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Isolation and Characterization of Bacteria Associated with Acute Appendicitis and Some Histopathological Changes

A dissertation submitted to Council of the College of Education Pure Sciences - Kerbala University, as a partial fulfillment of the requirements for the degree Doctorate of Philosophy in Biology-Zoology

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"Dedication"

I would like to dedicate this work to every holy drop of blood that fell down on this earth , to the souls of my parents and my family who were tired with me and supported me a lot at this stage. to every one wished me well.

Nada AL-karawi

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Nada AL-karawi

Summary

The current study included (135) samples , excluded only 35 patients with abdominal pain at any location and with no particular suspicion of appendicitis, other patients excluded were those that had peritonitis from ruptured appendix and those that had incidental appendectomy whereby appendix was removed during laparotomy for indication other than acute appendicitis , divided into two groups : group I , included (50) patients who suffered from fever, diarrhea , nausea, vomiting and abdominal pain which migrate to the right iliac fossa , to explain the presence of acute appendicitis , samples were collected from patients who were attending to Imam Hussein medical city in Holy Karbala during the period of January 2024- June 2024 . Patients samples included appendix and blood in compere with (50) blood samples collected from healthy (group II) that were identical to patients group but not suffered from any symptoms of acute appendicitis.

Current study demonstrates on demography pictures that include: age, sex and clinical feature. Our results showed that appendicitis effects all age group**,** samples collected from patients between (8 - 60) years old .The majority rate of appendicitis were recorded (16-30) years with a percentage 44% from the total cases , then (1-15) years with a percentage 40% , (31 - 45) years with 14% and the minority rate was 2 % which belongs to years (46 - 60) , also this study shows that appendicitis affects both sex with no significant differences between both groups but it effect males (54%) more than females
(46%). The present study recorded common clinical symptoms accompanying acute appendicitis such as: abdominal pain , vomiting

, nausea, fever and diarrhea 50(100%) , 38(76%) , 37(74%) , 13(26%) , 5(10%) respectively and show high significant difference between patients with acute appendicitis and associated symptoms.

Current study isolated types of bacteria habitation in human appendices and evaluating the sensitivity of some antibiotics against them, all samples showed positive growth . G+ bacteria were isolated at a lower rate (6%) than G− bacteria (94%) ,there where highly significant difference between G+ and G−bacteria (*p-value*= 0.00001**) . *Escherichia coli* which accounted for 29(58%) isolates, followed by *Klebsiella pneumoniae* isolates 8 (16 %) , 5(10%) isolates of *Psedomonas aeruginosa*, 2(4%) isolates of *Enterococcus faecalis*, 1(2%) isolates of *Enterobacter aerogenes*, 1(2%) isolates of *Salmonella typhi* and 1(2%) of *Proteus mirabilis* . *Staphylococcus aureus* 2(4%).Whereas *Staphylococcus epidermmidis* was accounted as 1(2%).

The antibiotics sensitivity test performed on all isolates; both Imipenem and Amikacin were effective antibiotics against G− bacteria; meanwhile, Vancomycin , Penicillin , Rifampicin and Ticarcillin were effective against G+bacteria, all species of gram positive bacteria had be isolated were resistance to Benzyl penicillin and Oxacillin, most G−isolates were resistance to Ticarcillin and Piperacillin 92.8%. G−isolates showed to have more resistance than G+ isolates.

The hematological results showed there were a high significant elevation in the count of total white blood cell (WBC) and neutrophils in the acute appendicitis patients group compared to the healthy group , there was non- significances decrease in count of the platelets in acute appendicitis patients group compared to the healthy group. Current

study showed a significant decrease in the lymphocyte, RBCs, and HCT count in patients compare with the healthy .

The serological results demonstrated that there were high significant difference for Procalcitonin , Interleukin-8(IL-8) , Interleukin-10(IL-10) , Interleukin IL-1Ra , Monocyte chemotactic protein- 1 (MCP-1) , Macrophage inflammatory protein (MIP)-1α (*p-value* = 0.0001^{**}) in the serum levels of appendicitis group and healthy Healthy group

Current study showed histopathological changes which included macroscopic and microscopic examination of all appendices . Some of these appendices were enlarged and tissue surrounded by vesicles , and some have fibrous and ulcerated walls of varying colors. The tissue changes showed changes in the histological structure of appendix, represented by expansion of its lumen and congestion of the venous blood vessels in the mucosa and sub mucosa , and increasing amount of lymphatic tissue spread through the layers of appendix , lymphoid follicular hyperplasia , infiltration of inflammatory cells that spread through the layers of appendix.

