 15 mmol/L NaN3, and protein.
   - EnVision™ FLEX Substrate Buffer 12 x 20 mL (SM803) (Code K8000,8002,8023). Buffered solution containing hydrogen peroxide and preservative.
   - EnVision™ FLEX Hematoxylin 45mL (Code K8008) (SM806) is recommended for counterstaining. The reagent provides a clear blue, nuclear staining.
   - EnVision™ FLEX Pepsin 4 x 6.0 mL (Code K5731), ready-to-use, Pepsin solution, pH 2.0; contains stabilizer and an antimicrobial agent.
   - EnVision™ FLEX Pepsin Diluent (10x) (Code K5731), 24 mL concentrated 10x. Dilution buffer, pH 2.0; contains an antimicrobial agent.
   - EnVision™ FLEX /HRP 3 x 40 mL (Code K8000, 8002, 8007, 8023) (DM831), ready to use, Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins. In buffered solution containing stabilizing protein and preservative.

- EnVision™ FLEX Wash Buffer (20x) (Code K8000, 8002, 8023) (SM802) 4 x 1 L 20x concentrated, Tris-buffered saline solution containing Tween 20, pH 7.6 (±0.1).
- EnVision™ FLEX Peroxidase-Blocking Reagent (SM801) 3 x 40 mL, ready-to-use, Phosphate buffer containing hydrogen peroxide, 15 mmol/L NaN3 and deterget
- EnVision™ FLEX DAB+ Chromogen 3 x 3 mL 3,3'-diaminobenzidine tetrahydrochloride in organic solvent.

3.8.2 Procedure of IHC Staining

1 Thin sections (4µm) were obtained from each 10% neutral formalin-fixed paraffin block by using manual microtome.

2. Sections were placed in oven at 56-60c^{o} for 1 hour, then deparaffinized in 2 jars of xylene (5x5 minutes), rehydrated in graded ethanol (99%, 80%, 70%, 3x3x3 minutes for each one) respectively.

3. Sections were washed with running tap water slowly to remove residual alcohol and rinsed in wash buffer WB solution for 2 minutes

4. Slides were wiped and the tissues were encircled with DAKO Pen (this pen was used to prevent the waste of valuable reagents by forming a water-repulsive barrier around the specimen which in turn creates surface tension to hold an antibody solution or detection reagent within the target area on the slide)

5. Cover section with pepsin working solution (1-2dropes) and incubated for 30 minutes at 37c^{o} in humidified chamber.

5. Allow sections to cool slowly for 10 minutes at room temperature.

6. Slides were rinsed in WB solution for 2 minutes

.7. Slides were rinsed in WB for 2 minutes ×2.

8. The sections were incubated with mouse anti-human eosinophil major basic protein (MBP) monoclonal antibody for 1 hour.

10. Sections were rinsed in WB for 2 minutes ×2.

11. Enough Peroxidase-Blocking Reagent was applied to cover all the tissue and was incubated for 10 minutes in order to block endogenous peroxidase activity.

12. Sections were rinsed in WB for 2 minutes ×2.

13. Sections were incubated with labeled polymer HRP for 30minutes

14. Sections were incubated with the prepared substrate-chromogen solution for 10 minutes which result in a brown colored precipitate at the antigen site.

15. Sections were rinsed with distilled water and counter stained with Mayer's hematoxylin then washed with running tap water.

16. Slides were dehydrated in graded ethanol (70%, 100%, 100%, 2x2x2 minutes for each one) respectively.

16. Mounting by DPX and cover then examined under light microscope.

3.9 Statistical Analysis:

The results were expressed as Mean ± Standard Error (SE) and analyzed statistically using one-way analysis of variance ANOVA. Calculations were done by Graph Pad Prism software version 9. Tukey's multiple comparisons post hoc test. The one way repeated measures analysis of variance (ANOVA) followed by Tukey's multiple comparisons test for making comparisons between stains. Unpaired t-test was used for analyzing the data between two groups (male and female) in CBC parameters. One sample t test used for analyzing the actual means and theoretical means of CBC parameters. Ordinary one- way ANOVA multiple comparison was used for analyzing the data between age groups and dimensions. Furthermore, Pearson correlation coefficient (r) was used find the relationships between the tissue eosinophils and (age, gender, appendix dimension and peripheral blood eosinophil). The results were considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. (* or # or +) represented ($P<0.05$), (** or ## or ++) represented ($P<0.001$) and (*** or ### or +++) represented ($P<0.0001$).

CHAPTER FOUR

RESULTS

4. The Results

This retrospective study included (50) cases with preoperatively clinical diagnosis of AA and treated by either laparoscopic or open appendectomy.

4.1 Age Distribution in Acute Appendicitis

This research revealed that the patients' ages ranged from 4 to 43 years (20.98 ± 1.217) and majority of the patients (30 cases) (60%) were between (4-20) years. Among the patients (28) were men (56%) and (22) were women (44%). Postoperative appendicular dimensions and weight were correlated with age of the patients. (Table 4.1).

Table 4.1: Showed (Mean ± SE) of appendicular weight and dimensions in study age groups

Age groups / years	**Height (cm) Mean ± SE**	**Length (cm) Mean ± SE**	**Width (cm) Mean ± SE**	**Weight (gm) Mean ± SE**
≤ 20 (n = 30)	0.6645 ±0.04920	7.231 ±0.2924	1.257 ± 0.1424	3.904 ±0.281
21– 30 (n =12)	0.5000 ±0.02981 ##	5.629 ±0.6707 *	0.933 ± 0.1364	5.829 ±0.663 *
≥ 30 (n = 8)	1.029 ± 0.2407 *	5.420 ±0.4375 **	0.750 ± 0.119	6.77 ± 1.077 **

Values were expressed as mean ± SE. Asterisks represent the significant change between all the groups that compared with ≤2years age. Hashes represent the significant change between 20 - 30 age that compared ≥30 age.

4.1.1 Height of Appendix

Postoperative fresh appendicular height ranged from 0.3 to 2.4 cm (0.7 ± 0.052). The height of the appendix was non-significantly higher in patients ≤ 20years age group when compared to (21-30years) age group. But, significantly (P<0.0276) it was lower when compared with ≥30 age group, however, there was significant (P<0.0053) decrease between 21-30 and ≥ 30 groups. (Figure 4.1).

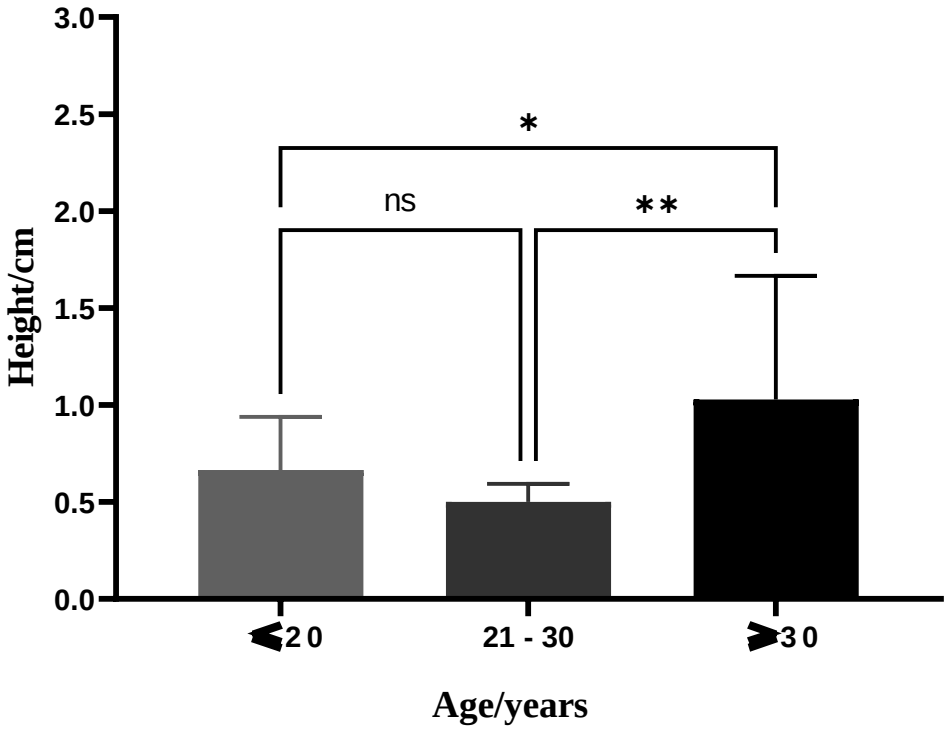


Figure 4.1: Appendicular height in the study age groups.

4.1.2 Length of Appendix

Postoperative fresh appendicular length ranged from 2 to 13 cm (6.824 ± 0.270). The length of the appendix was significantly (P<0.0429) higher in patients ≤20years age group when compared to (21-30years) age group and those ≥30years age group (P<0.0465). However, there was a non-significant difference between 21-30 years and ≥ 30 years. (Figure 4.2).

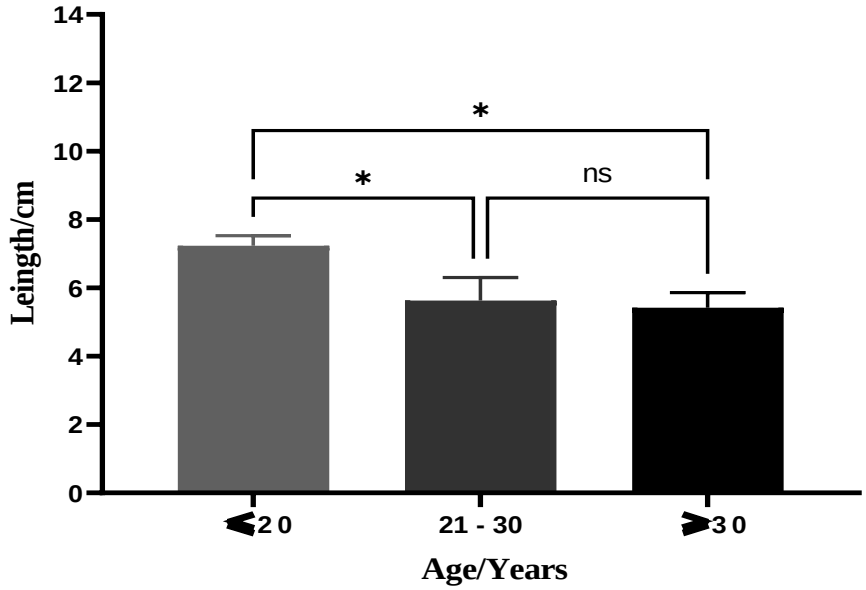


Figure 4.2: Appendicular length in the study age groups.

4.1.3 Width of Appendix

The results showed that postoperatively width of appendix was non-significantly increased between all the age groups as illustrated in (Figure 4.3).

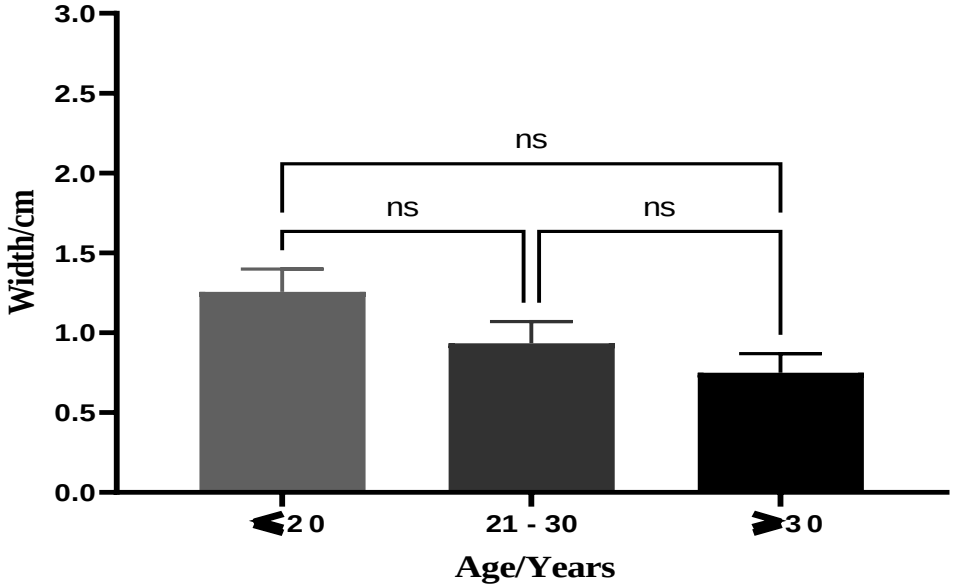


Figure 4.3: Appendicular width in the study age groups.