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Figures	Items
AA	Acute appendicitis
AK	Amikacin
AST	Antibiotics susceptibility testing
AZT	Aztreonam
BENPEN	Benzylpenicillin
BHI	Brain heart infusion
CBC	Complete blood cells
CEF	Cefepime
CIP	Ciprofloxacin
CLIN	Clindamycin
CRP	C-reactive protein
CTX	Cefataxime
EDTA	Ethylene Diamine Tetra Acetic Acid
ERY	Erythromycin
FUS	Fusidic Acid
GEN	Gentamicin
H&E	Hematoxylin and Eosin
HCT	Hematocrit
ID	Identification card
IL-1	Interleukin-1
IL-10	Interleukin-10

IL-1RA	Interleukin-1 receptor antagonist
IL-8	Interleukin 8
IM	Imipenem
LPS	Lipopolysaccharides
LYM	lymphocytes
MBC	Minimum bactericidal concentration
MCP-1	Monocyte chemo attractant protein 1
ME	Meropenem
MIC	Minimum inhibitory concentration
MIN	Minocycline
MIP-1 α	Macrophage inflammatory protein-1Alpha
MOX	Moxifloxacin
N.S	Normal saline solution
NK	Natural killer cells
OXA	Oxacillin
PBP	Penicillin binding protein
PCT	Procalcitonin
PCT	Plateletcrit
PIP	Piperacillin
PLT	Platelet
RBC	Red blood cell
RIM	Rifampicin
SIgA	Immunoglobulin secretory type A
SXT	Trimethoprim /Sulfamethoxazole
TCC	Ticarcillin/Clavulanic Acid
T-Cell	Lymphocyte that differentiate in the thymus

TEC	Teicoplanin
TET	Tetracycline
TIC	Ticarcillin
TIG	Tigecycline
TLRs	Toll like receptors
TOB	Tobramycin
TZP	Piperacillin /Tazobactam
UK	United Kingdom
VAN	Vancomycin
VIP	Vasoactive intestinal polypeptide
WBC	White blood cells
Zyvox	Linezolid

CHAPTER ONE

INTRODUCTION

1.1 Introduction

Acute appendicitis (AA) is one of the common causes in the emergency unit due to the abdominal pain and appendectomy is one of the most surgical procedure performed in the world (Benabbas *et al*., 2017), and it is a common gastrointestinal disease that affects all ages, with the highest incidence in the children and young adults. The incidence varies depending on race, gender, age, obesity, the time of year and country, especially in underdeveloped countries due to poor hygienic conditions, with the incidence being lower in populations that eats a high- fiber diet (Ferris *et al*., 2017). Approximately with an average lifetime risk of 7–8% (Bhangu *et al*., 2015).

The vermiform appendix is a tubular structure (blind tube, worm like structure) on the cecum's posterior wall, 1.7 cm from the ileum valve, where the colon's meets at the cecum and serves as a reservoir for intestinal microbial flora; it also plays a role in the correct development of lymphatic tissue in the stomach (Radenković *et al.*, 2021). Histologically, the four typical sub layers that form the appendix wall starting from the inside are mucosa, sub mucosa, muscularis external and serosa (Bandyopadhyay *et al*., 2022) .The presence of lymph nodes in the sub mucosa suggests their role in the immune system, appendix was previously considered a vestigial organ in humans, but this idea is incorrect since its role as a neuroendocrine and immunological factor has been demonstrated (Dimberg *et al.*, 2020). There is a neural proliferation in appendix associated with increased immunologic reaction to the vasoactive intestinal polypeptide (VIP) and to the peptide substance-P in the patient with clinical diagnosis of acute appendicitis (AA) without inflammatory reaction (Barroso *et al.*, 2015). Increase in these mediators in appendix can cause pain in the right iliac fossa in the presence of acute

appendicitis (Alshammari *et al*., 2020). Acute appendicitis is a high incidence disease that requires rapid and accurate diagnosis combined with laboratory investigations supplement by selectively focused imaging , delayed diagnosis of acute appendicitis could lead to complications such us perforated appendix, peritonitis, sepsis, increased morbidity and mortality; however its diagnosis remains challenging (Li *et al.*, 2021).

Two important components are attributed to the pathogenesis of acute appendicitis: obstruction and infection by pathogenic event in the most patient with acute appendicitis, the latter believed due to luminal obstruction which may result from a variety of causes, i.e., lymphoid hyperplasia, fecalith , foreign bodies and parasite (Shahmoradi *et al*., 2021). Bacterial infection is believed to be crucial for inflammation of appendix (Takahashi, *et al.*,2021). It is evident that some bacteria can pass through the intact appendix wall before perforation, while progressive infection and subsequent tissue damage with necrosis allow the bacteria to enter the abdominal cavity (Sakellaris *et al.,* 2023).

The gold-standard treatment for appendicitis was appendectomy, which was done by either an open or laparoscopic approach. (Teng *et al*., 2021). Appendectomies represented the most common emergency surgical procedure and the accurate diagnosis of appendicitis remains difficult and delays in the diagnosis and surgery might lead to many complications (Gignoux *et al.*,2018).

Acute appendicitis is connected to the production of cytokines, the cytokine profile varies depending on how long the infection has been present and whether or not it has had any repercussions (Wheeler, *et al.*, 2021) .