4.1.4 Weight of Appendix

Postoperative fresh appendicular weight ranged from 1.2 to 11.5 gram (5.126 ± 0.371) The weight of the appendix was significantly (P<0.0437) lower in patients ≤ 20years age group when compared with (21-30 years) age group and those ≥30years age group (P<0.0019). However, there was a non-significant difference between 21-30 years and ≥ 30 years patient samples. (Figure 4.4).

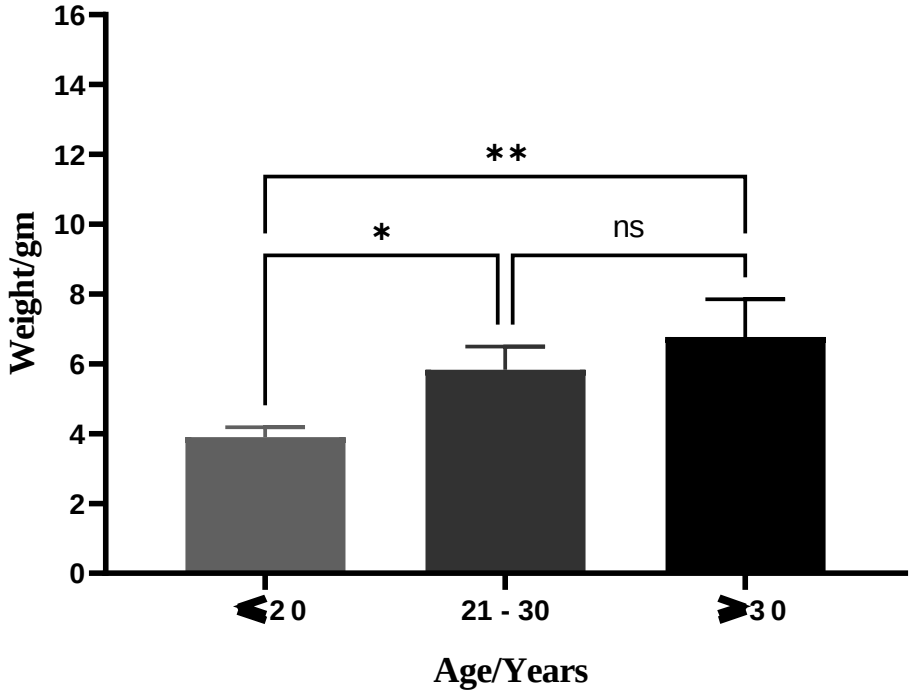


Figure 4.4: Appendicular weight in the study age groups.

4.2 Blood Parameters in Patients with Acute Appendicitis

4.2.1 Gender Distribution and Blood Parameters

The present study showed that male patients were more than females (n=28) and (n=22) respectively. The peripheral blood parameters and CRP had statistically no-significant differences between males & females, as seen in Table (4.2).

Table 4.2: Gender Distribution and Mean Values (Mean ±SE) of (some CBC Parameters and CRP) in Patients with Acute Appendicitis

Variable	**Male (n = 28) Mean ± SE**	**Female (n = 22) Mean ± SE**	**P value**
WBC **(10^3/µL)**	11.85 ± 0.7413	11.31 ± 0.9012	NS
Neutrophil **(10^3/µL)**	7.068 ± 0.8763	8.732 ± 0.8364	NS
Eosinophil **(10^3/µL)**	2.089 ± 0.3054	1.318 ± 0.2042	NS
Lymphocyte **(10^3/µL)**	2.289 ± 0.1640	2.350 ± 0.2074	NS
Platelet **(10^3/µL)**	264.1 ± 13.09	295 ± 14.53	NS
MPV (**fL**)	7.786 ± 0.1224	7.755 ± 0.1408	NS
CRP **(mg/L)**	19.60 ± 4.654	14.95 ± 3.073	NS

This study showed non-significant change between male and female in lymphocyte and mean platelet volume, but peripheral blood eosinophils, CRP and WBC of male was non-significantly increased when compared to female. However, there was non-significant increase in neutrophil and platelet of female when compared with male.

4.2.2 Some Peripheral Blood Parameters in Acute Appendicitis

There was a statistically significant difference between actual and theoretical means of peripheral blood parameters. (Table 4.3).

Table 4.3: Theoretical and Actual Means of (CBC Parameters and CRP) in Patients with Acute Appendicitis

Variable	**Theoretical Mean**	**Actual mean**	**P value**
WBC (**10^3/µL)**	13.35	11.62	0.0037
Neutrophil **(10^3/µL)**	13.16	7.800	0.0001
Lymphocyte **(10^3/µL)**	2.760	2.316	0.0011
PLT **(10^3/µL)**	315.6	277.9	0.0004
MPV **(fL)**	5.78	7.772	0.0001
N/L**%** (Neutrophils/Lymphocyte)	5.65	3.385	0.014
RDW **%**	14.17	13.09	0.0001
CRP **(mg/L)**	15.26	17.55	0.6

The actual mean of CRP was non-significantly (P<0.6) higher than theoretical means. But the actual means of WBC, lymphocytes and N/L were significantly decreased (P<0.0037), (P<0.0011), (P<0.014) respectively, compared to theoretical means. However, the actual means of neutrophils, PLT and RDW were highly significantly decreased (P<0.0001), (P<0.0004), (P<0.0001), respectively compared to theoretical means. But the actual mean of MPV was highly significantly (P<0.0001) increased compared to theoretical mean.

4.3 Eosinophils Demonstration

4.3.1 Demonstration of Tissue Eosinophil

Histologic demonstration of eosinophils in all postoperatively tissue sections were varied according to the stains used in this study, as illustrated in figure (4.5). Tissue sections stained by IHC (14.73 ± 1.05) and Congo red (3.50 ± 0.9540) revealed that tissue eosinophil count was highly significantly (P < 0.0001) increased when compared to H&E (4.082 ± 0.3769) and MGG (5.136 ± 0.3602), while the IHC significantly (p < 0.05) yielded a higher eosinophil counting than Congo red (Table 4.4).

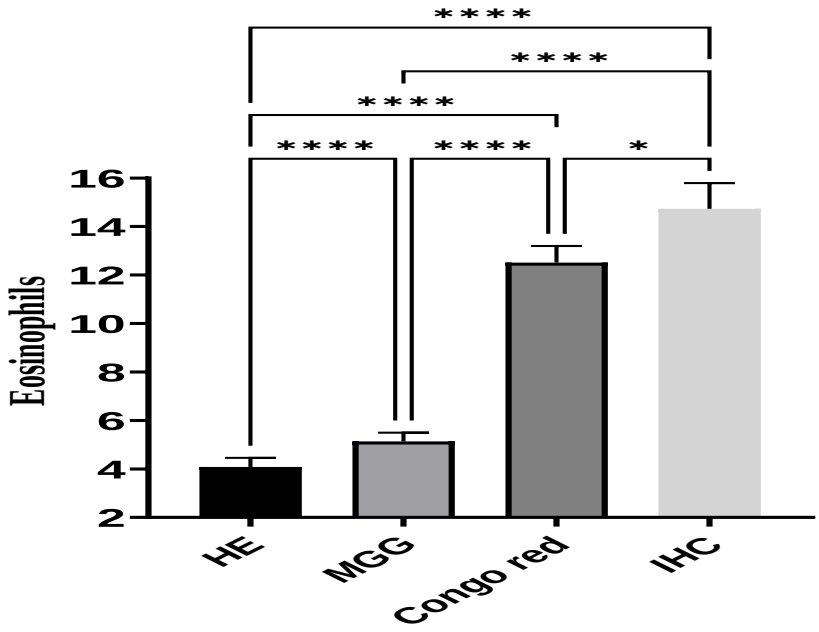


Figure 4.5: The significantly differences in tissue eosinophils count according to H&E, MGG, Congo red and IHC stains

Table 4.4: The mean values (Mean ± SE) of patient tissue eosinophils stained by H&E, MGG, Congo red and IHC stains.

Name of Stains	Eosinophil in tissue Mean ± SE
IHC	14.73 ± 1.05
Congo red	13.50 ± 0.9540*
MGG	5.136 ± 0.3602 ****####
H&E	4.082 ± 0.3769 ****####++++

Values were expressed as mean ± SE. Asterisks represent the significant change between all the groups that compared with IHC. Hashes represent the significant change between Congo red stain with MGG and H&E stains, Plus, represent the significant change between MGG and H&E stains.

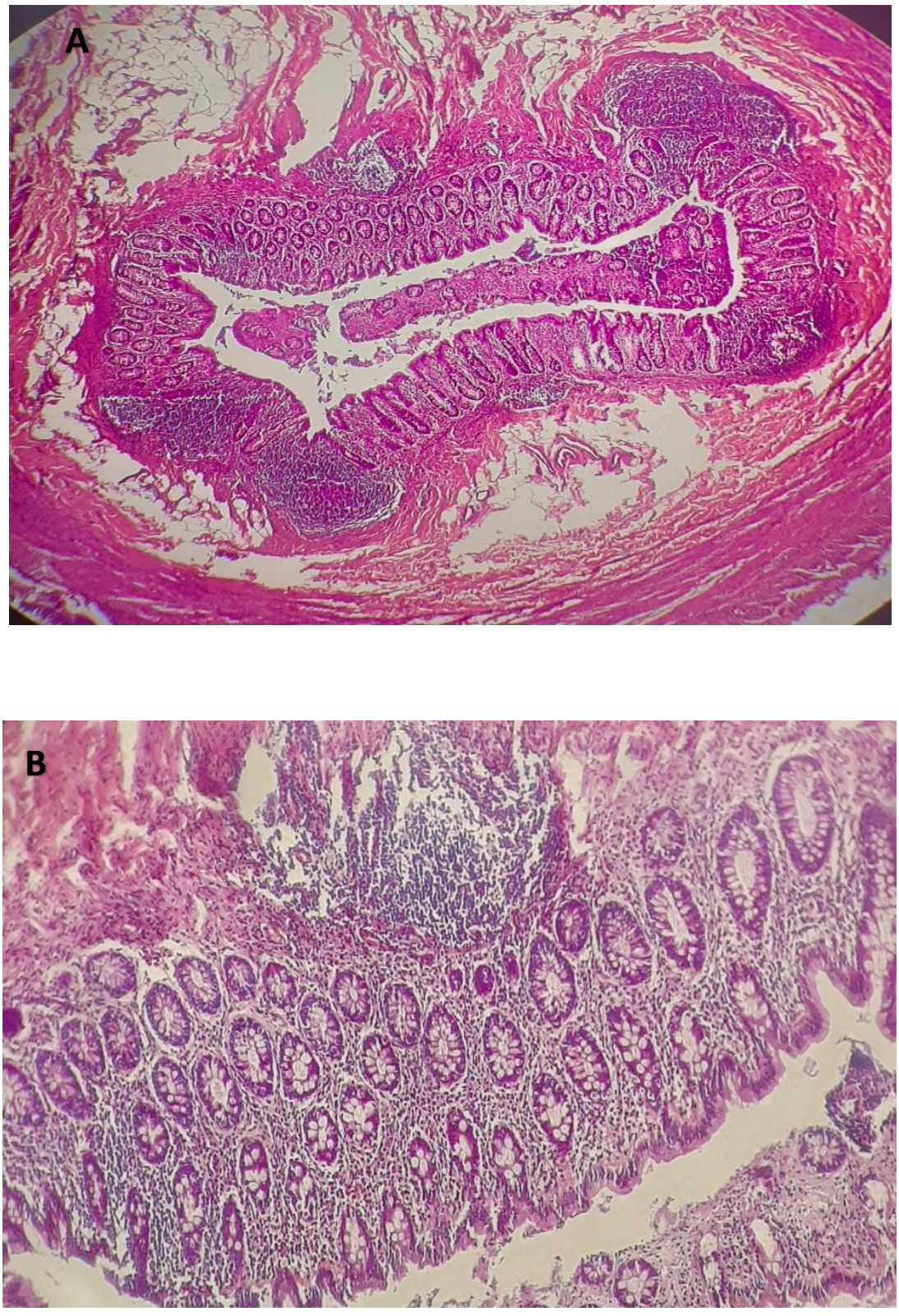


Figure 4.6: Appendicitis with demonstration of eosinophils (arrows) infiltrating the lamina propria layer. (A) Cross section 40X, (B) Cross section 100X (H&E) stains.