Studies on acute appendicitis are few, as studies related to identifying bacterial isolates associated with acute appendicitis and their role in increasing the complications of inflammation are limited, so the following study was conducted :

1. Isolation and identification of bacterial profiles are associated with appendicitis.

2. Study of the antimicrobial susceptibility of the isolated bacteria to different antibiotics.

3. Investigation and comparision the level of some immunological factor (IL-8 , IL-10, IL-1Ra , IL MCP-1 , IL MIP -1α & Procalcitonin in peripheral blood of patients with acute appendicitis and healthy control and identified the significant level to be used as a diagnostic test for acute appendicitis.

4. Hematological study of some cell blood counts test as: white blood cells count (WBC), lymphocyte count , red blood cells count (RBC) , hematocrit (HCT) , platelets count (PLT) and neutrophils.

5. Histopathological evaluations of the inflamed appendix to confirm acute appendicitis.

CHAPTER TWO

LITERATURES REVIEW

2.1 Anatomical and Histological Structure of Appendix

The appendix is the only organ in the body that does not have a fixed anatomical position. It gets its name from its worm-like appearance. It is located in the right lower quadrant of the abdomen (Jagdish & Ashoka, 2018). The position varies from person to person (M. A. Rahman *et al.*, 2019). It has been suggested that the location of the appendix is influenced by age, sex, race, and other demographic characteristics (Zacharzewska-Gondek *et al.,* 2018). The appendix is a worm-like tube filled with lymphoid follicles (Mohammadi *et al.*, 2017). It has a thick wall and a limited lumen because there are many lymphoid follicles in its wall (Rahman & Karim, 2019). One crucial component of this structure at an early age is the formation of large and numerous lymphoid follicles in both the mucosa and sub mucosa layers (Salih & Abdullah, 2020). The appendix has four coats that are visible from the outside inward: serous, muscular, sub mucous, and mucous (Bornali & Sekhar, 2016). Because lymphoid follicles can be seen in lamina propria and sub mucosa of the appendiceal wall, we can infer that quantity, existence, and function of cells in these layers differ between the colon and the appendix (Kooij *et al.,* 2016). A triangular fold, called the mesoappendix, connects the appendix to the lower portion of the ileal mesentery. The ileocolic artery is called appendicular artery , supplies blood to the organ through a free border in the mesoappendix and is a section of the colon with a free tip and the ability to be retrocecal, subcecal, pre postileal or pelvic. with a base attached to the posterolateral surface of caecum just below the ileocecal junction. Surgeons need to know the specific anatomical location of the appendix to quickly identify and treat acute appendicitis (Shaikh & Gurukkal, 2018). The appendix has several characteristic features and different cells. The various layers of the appendix include a

sub mucosa with an external muscularis and serosa located externally, surrounded by an internal mucous membrane lined with columns of enterocytes, goblet cells, enteroendocrine cells and Paneth cells. (Bandyopadhyay *et al.*, 2022). It is (2–15) cm long and is most commonly seen intraperitoneally, retrocecally (65%), or in the pelvis (30%) (Jagdish &Ashoka, 2018). The length and location of the appendix could alter the clinical presentation of appendicitis and its susceptibility to acute inflammation (Khatun, Thakur, & Shah, 2019). The appendix is most likely a specialized tissue and is neither degenerated nor atrophied due to its extensive vascularization and histological differentiation (Sarkar *et al.,* 2015). The lymphatic tissue of the appendix consists of several lymphoid follicles containing B lymphocytes and a modest number of T lymphocytes, as well as macrophages and dendritic cells. According to some authors, The enteric nervous system may play a role in controlling the immunological activities of gut associated lymphoid tissue, including the appendix (Radenković *et al.*,2021).

1) **The mucous membrane consists of three layers that run from the inside to the outside**

- The mucosal epithelium consists of simple columnar epithelium, the intestinal glands (crypts of Lieberkühn) embedded in the surface epithelium, which are mainly lined by goblet cells, and about absorptive cells (Girard *et al*., 2018). In the lower part of the glands, there are some paneth cells, which produce antibiotics peptides, and numerous endocrine cells (O'Dowd *et al*., 2020). These crypts are irregular in shape and their walls are composed of a small number of argentaffin cells. However, between the crypts and the muscularis mucosae , there are neuroendocrine complexes that represent part of the parasympathetic nervous system (Soybel, 2008).

- The lamina propria is formed by loose connective tissue (Co. T), which is characterized by the presence of a mass of lymphoid tissue (Girard *et al*., 2018).

- The muscularis mucosa consists of a thin layer of smooth muscle that separates the mucosa from the loose vascular sub mucosa beneath ,the muscle mucosa is influenced by the lymphocytes surrounding the follicles (Vitetta *et al.*, 2019).

2) The sub mucosa is a relatively thick layer rich in blood and lymphatic vessels and nerves and housed in loose connective tissue (Kahai *et al*., 2020).

3) The muscularis externa, this sub layer, consists of two complete muscle layers : an internal circular smooth muscle located around the appendix and an external longitudinal smooth muscle (Standring, 2016).

4) The serosal layer (the visceral layer of the peritoneum) contains the

major blood vessels and merges with the serosa of the mesoappendix (George *et al.*, 2007).

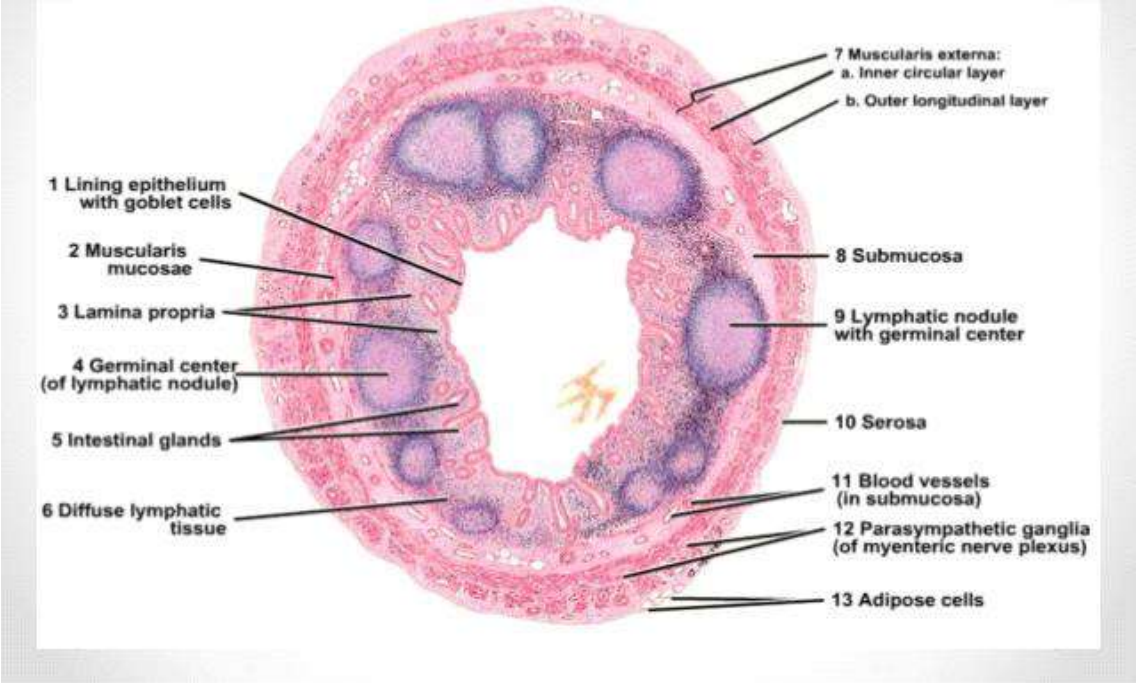


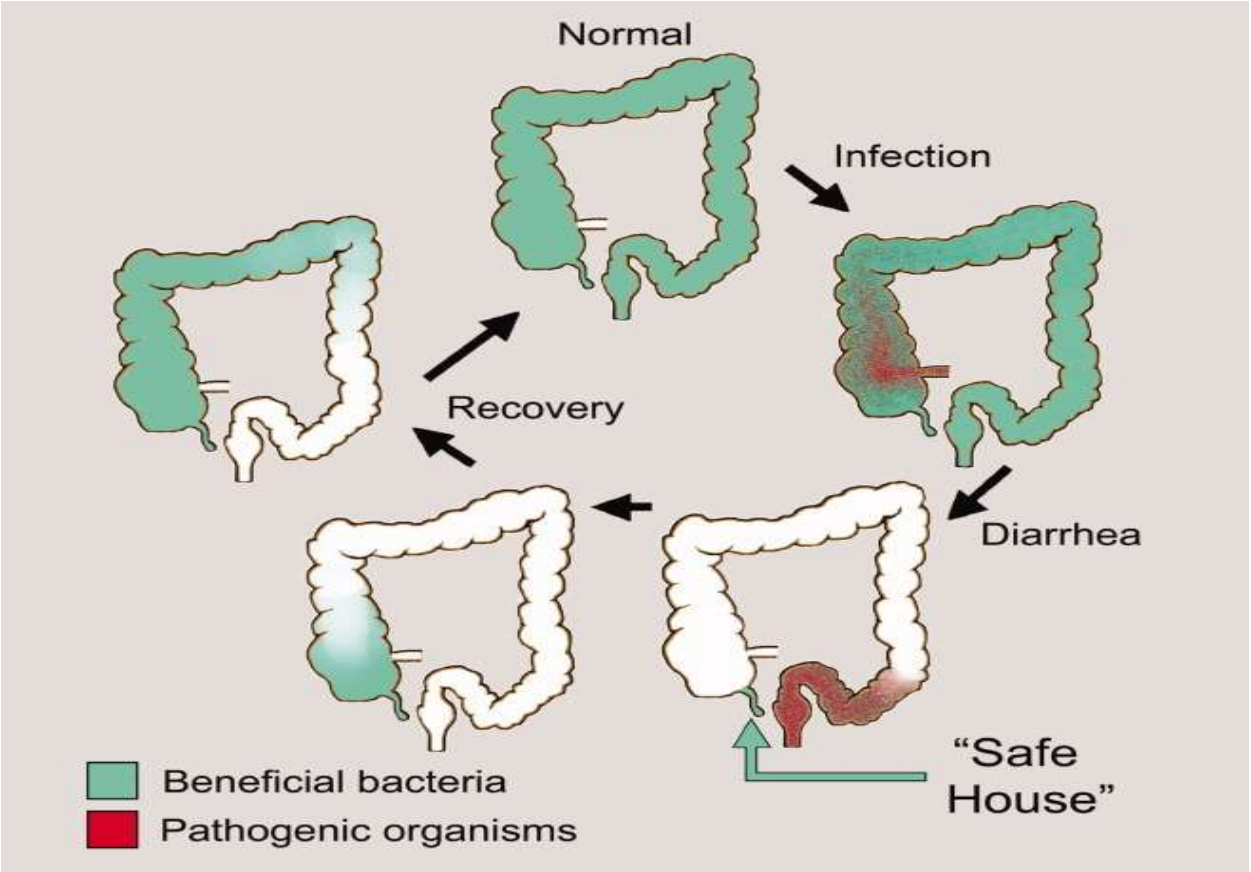
Fig. (2.1) Cross section in a normal or healthy human appendix demonstrates the conventional sub-layers of the GIT (Manzoor, 2015).