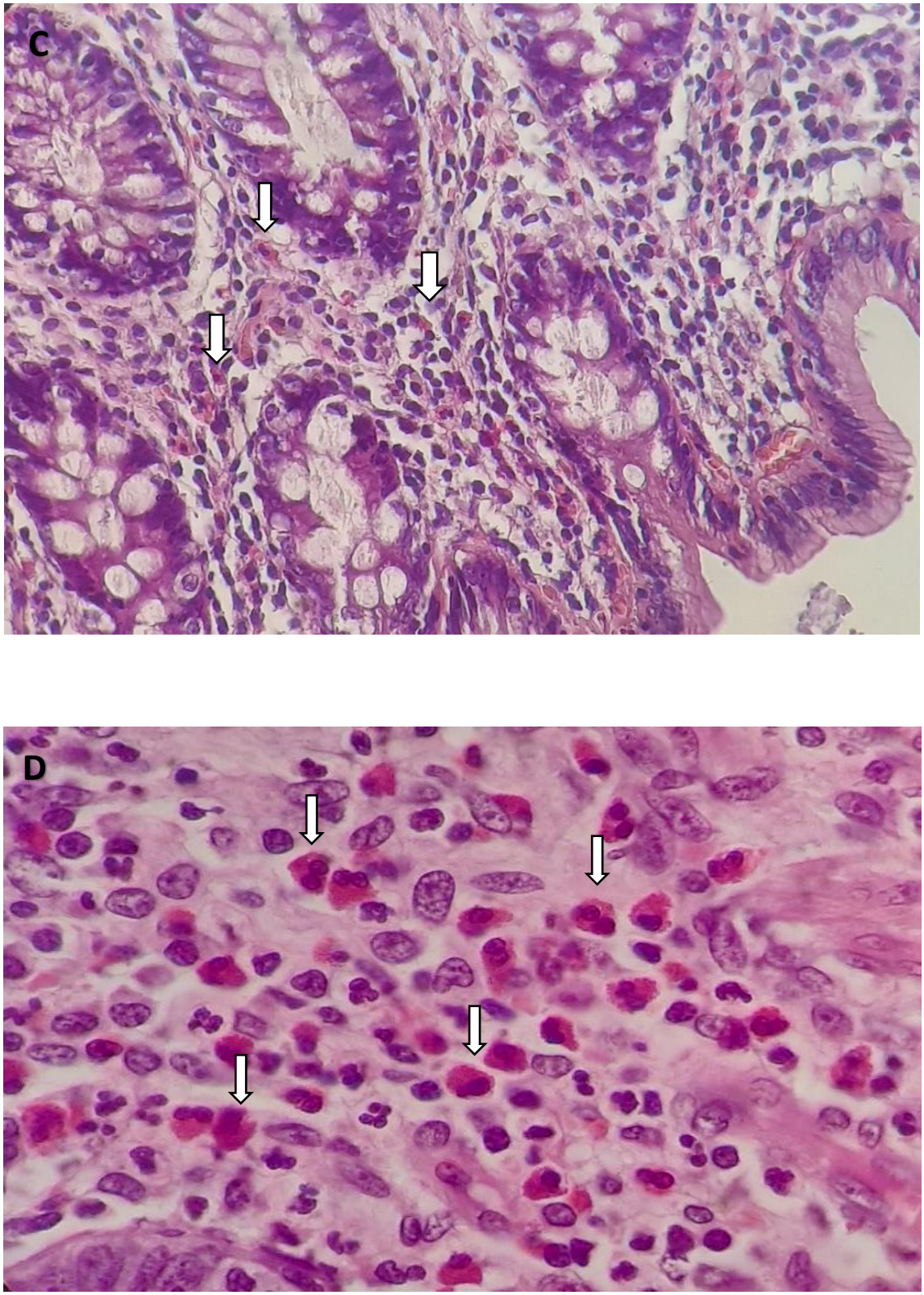


Figure 4.7: Appendicitis with demonstration of eosinophils (arrows) infiltrating the lamina propria layer. (C) Cross section 400X, (D) Cross section 1000X (H&E) stains.

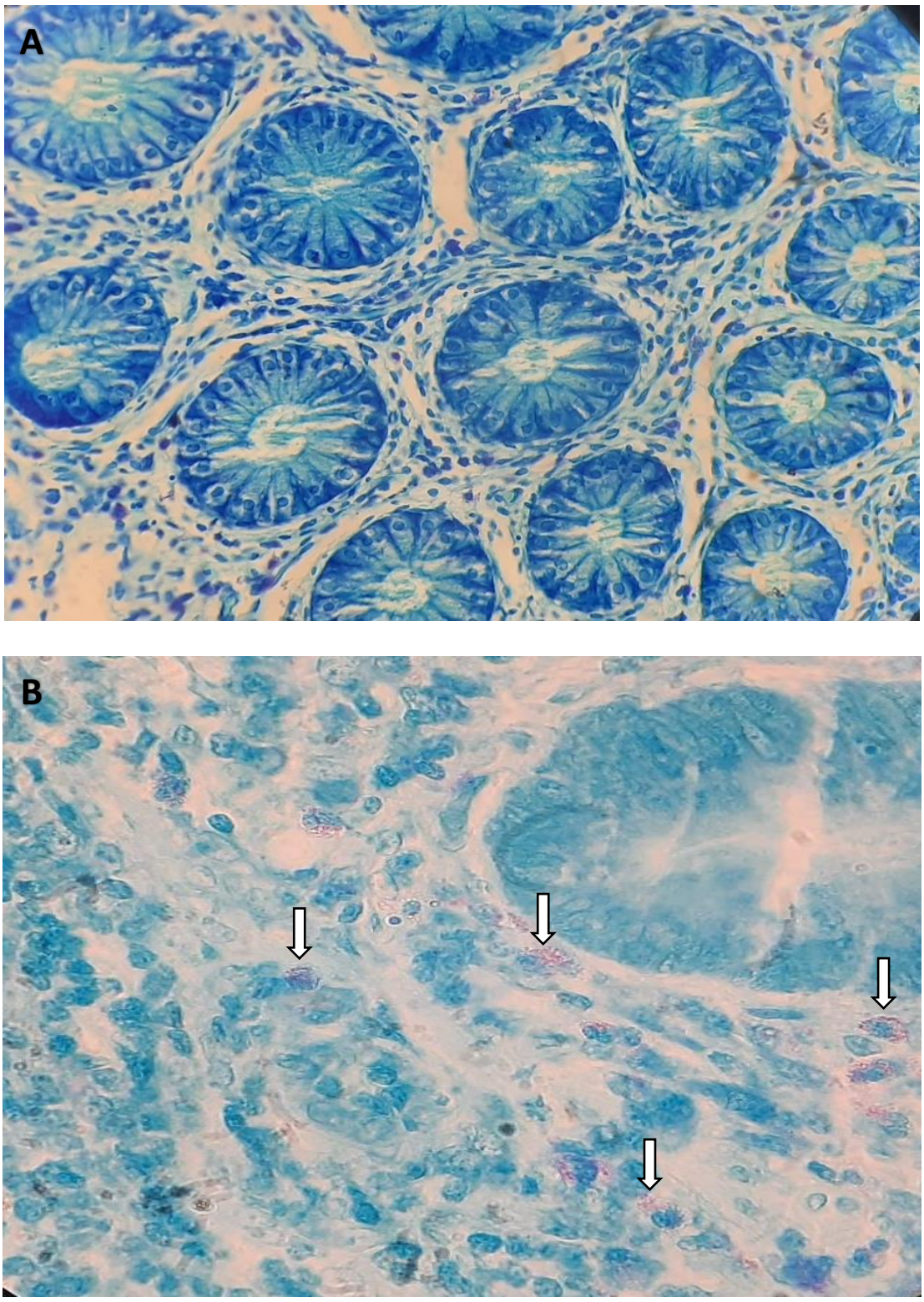


Figure 4.8: Appendicitis with demonstration of eosinophils (arrows) infiltrating the lamina propria layer. (A) Cross section 400X, (B) Cross section 1000X (MGG) stains.

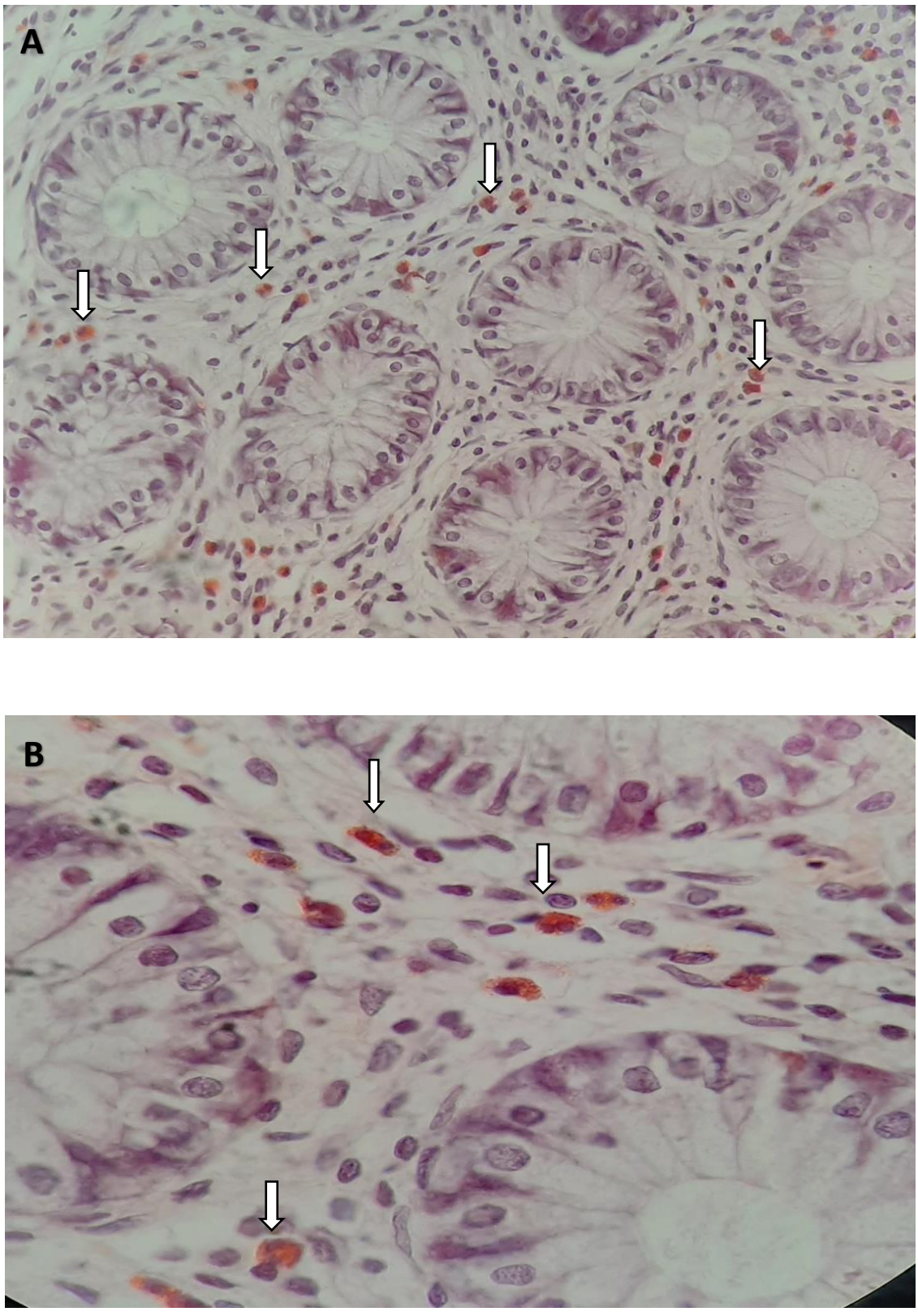


Figure 4.9: Appendicitis with demonstration of eosinophils (arrows) infiltrating the lamina propria layer. (A) Cross section 400X, (B) Cross section 1000X (Congo red) stains.