2.2 Appendix function

From its identification over 400 years ago until the 21st century, the function of the appendix in humans was not known, with numerous. evidence from medicine suggesting that there was no function. However, a major obstacle to understanding appendix was overcome in 2003 when it discovered that the immune system supports the growth of beneficial bacteria in the gut of mammals (Bollinger,*et al.,* 2003). This important change in thinking enabled the conclusion that the human appendix is a well-adapted (safe-house) for the maintenance of the mutualistic gut bacteria . An apparent function of the identified human appendix is well adapted to facilitate the maintenance of biofilms (adherent bacterial colonies growing in an extracellular matrix) containing a mutualistic intestinal flora. The appendix appears to play an important role in the intestinal microbial ecology (Prestwich *et al.*,

2018).In addition, biofilms were found in the epithelial lining of the mucosal epithelium . The concentration of biofilms in the appendix has been found to be higher than in any other area of the intestine (Arjomand *et al.*, 2023). The structure of the appendix is expected to enhance the protective role that biofilm formation has for commensal microorganisms. Additionally, it has been shown that this type of biofilm growth in the appendix not only increases the typical intestinal microorganisms, but also prevents harmful organisms from adhering to the surface of the large intestine (Arias *et al.*, 2021). A multitude of experimental findings suggest that secretory IgA (sIgA) and mucin, two of the body's most widely generated macromolecules, do both support the development of biofilms on the intestinal epithelium (Liu, *et al.*, 2021). Both the mucin and sIgA assist in the formation of biofilm by promoting the adhesive growth of the agglutinated gut flora, (Everett *et al.*, 2004) . While sIgA stimulates the agglutination of bacteria, mucin in turn binds these bacteria to the mucus layer. Overall, the appendix shows a high density of mucin and sIgA, which are produced by B cells in the mucous membrane. Therefore, the outer layer of loose mucus of the appendix has a promicrobiotic environment, which in turn supports its function as a "safe house" (Bollinger *et al.*, 2007). Consequently, the acceptable conclusion is that the appendix may serve as a "safe house" for commensal bacteria. This reservoir of intestinal flora could serve to repopulate the digestive system after an infection such as dysentery or cholera (Everett *et al.*, 2004; Paterson-Brown, 2012). Figure (2.2) shows the cycle of infection, purging of the gut by diarrhea and re inoculation of the gut by commensal organisms from the appendix. The appendix also performs some functions related to the gastrointestinal tract, the main function being immunological in nature (Girard *et al.*, 2018). The lymphatic tissue of the appendix develops during the first year of life,

Some atrophy is observed during the teen , but the appendix has an immunological function throughout life, although with an activity that gradually declining activity (Kooij *et al.*, 2016). It has also been stated that appendix acts like tonsil , as the tonsil protects upper alimentary tract from bacteria, the appendix also protects the small intestine from the bacteria present in the large intestine (Marniok, *et al.*, 2004; Ahmed, *et al.*, 2007). Mesenchymal stem cells are cells that can be produced in the appendix during infancy and old age, as demonstrated by research by De Coppi and colleagues. These cells have the potential to become myoblasts, osteoblasts, and lipoblasts , according to the stimulus. They came to the conclusion that the appendix serves as a storehouse for stem cells that can mend intestinal damage at any time in a person's life (De Coopi, *et al.*, 2006).



(Fig-2.2)The cycle of infection purging of the gut by diarrhea and re inoculation of the gut by commensal organisms from appendix (Laurin, *et al.*, 2011)

2.3 Appendicitis

2.3.1.Acute Appendicitis(AA)

Acute appendicitis is one of the most common causes of abdominal pain requiring surgical intervention to exclude suspected complications (Akbulut *et al.,* 2020).Approximately 7% of the population develops acute appendicitis during their lifetime ((Téoule *et al.,* 2020). The etiology of acute appendicitis can vary greatly (Al-Saeedi & Al-Nasiri, 2019).The most important factor is lumen obstruction; The most common cause of obstruction is said to be fecolith (Majed and Al Bakri, 1984). However, a foreign body may be present (Sharma *et al.,* 2017). Appendicitis occurs when cellular capacity is reduced and bulk diet is reduced due to excessive protein intake (Myageri *et al.,* 2019). Although the existence of chronic appendicitis is controversial, most physicians have treated patients with acute abdominal pain that resolved with appendectomy. In some cases, the history consists of a short-term pain in the lower right abdomen and actually indicates chronic changes associated with recurrent inflammation and fibrosis. Some surgeons have suggested that detecting that the appendix is not filled or partially filled is different from an enema or computer tomography and the failure of contrast to drain from the appendix after several days following contrast administration, It indicates chronic or recurrent appendicitis in a patient with chronic abdominal pain. However, the patient with chronic abdominal pain should be evaluated before surgery (Kim *et al.*, 2016).

2.4 Incidence

2.4.1 Age Incidence

Appendicitis is rarely encountered before the age of two years. It is unusual encountered the age of one year and more common in childhood and adolescence. The highest incidence is in the 20 to 30 years, after which there is a gradual decline, but no age is excluded (Pathan *et al.*, 2018; Sarkar, 2015).

2.4.2. Sex Incidence

While the sex ratio in acute appendicitis is approximately 1:1 in males before puberty, the male/female ratio increases to 2:1 between the ages of 15 and 25. This ratio gradually decreases until the genders become equal again . While the incidence of appendicitis is 1.4 times higher in men than in women, the incidence of primary appendectomy is similar in both genders (Sarla , 2018). Primatesta and Coldacre (1994) examined the statistical and temporal characteristics of emergency appendectomy according to age and sex rate and divided appendectomy into three categories.

1. Emergency appendectomy for acute appendicitis **is** more common in the males that in females peaked in 10-19 year age group and declined overtime.

2.Emergeney appendectomy as the main operation without appendicitis recorded a diagnosis was more common in women than men (Female male ratio 1.9:1) peaked at the age 15-17 years and did not decline overtime.

3. Incidental appendectomy with other operations but without appendicitis was more common in women (female male ratio 3:1). Peaked al older ages than the first two groups and decreased over time. significantly overt

2.5 .Etiology of appendicitis

2.5.1.Obstruction

Obstruction of appendix lumen has been widely held to be important and some forms of luminal obstruction either by faecolith or structure found in the majority of cases. Obstruction of the appendiceal orifice by the tumor, particularly carcinoma of the caecum is an occasional causes of acute appendicitis in middle age and elderly. Appendicitis is clearly associated with bacterial proliferation within appendix, no single organism is usual (Childers *et al*., 2019).

2.5.2. Bacterial Appendicitis

Bacterial infection appears to play an important role in appendicitis. It occurs when bacteria from the intestinal tract grow on the closed appendix, causing swelling and infection. If the infection progresses, the swelling can cut off blood flow to the appendix, which can lead to death. The walls of the appendage may rupture, causing the appendix to rupture. peritonitis (Shelton *et al.*, 2003). There are many types of bacteria that contribute to appendicitis. Aerobic and anaerobic bacteria have been identified in appendicitis, with the most common anaerobe being *Bacteroid fragilis*. The importance of beta-hemolytic streptococci in appendicitis has been studied by (Jakobsen *et al*., 1987) reported that only six cases were identified as streptococci. (Okoro ,1988) reported that *Yersinia enterocolitica* constitutes 13.8% of the pathogens responsible for appendicitis. A retrospective study by (Guasco *et al* ., 1991) on the microbiology of abdominal pus due to acute appendicitis or peritonitis found that 45 samples (84.4%) were more positive for bacterial diagnosis with polymicrobials (76.3%) than others. Species represented are *E.coli* (28.4%), *Bacteroid fragilis* (78%), *Streptococcus milleri* (7.8%) *and Ps. aeruginosa* (3.9%) Polymicrobial is mainly represented by

Enterobacteriaceae *and Streptococcus* spp. A study on inflamed appendices revealed mixed infections , with the most common organisms being *E.coli, Enterococci*, anaerobic *streptococci* and *Clostridum prfringenes* (Rains & Capper 1988). . A bacteriological study involving 110 pediatric emergency cases was reported by (Barker ,1985). However, during appendicitis, anaerobic bacteria have been observed to colonize the appendix and ileum (Bennion *et al.*, 1990; Park homenlco *et al.*, 1991). They examined the virulence properties of bacteria in the appendix of children with appendicitis. (Baron *et al.*,1992) reported that bacteria obtained from appendix specimens of patients with acute appendicitis were fewer in number and less specific in terms of species compared to specimens from patients with severe disease. Some appendicitis has been found to be caused by *Salmonella* species. (Kazlow *et al.*, 1991). Additionally, (Parkhomenko ,1998) isolated *Yersinia enterocolitica a*nd *Citrobacter frundii* from some appendicitis cases.