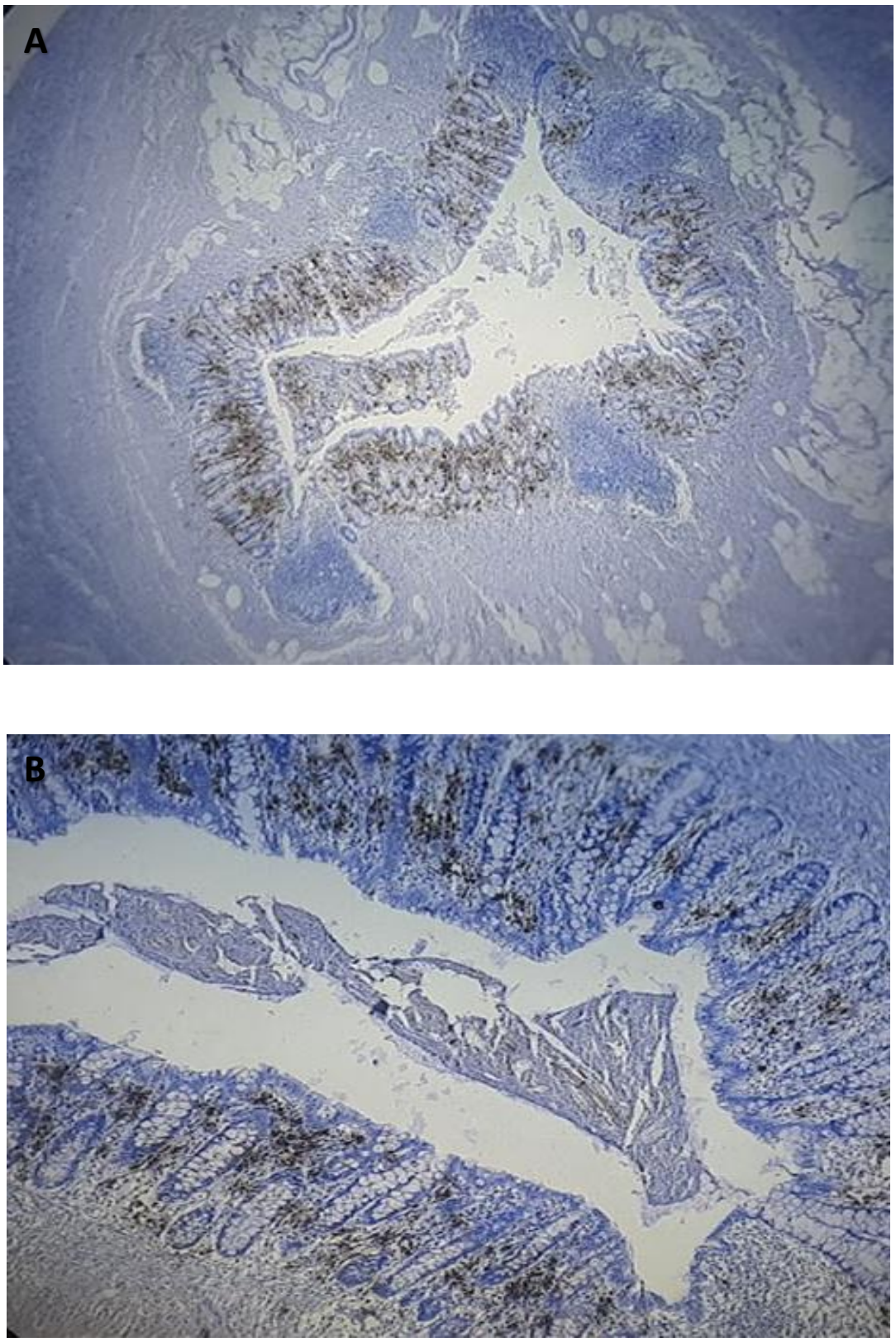


Figure 4.10: Appendicitis with demonstration of eosinophils infiltrating the lamina propria layer. (A) Cross section 40X, (B) Cross section 100X (IHC) stains.

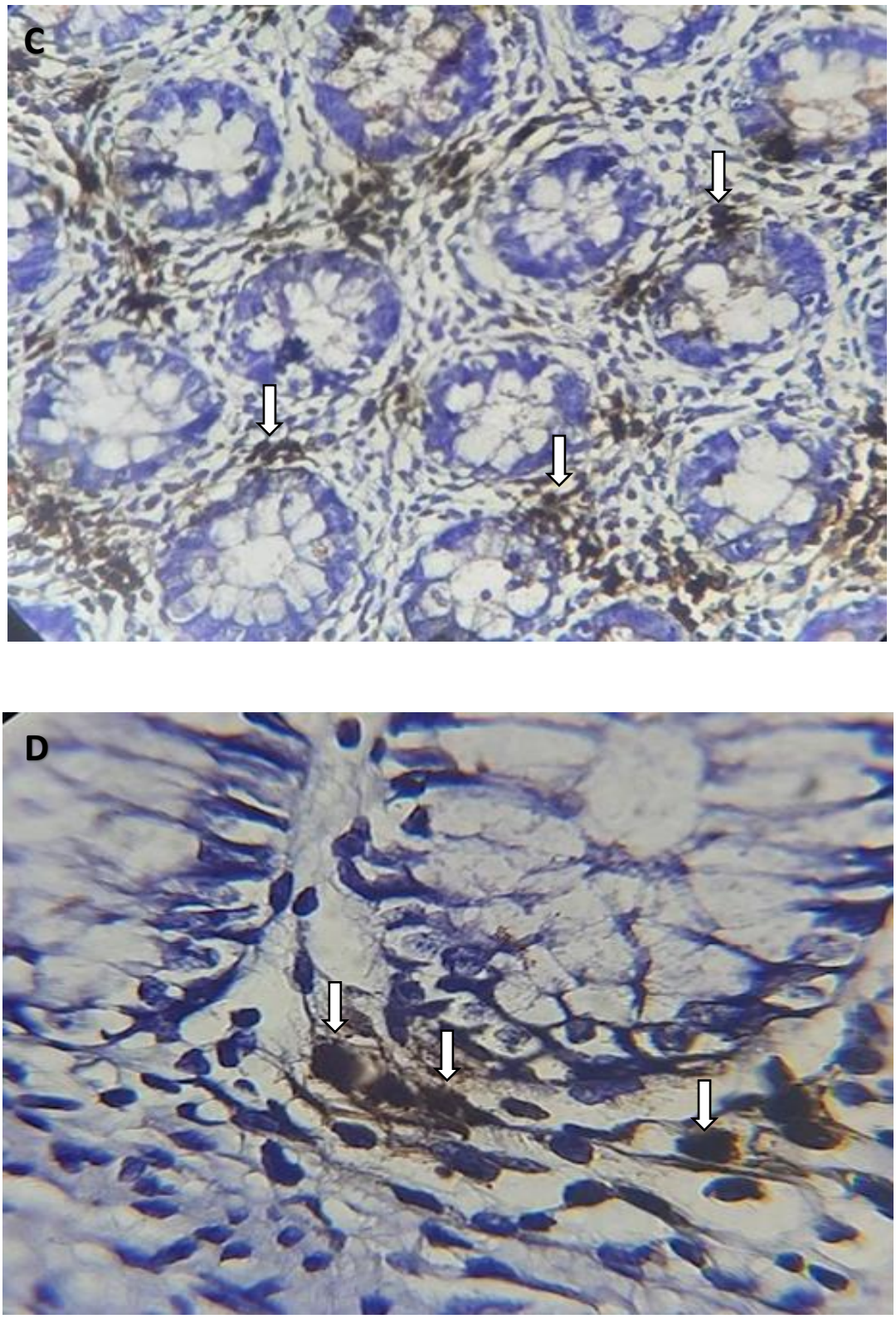


Figure 4.11: Appendicitis with demonstration of eosinophils (arrows) infiltrating the lamina propria layer. (C) Cross section 400X, (D) Cross section 1000X (IHC) stains.

4.3.2 Demonstration of Peripheral Blood Eosinophil

Eosinophils were slightly larger than polymorphs, and the nucleus is usually bilobed. Their defining characteristic is the presence of orange-red granules in the cytoplasm (Figure 4.12).

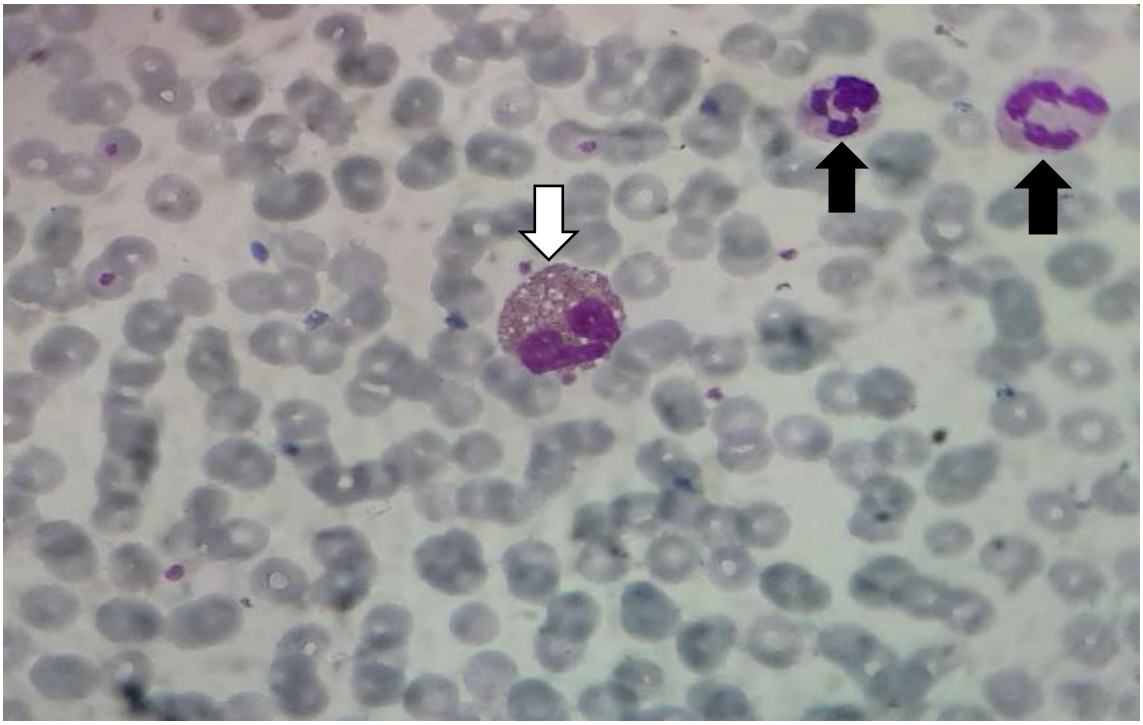


Figure 4.12: Eosinophil (white arrow) in peripheral blood smear and two neutrophils (black arrows) (Giemsa stain; magnification 1000×).

4.4 Correlations of Tissue Eosinophil

There was a correlation (using spearman correlation test) between tissue eosinophil as dependent variable when compared to age, sex, weight and dimension of appendices as independent variable in patients with acute appendicitis (Figure 4.13 and 4.14). Between tissue eosinophil and peripheral blood eosinophil count, a significant positive correlation was discovered. ($p = 0.0141$). Age and tissue eosinophils were non- significantly correlated with one another ($p = 0.1765$), but a negative correlation was detected between tissue eosinophil and (sex and length) which were non-significant ($p = 0.5436$ and $p = 0.7669$), respectively. There was negative highly significant correlation between tissue eosinophil and gross appendicular parameters (weight, width, height and total WBC) ($p = 0.0025$, $p = 0.0035$ and $p =0,0093$) ($p = 0.0141$) respectively, as seen in Table (4.5).

Table 4.5: Correlation Between Tissue Eosinophil as Dependent Variable with the (Age, Sex, WBC, Peripheral Blood Eosinophil, Appendicular Weight and Dimensions) as Independent Variables.