2.6 Risk Factors for Acute Appendicitis

According to current knowledge, there is no direct cause of appendicitis. Although some things seem to be somehow related to appendicitis or make infection more likely, they do not always cause infection. (Sharma and Gupta, 2004). The following factors have been suggested to treat acute appendicitis in various areas, including high fiber diet, good carbohydrates, amebiasis, viral gastroenteritis and bacterial gastroenteritis .According to current knowledge, there is no direct cause of appendicitis. Although some things seem to be associated with
appendicitis or seem to make it more likely to cause infection, they do not always cause infection (Sharma and Gupta, 2004).

2.6.1. Diet

Supporting evidence from economical and geographical studies has shown that changes resulting from a high-residue diet are associated with an increased incidence of appendicitis (Ahmed *et al* 2018). Short (1920) showed that when people from the African region migrated to urban areas, appendicitis was more common. This may be due to departure from a simple fiber-rich diet to a meat-rich diet, but this may not be the entire explanation, as acute appendicitis can occur in both vegetarians and breast-feeding children (Nicola *et al*., 2021). Reducing dietary fiber not only slows fecal contamination, but also results in hard, solid stools that are significantly smaller in size and more prone to stool formation. This leads to high intra luminal pressure (Soliman, 2019). Urban black have been reported to be free of appendicitis **(**Siraj *et al*., 2020).

2.6.2 Genetic Factors

Understanding the genetic factors that influence Appendicitis could lead to a better understanding of the disease etiology (Orlova *et al.*, 2019).Other studies have shown that most people with appendicitis have close relatives (brothers and sisters). parent or both not seen in a control group underwent abdominal operations other appendectomy. The explanation for this family trend is unclear, but possible causes may include dietary habits , a genetic difference in resistance to bacterial infections or the inheritance of the fibrous tissue of peritoneum which kinks the base of appendix (Fig.2-3) , therefore partially occluding the lumen and thereby predisposing to appendicitis (Simó Alari *et al*., 2017). From the evidence sometimes found today, it can be said that in some cases certain genetic factors play an etiological role. In (1990) Blandino *et al.* demonstrated the role of an

important gene. A follow-up study from the same group examining three generations of families using a small-scale analysis of variance was supplemented by a multivariate model. There is no definitive evidence to support a dominant gene, but it cannot be ruled out that a rare gene is the cause of some cases. Specific investigations into genetic and environmental factors in this serious disease appear valuable, but at present a positive family history of appendicitis may be associated with other symptoms leading to a clinical diagnosis of appendicitis. (Yaseen *et al*., 2018).