Variable	r	CI	P value
Age **(years)**	0.1942	- 0.09730 to 0.4551	0.1765
Sex	-0.08795	- 0.3649 to 0.2033	0.5436
Weight **(gm)**	-0.3168	- 0.5528 to 0.03369	0.0025
Length **(cm)**	-0.043	-0.3251 to 0.2462	0.7669
Width **(cm)**	-0.2981	- 0.53883 to 0.01312	0.0035
Height **(cm)**	-0.2398	- 0.4922 to 0.04975	0.0093
WBC **(10^3/µL)**	-0.4289	- 0.6368 to 0.1627	0.0019
Blood Eosinophils **(10^3/µL)**	0.3452	0.6557 to 0.5746	0.0141

(r) means correlation coefficient
(CI) means confidence interval

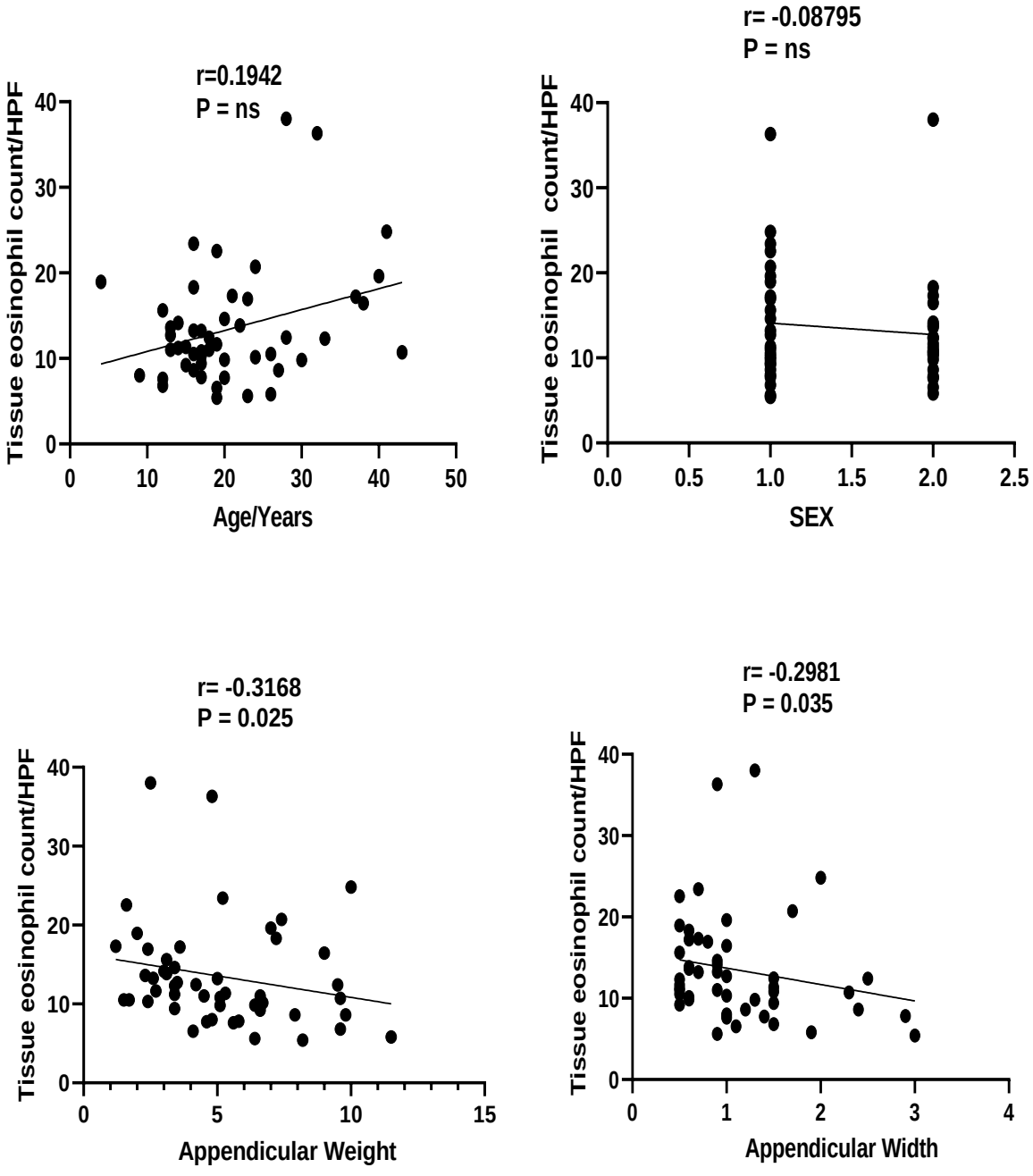


Figure (4.13): Scatter plot for the study of the correlation between Tissue Eosinophil as dependent variable with the (Age, Sex, Appendicular weight and Appendicular width) as independent variables.

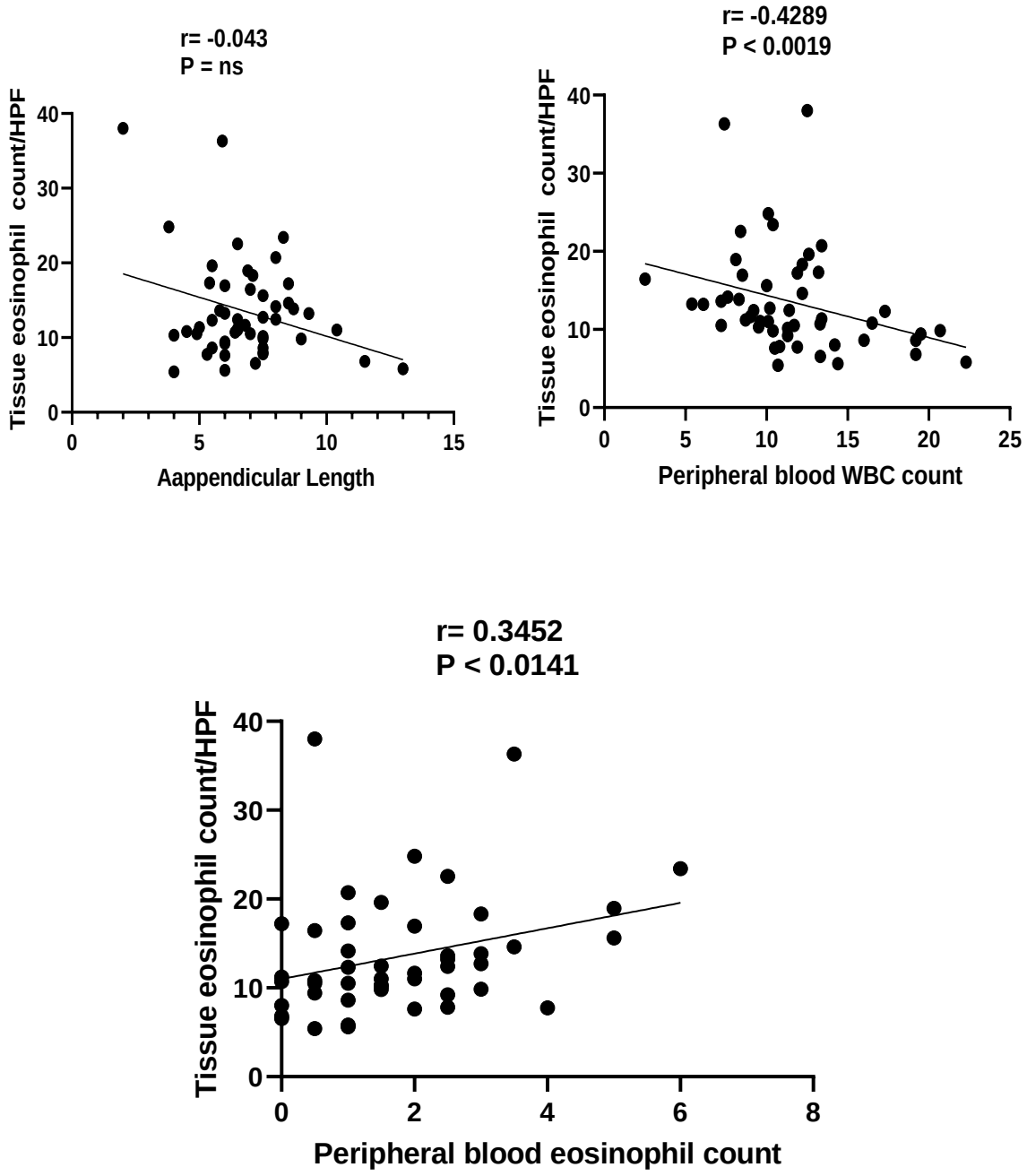


Figure (4.14): Scatter plot for the study of the correlation between Tissue Eosinophil as dependent variable with the (WBC, Peripheral blood eosinophil and Appendicular length) as independent variables.

CHAPTER FIVE

DISCUSSION

5. Discussion

The most prevalent type of surgical emergency in the world is AA, which is defined by the development of localized inflammation followed by a more broad response to inflammation (Dixon and Singh, 2020). The majority of AA cases, despite some persistent controversy, are thought to be caused by luminal obstruction, which results in distention and increased pressure within the lumen, increased intraluminal pressure, appendix ischemia and mucosal hypoxia, ulceration, a breach of the mucous barrier, and the development of necrosis (Sazhin *et al*., 2021). Furthermore, (Garza-Serna *et al*., 2016) showed that the development of AA may also be influenced by environmental, regional, nutritional, genetic predisposition, and infections factors. Moreover, (Aggelidou *et al*., 2019) proposed allergic etiology for AA, the discovery of eosinophil infiltration of the muscle layer together with splitting of muscle fibers as a result of intramuscular edema in instances of AA raised the hypothesis that a type 1 hypersensitivity reaction was responsible for the disease's development.

In the present research 50 patients were examined in total, and the findings revealed that men were affected more than females, with 28 (56%), and 22 (44%), patients, respectively. Our findings agree with (Oguntola *et al*., 2010) who found an increase in the frequency of appendicitis in men; this may be attributable to differences in male and female body physiology and male have higher body immunity than female so male have higher body response. This was also verified by different research, who found that the male ratio was higher than the female ratio (Ohene-Yeboah and Abantanga, 2009, Memon *et al*., 2013, Danish, 2022).

The AA incidence is significantly influenced by age (Al-Mulhim, 2011). It is uncommon in newborns, but it increases in frequency throughout childhood and the early years of adulthood, peaking in the adolescent and early 20s. The likelihood of getting appendicitis decreases after middle age (Williams *et al*., 2008). Typically, it closely resembles lymphoid development (Akbulut *et al*., 2011). In the current research, the incidence was found to be greater 30 (60%) among patients whose ages ranged from (≤20) years. It is believed that the high incidence of the disease is caused by the peak in lymphoid tissue development that occurs around adolescence, which increases the appendix's capacity to block. This was in line with studies (Kolur *et al*., 2014) who showed a greatest number of occurrences during the first and second decades of life, with a decline in incidence after the third decade. Furthermore (Rahman *et al*., 2008) reported

that in 60% of cases, appendicitis was caused by lymphoid hyperplasia that resulted in occlusion of the appendix's interior, indicating that the appendix is usually subject to abnormal lymphoid tissue proliferation. The findings of these studies are comparable to those of a study conducted in Sulaimani City, Iraq by (O.Ahmed, 2006).

The present research showed that the weights and dimensions of appendices varied significantly depending on the age groups. The weight of appendix was higher in group (≥ 30) than group (21– 30), (6.7 gm) and (5.8gm), respectively, so there was a gradual increased with age groups and this was agreeing with study conducted by (Mohammadi *et al*., 2017) who reported the highest weights of appendix was (7.04gm) in group (30-39) and the lowest was (2.65gm) in group (< 10). But in contrast (Salih *et al*., 2020) found that the highest weights of appendix were (7.64gm) in group (20-29) and the lowest was (4.13gm) in group (60-69) because of the individual differences in immune response strength and the probable decline in the number of lymphatic follicles, their replacement by connective tissue.

Knowledge of variation in appendiceal dimension is important during appendectomy, the association between age and appendiceal dimensions were statistically changed in existing study the length, width and height was significantly decreased gradually with age. The appendix was longer in age group (≤ 20) than age group (20-30) and (≥30) (7.2cm), (5.6cm) and (5.4cm) respectively. probably the appendix achieves its adult sizes after an initial development phase that lasts from early infancy to around three years and does not continue to grow throughout childhood (Searle *et al*., 2013), This result was comparable to that of another research done by (Patel and Naik, 2016) according to their findings, the vermiform appendix is longer in young adults and children, and as people age, it progressively becomes shorter. Furthermore, the findings of the present study were similar to the results of (Paul *et al*., 2011), but in contrast, (Ghorbani *et al*., 2014) found that the length of the appendix is longer in elderly people. Also there are some different findings in other study (Salih *et al*., 2020) who reported that the vermiform appendix's length gradually reduced with age and that males' vermiform appendixes were somewhat longer than females.

Delay in treatment of AA may causes perforation. Therefore, achieving the correct diagnosis is crucial to decrease the chance of perforation and negative appendectomy (Andersson, 2007). In addition to the history, physical exam and radiographic studies, inflammatory indicators are

helpful in determining the diagnosis of appendicitis. Yet, they can't be employed by themselves because of their limited sensitivity and specificity (Ahmed *et al*., 2019).

The CBC parameters were a highly valuable and cost-effective basic laboratory test. Also, (Birchley, 2006) confirms that the evaluation of CBC parameters as proven by numerous studies may be helpful to the identification and diagnosis of AA. In the present study CBC Parameters had a statistically non -significant differences between male & females, but there was statistically significant difference between actual and theoretical means of CBC parameters. The actual means of WBC and lymphocytes significantly decreased compared to the theoretical means. Nevertheless, the actual means of neutrophils and platelets were highly significantly decreased compared to the theoretical means.

However, the actual means of WBC and neutrophils were less than the theoretical mean. Even so a rise in neutrophil and WBC counts was one of the initial signs of inflammation in AA and may aid in the identification of the condition. These findings were comparable to that seen in a study conducted by (Panagiotopoulou *et al*., 2013). Although (Ulukent *et al*., 2016, Al-Jawdah and Kamal, 2022) reported that the WBC and nutrophil count were significantly higher in AA. In contrast to other results conducted by (Imad Wajeh *et al*., 2010), they have showed that WBC can provide information about the diagnosis of AA. However, it is not always useful as approximately one-third of cases do not exhibit elevated WBC counts. Furthermore, (Shogilev *et al*., 2014) who reported that the early signs of inflammation in AA are increasing of WBC and neutrophil count. Because they are frequently elevated in individuals with various inflammatory disorders they are not a specific sign, therefore this should be considered for the differential diagnosis.

In the current study, the actual mean of lymphocytes was decreased significantly compared to theoretical means. There was a statistically significant correlation between a reduced lymphocyte count and the clinical diagnosis of AA (Al-Jawdah and Kamal, 2022), while (Virmani *et al*., 2018) reported that Increased risk of complications is linked to low lymphocyte count. This finding can emphasize the value of lymphocyte count in predicting complicated appendicitis but its inaccuracy in predicting the histological outcome of appendectomies.

The N/L is a helpful, easy and economical indicator of inflammation that can be determined using a complete count of blood cells (Shimizu *et al*., 2016). In addition (Goodman *et al*., 1995)

reported that Compared to the overall amount of leukocytes, N/L is a more effective parameter in the diagnosis of AA.. The findings of the current study revealed found that actual mean of N/L significantly decreased when compared with theoretical mean and contribute to diagnosis of AA this agreed with (Al-Jawdah and Kamal, 2022) who found that N/L is highly accurate and reliable for diagnosing acute appendicitis and that it may be utilized as a help in making difficult diagnoses to lower the percentage of negative appendectomies. Furthermore, (Jung *et al*., 2017) suggested that the initial N/L in the older individuals is the most effective indicator for the diagnosis of AA perforation. elevated N/L is useful biomarkers for the diagnosis of AA..

Both the function and activation of PLT are related to MPV. Different outcomes were seen while using MPV to diagnose AA. According to some research, these values rise, however in other studies they fall or remain the same (Saxena *et al*., 2015). In the present study, the actual mean of MPV was highly significantly increased compared to the theoretical mean and the actual mean of MPV was more than the theoretical mean, even so, MPV level was not an accurate indicator for the diagnosis of AA. So this matched with (Daldal and Dagmura)2020 who revealed that the diagnostic power of MPV is not high for the diagnosis of AA. Another study conducted by (Akbulut *et al.*, 2019) who found that despite being an inflammatory marker, studies have not yet supported the use of MPV in the diagnosis of appendicitis. These research' results are entirely compatible to findings of the present study.

Although, (Dooki *et al*., 2022) results matched with the results of the present study, who showed that MPV is ineffective in the diagnosis of AA; but a significantly lower amount has been observed in children with acute appendicitis. It contrast (Tanrikulu *et al*., 2014) demonstrated higher WBC and lower MPV in AA patients. Moreover, (Tullavardhana *et al*., 2021), demonstrated reduced MVP is an important diagnostic indicator for AA.

In this study, it was found that the actual mean of RDW was highly significantly decreased compared with theoretical mean but RDW is not useful as an AA biomarker and this is consistent with a research done by (Anand *et al*., 2022). the findings of the present study matched with (Sengul *et al*., 2020) who revealed that RDW and AA have no relation to one another. In contrast the present result was not consistent with the results of (Haghi *et al*., 2020) who reported RDW indices may be used to differentiate between acute and perforated appendicitis. Besides, (Tartar *et al*., 2020) have showed that an elevated prevalence of RDW in complicated cases.

The CRP is an important inflammatory marker in AA (Ramrao *et al*., 2020) and it has been found to be an effective independent indicator of complicated AA (Ribeiro *et al*., 2022). In this study, however, the actual mean of CRP was non-significantly higher than the theoretical means. But patients with acute appendicitis have elevated CRP levels, which is a marker for the condition (Narci *et al*., 2013). Although (Kareem and Karim, 2014) suggested that Preoperative CRP % is a helpful indicator of the severity of AA pathological alterations that need early surgical treatment and prevent unneeded complication (Withers *et al*., 2019).

Presence of inflammatory cells such as eosinophils within the wall of the appendix is required for histological diagnosis of AA and sometimes unusual type of appendicitis is called AEA that may be caused by allergic reaction (hypersensitivity reaction type 1 or parasitic infestation) and diagnosed by edema infiltration of muscularis layer by eosinophils , and these can be demonstrated by different routine & special stains e.g. H&E , Congo red, MGG, IHC and gimsa stains for blood smear (Kinoshita *et al*., 2019).

The H&E stains are a common, simple, affordable, and reliable procedure that are used in many labs (AbdEl-Latif *et al*., 2016). In this study, histologic demonstration of eosinophils varied according to H&E when compared to MGG, Congo red and IHC stains. The results revealed that H&E highly significantly decreased compared to MGG, Congo red and IHC. The cytoplasm of eosinophils is stained deep red (Figure 4.6 and 4.7) due of the granules, but the cytoplasm of neutrophils is stained pale red because eosin may stain the cytoplasm of all cells to varied degrees of red non-specifically. As a result, it may become challenging to distinguish between eosinophils and neutrophils, and viewing many of slides could easily make one visually tired. This result matched to a study done by (Ikeda *et al*., 2022) who determind that the number of counted eosinophils was higher with IHC and Congo red compared to H&E staining.

Moreover, (Meyerholz *et al*., 2009) reported that eosinophil identification with H&E may be successful. The presence of overlapping staining structure and morphological characteristics in eosinophils and neutrophils, however, might provide challenges for certain researchers. Although (Joshi and Kaijkar, 2013) results matched the result of the present study that showed that eosinophils are often simple to identify in standard H&E sections, occasionally these

granulocytes take on an unusual form, particularly in fibrous tissue and inflammatory infiltrate, making their diagnosis in routinely stained sections quite challenging.

Due to its simplicity and consistency, MGG seems to be preferable to H&E in that it stained eosinophil granules pink (figure 4.8) with less background staining of other tissue structures (Lee and Kim, 2015). MGG staining was better than H&E staining in terms of making eosinophilic granulocytes visible under a microscope because they were more distinct (Lin *et al*., 2005). Despite the background staining and poor staining intensity but the yield of MGG as a diagnostic tool was significant as compared to H&E. Another study conducted by (Samoszuk, 1997) reported that MGG staining usually makes the intensely red granules of eosinophils considerably easier to see within tumor tissue.

One of the simplest, least costly, and most accurate procedures is Congo red staining. Due to its special ability to bond with eosinophils, it has also been demonstrated to be an effective diagnostic technique for eosinophil identification (Jain *et al*., 2014). In this study, Congo red staining was quite effective at demonstrating eosinophils compared to MGG and H&E staining and the results were statistically highly significant. With reduced background staining compared to MGG and H&E, Congo red dyed eosinophils' orange-red color (figure 4.9) clearly contrasts with other cellular components. Even (Sujatha *et al*., 2022) who reported that histochemical analysis of tumor associated tissue eosinophilia (TATE) in sral Squamous cell carcinoma using Congo red can help highlight the eosinophils for better identification and counting. Furthermore, (Song *et al*., 2018), reported that for more accurate eosinophil counts in nasal polyps, Congo red and Chromotrope 2R are both more suitable due to their specificity and reproducibility. In contrast, (Debta *et al*., 2012, Debta *et al*., 2010) reported showing the presence of tissue eosinophils in tumor stroma, carbol chromotrope stain is preferable to Congo red.

Although MBP is a marker for eosinophils and is used for observing eosinophil distribution and degranulation. Therefore, tissue eosinophils are also detected using an IHC stain against MBP (Du *et al*., 2016). This study found that eosinophil counts were considerably greater when MBPmAb IHC labeling was used instead of Congo red. Therefore, MBPmAb IHC had reduced background staining than Congo red and was more selective in identifying eosinophils (figure 4.10 and 4.11). This result is matched with (Zhu and Zimmermann, 2022) who reported that IHC significantly improves the identification of eosinophils and their secreted granule proteins. In

contrast, (Song *et al*., 2018), reported that MBPmAb IHC may not be appropriate method for the quantification of eosinophils.. It is found that the nuclei may get contaminated in MBP-positive tissue, making it challenging to count the number of cells. Even though the eosinophils are interconnected, the nucleus is not entirely covered by the other histochemical positive signs. IHC may also stain peripheral cells during large degranulation, increasing the number of cells present.

According to current study results, neither Congo red nor MBPmAb IHC labeled neutrophils, lymphocytes, plasma cells, or mast cells, showing that these stains only stain eosinophils. In contrast, to MGG and H&E, results with Congo red and MBPmAb IHC revealed reduced background staining. Hence, are more accurate for counting eosinophils in AA and are specific, reproducible and suitable.

In this study, in comparison of eosinophils in particular layer (mucosa and submucosa) of the appendectomy specimen with (age, sex, dimension, WBC and peripheral blood eosinophils) it was found that there is non-significant correlation between eosinophils infiltration and (age, sex and length). However, a negative (Inverse) correlation between eosinophil infiltration and (weight, width, height and WBC) found which is statistically significant. As well there was a positive (Direct) correlation between eosinophil infiltration and peripheral blood eosinophil, which is statistically significant with and this was matched with a study conducted by (Carvalho *et al*., 2022).This positive (Direct) correlation means as there is increase in the number of eosinophils in tissue, there is also increase in the number of peripheral blood eosinophils.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has concluded that:

1. Males were more affected than females by AA.
2. The age group less than 20 years was the highest incidence of AA.
3. The actual means of CBC parameters in AA patients were different from theoretical means and it could be used as laboratory diagnosis for AA in addition to the history, physical examination and radiographic studies.
4. In AA patients the peripheral blood eosinophil count increased as the tissue count increased (positive correlation).
5. Demonstration of tissue eosinophils by Congo red and IHC staining were more effective than MGG and H&E, and IHC significantly ($p < 0.05$) yielded a higher tissue eosinophil count than Congo red.

6.2 Recommendations

Based on the result obtained from the present study, the following points can be recommended for the further studies and investigation:

1. A broader spectrum study should be conducted to include a larger number of patients and healthy individuals of different age groups to determine the exact incidence of acute appendicitis in our local population.

2. Giemsa stain should be considered as a routine adjuvant to H and E staining for demonstrating tissue eosinophilia in patients with AA.

3. Comparison & testing the efficacy of other special stains e.g. Carbol chromotrope to demonstrate the presence of tissue eosinophils.

CHAPTER SEVEN

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Appendix 1: Sex difference of Blood parameters (MPV, PLT, LYM and Eosinophil) in patients with acute appendicitis

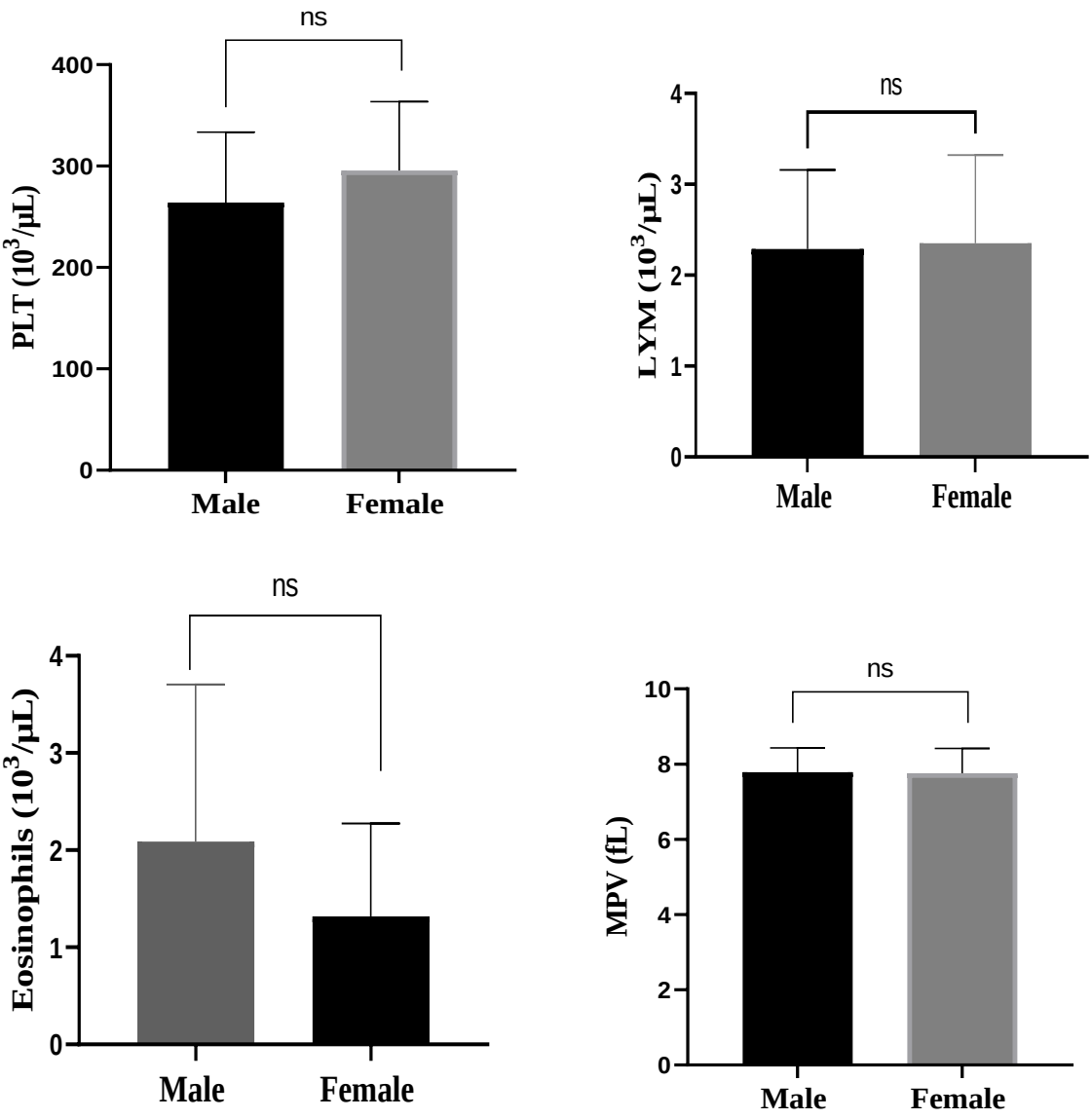

Figure 1: Non-significantly sex difference of Blood parameters (MPV, PLT, LYM and Eosinophil) in patients with acute appendicitis.

Appendix 2: Sex difference of Blood parameters (WBC, Neutrophil and CRP) in patients with acute appendicitis

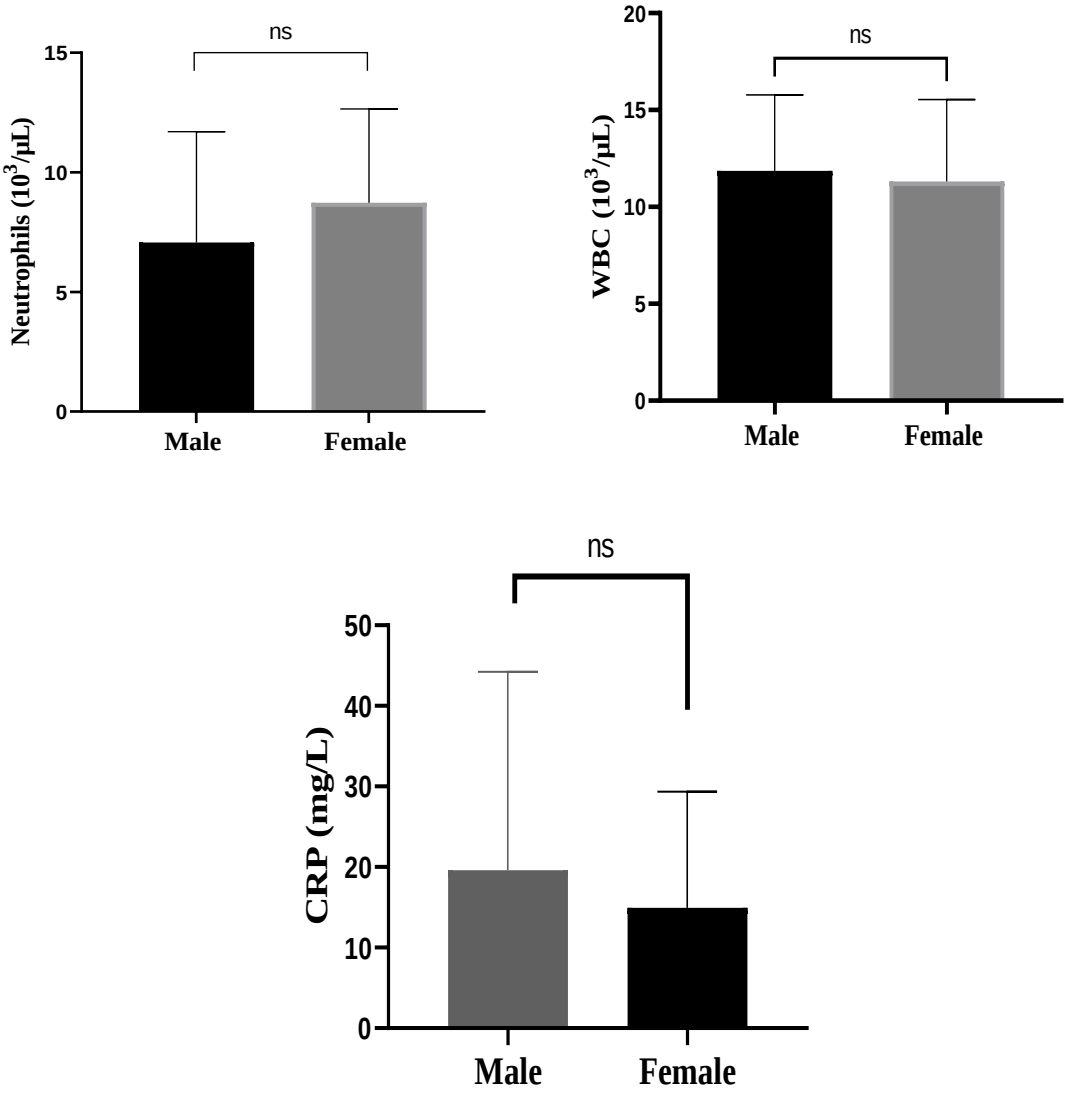

Figure 2: Non-significantly sex difference of Blood parameters (WBC, Neutrophil and CRP) in patients with acute appendicitis.

Appendix 3: Questionnaire form template

Questionnaire form	
Name of hospital:	City:
Sample collection date:	Sample code:
من شەهلە جەواد حسێن، خوێندکاری ماستەر لە زانکۆی کۆیە، بەشی بایۆلۆجی، توێژینەوەیەک ئەنجام دەدەم، ئایە ئامادەیت بەشێوەیەکی خۆبەخش بەشداربیت لە توێژینەوەکەمدا، بەمەرجێك کۆی تێجوی پشکنینەکان و دەرهاویشتەکان لەئەستۆی توئژەرە؟	بەڵێ ☐ نەخێر ☐
Name of patient	
Gender	
Age	
Occupation	
Marital status	
Place of residence	
Chronic illness	
Blood group	
Ultrasound	
Hematological parameters (WBC, Neutrophil, Lymphocyte, Eosinophil, PLT, MPV, RDW)	
CRP	
General Urine Examination	
Chief complain	
Onset and Duration of pain	
Body Temperature	
Nausea and Vomiting	

Appendix 4: Acceptance letter

Salahaddin University-Erbil
Division of Scientific Publications
Zanco Journal

زانکۆی سەلاحەدین-هەولێر
هۆبەی بڵاوکراوە زانستیەکان
گۆڤاری زانکۆ

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Dears:

Shahla J. Hussein / Department of Biology, Faculty of Science and Health, Koya University, koya KOY45, Kurdistan Region-F.R. Iraq.

Sarmad Raheem Kareem / Faculty of Medicine, Koya University, koya KOY45, Kurdistan Region-F.R. Iraq.

Acceptance of Research for Publication

Greetings…

As a result of review and revisions, we are pleased to inform you that, your following paper titled:

Demonstration of tissue and peripheral blood eosinophils in patients with acute appendicitis

was formally accepted for publication in one of the upcoming numbers of the **(Zanco Journal of Pure and Applied Sciences).**

Thank you for your contribution to our journal and we are looking forward to your future participation.

With our best regards...

Prof.Dr. Mustafa Saber Al- Attar
Editor-in-Chief

Prof. Dr. Asaad Hamid Ismail
Editor Secretary

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الخلاصة

التهاب الزائدة الدودية الحاد (AA) هو أكثر حالات الطوارئ الجراحية شيوعًا في منطقة البطن على مستوى العالم ، وتتطور معظم الحالات نتيجة انسداد التجويف الدودي. يعتمد حدوث التهاب الزائدة الدودية الحاد بشدة على العمر ويصل إلى ذروة الإصابة في سن المراهقة وأوائل العشرينات. يتم تشخيصه سريريًا بشكل أساسي بناءً على التاريخ المرضي, الفحص البدني, التصويرالطبي, و فحوصات مختبرية مساعدة ، ولكن يظل التحليل النسيجي هو المعيار الذهبي لتشخيص التهاب الزائدة الدودية الحاد.

الأكتشافات النسيجية في التهاب الزائدة الدودية الحاد هي وجود الخلايا النيوتروفيلية (العدلات) في الطبقة العضلية الملساء. ومع ذلك ، هناك نوع غير عادي من التهاب الزائدة الدودية, التهاب الزائدة الدودية الحاد الناجم عن الخلايا اليوزينية (الحمضات) (AEA) الذي يحدث فيه علامة مميزة مرضية وهي تسلل وتسرب الخلايا اليوزينية (الحمضات) الى الطبقة العضلية الملساء مع وجود وذمة تفصل الألياف العضلية دون وجود الخلايا النيوتروفيلية ويعتبر فرط الحساسية من النوع الأول هو المسؤول عن تطور هذا المرض لذلك ، كان الهدف من هذا البحث هو إيجاد طريقة بسيطة وسريعة لتشخيص شكل غير عادي من التهاب الزائدة الدودية الحاد الذي قد يتطلب علاجًا خاصًا بعد الجراحة.

أجريت دراسة مستقبلية في مستشفى شهيد د. خالد التعليمي في (مدينة كويسنجق / إقليم كردستان / العراق) ، تضمنت مجموعة عشوائية من 50 عينة زائدة دودية مستأصلة جراحيا خلال فترة ستة أشهر من 1 تشرين الثاني 2021 إلى 1 أيار 2022 من المرضى الذين تم ادخالهم في قسم الطوارئ و يشتبه في إصابتهم بالتهاب الزائدة الدودية الحاد على أساس التاريخ والفحص البدني والفحوصات المختبرية وفحص الموجات فوق الصوتية للبطن. تم أخذ عينات دم من جميع المرضى قبل اجراء الجراحة لتثبيت تعداد الدم الكامل CBC, بروتين سي التفاعلي C reactive protein و وجود الخلايا اليوزينية (الحمضات) في الدم المحيطي. بعد استئصال الزائدة الدودية ، تم فحص جميع العينات بشكل إجمالي (الوزن والأبعاد) وتم تصويرها. بعدها تم تثبيت العينة في الفورمالين بتركيز 10% وتركت حتى صباح اليوم الثاني حيث تمت معالجتها بالبارافين و الكحول وصبغت بواسطة صبغة هيماتوكسيلين ايوسين التقليدية, صبغة جيمسا, صبغة الكونغو الأحمر, وصبغة الكيمياء النسيجية المناعية.

في الدراسة الحالية كان هناك 28 مريضًا من الذكور (56٪) و 22 مريضة من الإناث (44٪). تراوح عمر المرضى من (4 إلى 43 سنة) بمتوسط (20.98) سنة.. ، وغالبية الحالات (60٪) تقع في الفئة العمرية (≤ 20) سنة. المقاطع النسيجية المصبوغة بصبغة الكيمياء النسيجية المناعية IHC (SE 1.05 ± mean 14.73) وصبغة الكونغو الأحمر Congo Red (SE 0.9540 ± mean3.50) أظهرت تعداد

أكثرللخلايا اليوزينية (الحمضات) وكانت النتائج ذات دلالة إحصائية عالية (P<0.001) مقارنة بالمقاطع النسيجية المصبوغة بصبغة الهيماتوكسيلين ايوسين التقليدية H&E (mean 4.082 ± 0.3769 SE) و صبغة جيمسا(mean 5.136 ± 0.3602 SE) MGG في حين أن صبغة ال IHC أظهرت تعداد خلايا يوزينية أعلى بشكل ملحوظ ($p < 0.05$) مقارنة بصبغة الكونغو الأحمر Congo Red. كانت هناك زيادة تدريجية في وزن الزائدة الدودية مع ازدياد العمر ولكن الأبعاد انخفضت بصورة ملحوظة. ايضا كانت الوسائل الفعلية لتعداد الدم الكامل (CBC) مختلفة بشكل كبير عن الوسائل النظرية ، ولكن كان هناك اختلاف غير مؤثر بين الجنسين في تعداد الدم الكامل CBC. على الرغم من وجود ارتباط وعلاقة سلبية بين تسلل وتسرب الخلايا اليوزينية (الحمضات) وتعداد خلايا الدم البيضاء WBC والتي كانت ذات دلالة إحصائية عالية ($P < 0.0019$) ولكن كان هناك ارتباط ايجابي بين تعداد الخلايا اليوزينية (الحمضات) في الأنسجة و الدم المحيطي وكانت العلاقة ذات دلالة إحصائية عالية ($P< 0.01$)

في الختام ، تمكنت هذه الدراسة و بشكل فعال جدا من تبيان وجود الخلايا اليوزينية في الأنسجة بأستعمال صبغة الكيمياء النسيجية المناعية IHC و صبغة الكونغو الأحمر Congo Red مقارنة مع صبغة الهيماتوكسيلين H&E و صبغة جيسما MGG بينما تمكنت صبغه الكيمياء النسيجية المناعية IHC من اظهار وجود الخلايا اليوزينية في الأنسجة بشكل أكثر من صبغة الكونغو الأحمر Congo Red وكان هناك ارتباط إيجابي بين وجود الخلايا اليوزينية (الحمضات) فى الأنسجة و في الدم المحيطي.

جمهورية العراق الفدرال
حكومة إقليم كوردستان
وزارة التعليم العالي والبحث العلمي
جامعة كوية

التهاب الزائدة الدودية الناتج عن الحبيبوم الحمضي,دراسة كيميائية نسيجية مناعية

رسالة مقدمة الى مجلس كلية العلوم والصحة في جامعة كوية وهي جزء من متطلبات نيل شهادة الماجستير في (اختصاص علوم الحياة)

من قبل

شهلة جواد حسين

بكالوريوس في علوم الحياة
كلية العلوم والصحة / جامعة كوية

بإشراف :

ا. م . د. سرمد رحيم كريم

١٤٤٤هجري

پوختە

هەوکردنی توندی ڕیخۆڵە کوێرە (AA) بریتیە لە باوترین حاڵەتی فریاگوزاری نەشتەرگەری سک لە جیهاندا، زۆربەی حاڵەتەکان لە ئەنجامی گیرانی ڕیخۆڵە کوێرە دروست دەبن. ڕوودانی هەوکردنی توندی ڕیخۆڵە کوێرە بە زۆری بەستراوە بە تەمەن و لە هەرزەکاران و سەرەتای بیستەکانی تەمەن دەگاتە لوتکە. بە شێوەیەکی سەرەکی، لە ڕووی کلینیکیەوە بە پشتبەستن بە مێژوو، پشکنینی جەستەیی، سۆنەرو وە تاقیکردنەوەی تاقیگەیی یارمەتیدەر دەستنیشان دەکرێت، بەڵام پشکنیکی هیستۆپاتۆلۆژی وەک پێوەرێکی زێڕین دەمێنێتەوە بۆ دەستنیشانکردنی هەوکردنی توندی ڕیخۆڵە کوێرە.

بوونی نیوترۆفیلەکان یەکێکە لە نیشانەکانی پشکنیکی هیستۆپاتۆلۆجی هەوکردنی توندی ڕیخۆڵە. بەڵام هەوکردنی توندی ئێۆزینۆفیلیکی ڕیخۆڵە کوێرە (AEA) کە جۆرێکی دەگمەنی هەوکردنی ڕیخۆڵە کوێرەیەو بریتیە لە دزەکردنی ئێۆزینۆفیل بۆ جینی (muscularis propria) وە جیابونەوەی ڕیشاڵی ماسولکەیی بەهۆی ئاوسان بەبێ بوونی دزەکردنی نیوترۆفیل وەک نیشانەی نەخۆشی . هەستیاری زۆری جۆری I بەرپرسە لە دروستبوونی نەخۆشییەکەی.

کەواتە، ئامانجی ئەم توێژینەوەیە بریتییە لە دۆزینەوەی ڕێگەیەکی سادە و خێرا بوو بۆ دەستنیشانکردنی جۆرێکی نائاسایی هەوکردنی توندی ڕیخۆڵە کوێرە کە ڕەنگە پێویستی بە چارەسەری تایبەتی دوای نەشتەرگەری هەبێت.

ئەم توێژینەوەیە لە نەخۆشخانەی فێرکاری شەهید د. خالید لە (شاری کۆیە/ هەرێمی کوردستان/ عێراق) ئەنجامدرا، کە بریتی بوو لە کۆکردنەوەی هەڕەمەکی 50 نمونەی لابردنی ڕیخۆڵە کوێرە لە ماوەی شەش مانگ لە 1ی تشرینی دووەمی. ٢٠٢١ تا ١ی ئایار٢٠٢٢. لەو نەخۆشانە وەرگیراوە لە بەشی فریاکەوتن کە گومانی هەوکردنی توندی ڕیخۆڵە کوێرەیان لەسەرە لەسەر بنەمای مێژوو، پشکنینی جەستەیی، لێکۆڵینەوە و سۆنەری سک. نمونەی خوێن پێش نەشتەرگەری لەم نەخۆشانە وەرگیرا بۆ پشکنینی (پێکهاتەکانی خوێن CRP, CBCوە ئێۆزینۆفیلەکان لە فیلمی خوێندا. دوای لابردنی ڕیخۆڵە کوێرە، هەموو نموونەکان بە شێوەیەکی بینینی پشکنینیان بۆ کرا (کێش، ڕەهەند و وێنەگرتن) دەستبەجێ لە 10% فۆرمالینی سروشتی بافەرکراودا بۆ ماوەی شەوێک پاشان پرۆسێس کران بە کهول و پارافین ئنجا ڕەنگ کرا بە H&E ڕۆتینی ، Giemsa، Congo Red وە IHC .

بەپێی ئەنجامەکان، لە توێژینەوەی ئێستادا ٢٨ نەخۆش لە ڕەگەزی نێر بوون (٥٦%)، و ٢٢ نەخۆش لە ڕەگەزی مێ بوون (٤٤%). تەمەنی نەخۆشەکان لە نێوان (4 بۆ 43 ساڵ) بوو، لەگەڵ (mean 20.98)

ساڵ، و زۆربەی حاڵەتەکان (60%) دەکەوێتە گروپی تەمەنی (≤20) ساڵدا. ئیۆزینۆفیلەکانی شانە زۆر کاریگەرانە (P< 0.0001) دەرکەوتن بە ڕەنگکردنی IHC (mean 14.73 ± 1.05 SE) و Congo Red((mean3.50 ± 0.9540 SE بە بەراورد لەگەڵ ڕەنگکردنی (mean 4.082 ± 0.3769 SE) H&Eو MGG (mean 5.136 ± 0.3602 SE) ، لە کاتێکدا IHC بە شێوەیەکی بەرچاو(p 0.05) >ژمارەی ئیۆزینۆفیلێکی بەرزتری بەدەستهێنا بە بەراورد بە Congo red. هەروها زیادبوونی بەرچاوی لە کێشی ڕیخۆڵە کوێرە لەگەڵ گروپی تەمەنیدا هەبوو، بەڵام ڕەهەندەکان بە شێوەیەکی بەرچاو کەمبوونەوە. جیاوازییەکی بەرچاو هەبوو لە نیوان (actual mean) وە (theoretical mean)ی پێکهاتەی خوێنی تەواو (CBC) ، بەڵام جیاوازی ڕەگەزی نەبوو لە پێکهاتەی خوێنی تەواو دا (CBC). هەرچەندە پەیوەندییەکی نەرێنی لە نێوان دزەکردنی ئیۆزینۆفیل و WBC دۆزرایەوە (P<0.0019) کە لە ڕووی ئامارییەوە زۆر بەرز بوو، بەڵام پەیوەندییەکی ڕاستەوخۆ لە نێوان ئیۆزینۆفیلی شانە و ئیۆزینۆفیلی خوێنیدا هەبوو(P<0.01) .

لە کۆتاییدا، ئەم توێژینەوەیە گەیشتە ئەو دەرئەنجامەی کە ئیۆزینۆفیلەکانی شانە زۆر کاریگەرانە بە ڕەنگکردنی IHC وCongo Red نیشاندرا بە بەراورد لەگەڵ ڕەنگکردنی H&E و MGG لە کاتێکدا ڕەنگکردنی IHC بە شێوەیەکی بەرچاو بەرزتر بوو بە بەراورد بە Congo Red و هەروها پەیوەندییەکی ئەرێنی لە نێوان ئیۆزینۆفیلی شانە و خوێنی بینرا.

كۆماری فیدراڵی عێراق
حکومەتی هەرێمی کوردستان
وەزارەتی خوێندنی باڵا و توێژینەوەی زانستی
زانکۆی کۆیە

هەوکردنی ڕیخۆڵە کوێرە بەهۆی ئیۆزینۆفیلکان, لێکۆڵینەویکی کیمیائی شانەی بەرگری

ماستەرنامەیەکە پێشکەشکراوە بە فاکەڵتی زانست و تەندروستی لە زانکۆی کۆیە وەك بەشێك لە پێداویستییەکانی بەدەستهێنانی بڕوانامەی ماستەر لە (بواری زیندە زانی)

لەلایەن

شهله جواد حسین

بەکالۆریۆس لە زیندە زانی
فاکەڵتی زانست و تەندروستی/ زانکۆی کۆیە

بە سەرپەرشتی:

پ. ی. د. سەرمەد رحیم کریم

٢٧٢٣ کوردی