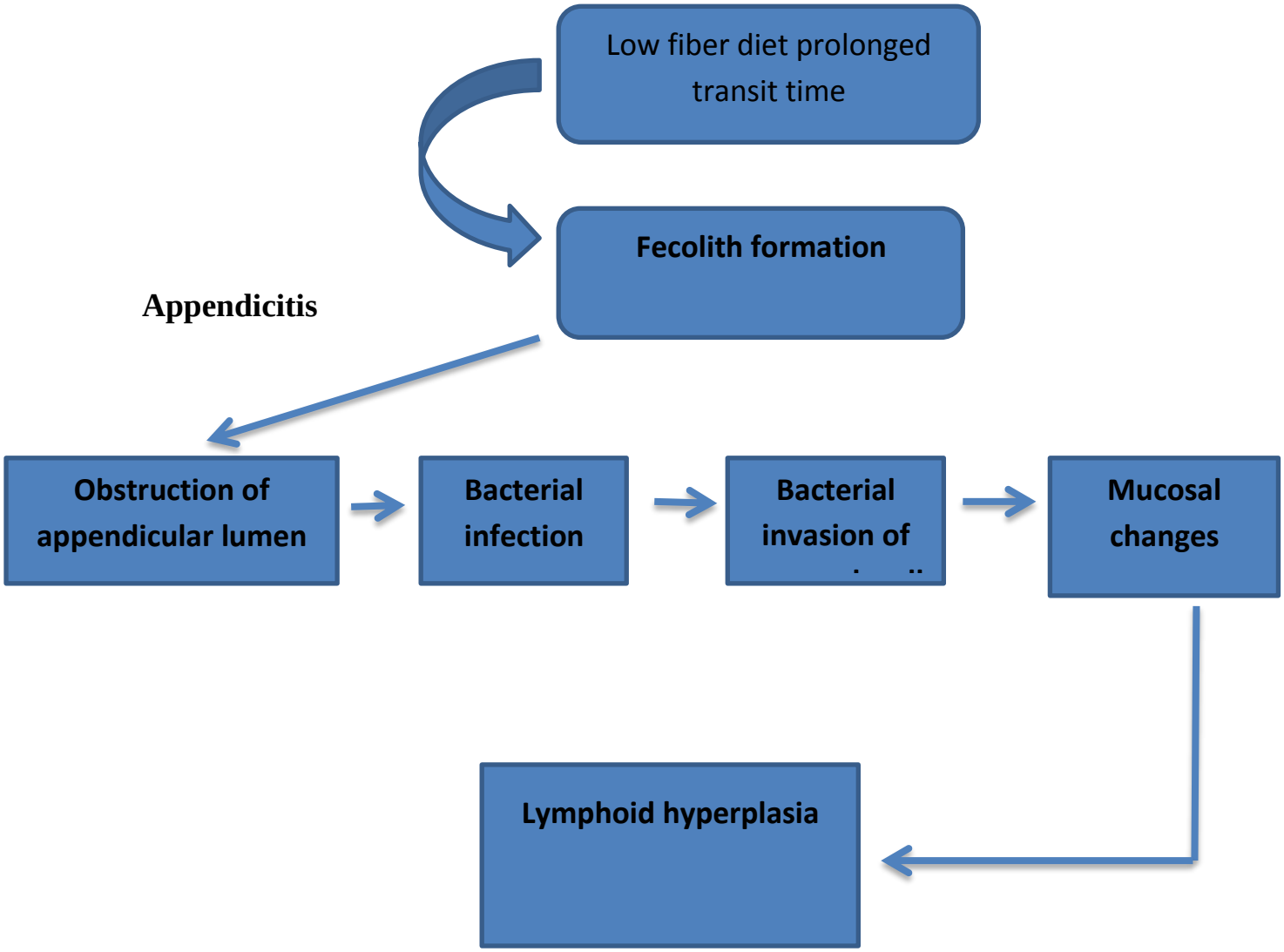


Fig. (2.3) Different theories for the etiology of appendicitis

2.7 Bacterial species isolated from appendicitis

2.7.1 *Escherichia coli*

Gram-negative bacilli, some species have both pili and antigenic antigens (CFA/I, CFA/II, CFA/III). Pili are responsible for binding to bases and play an important role in pathogenesis (Pakbin *et al.* 2021). *E. coli* capsule expresses K antigen, which helps bacteria attach to epithelial cells (Arredondo *et al* ., 2023). In addition to virulence factors, *E. coli* can also produce α- and β-hemolysins (Nielubowicz *et al.*,2010) . Colonization of the gut by virulent *E coli* type 1c- fimbriated, P - fimbriated O_{25}: K5: H1 serotype lead to perquisite for development of appendicitis, the producing of green metallic sheen of colonies EMB the specific diagnostic character for this species, agar is the specific diagnostic character for this species.

2.7.2 *Klebsiella* spp.

Klebsiella spp. are non-motile gram-negative bacilli characterized by the formation of long , highly mucoid colonies. The virulence of *Klebsiella* is not well understood but the anti-phagocytosis capsule plays a role in the infection by preventing the phagocytosis. It is believed to be aerobactin, an iron binding protein and production of β- lactamase enzyme which is involved in pathogenesis and antibiotic resistance (Paczosa *et al* .,2016).

2.7.3 *Proteus* spp.

This organism is characterized by active motility, which h results in what it called swarming phenomena , it can cause a disease only when leaving the intestine and it produces the urease enzyme. Endotoxins released during this process may play an important role in bacterial virulence (Chakkour *et al.*,2024).

2.7.4 *Enterobacter* spp.

Gram negative bacilli has many virulence factor, small capsule and siderophore production contribute to infection and they can also produce enzymes and some endotoxins that play important roles in virulence and antibiotic resistance (Klebba *et al.,*2021). *Enterobacter* species can produce antagonistic substances with antibacterial activity against many bacteria except *Acinetobacter* and *Pseudomonas* (Davin *et al.,* 2019).

2.7.5 *Salmonella* spp.

It is gram negative bacilli, motile and peritrichous flagella. *Salmonella* spp can penetrate the intestinal mucosa and cause intestinal infections that can eventually lead to typhoid symptoms. Capsules of these organisms can cause serious complications in immunosuppressed individuals (Lamichhane *et al.,* 2024).

2.7.6 *Pseudomonas aeruginosa*

It is gram negative and has many virulence factors that are important in causing disease. These include capsule which associates in adherence and effectively protect the bacteria from phagocytosis. The production of extra cellular protease , hemolysin and cytotoxins play important roles in virulence as well as siderophore that support the growth of the organism . The soluble and media diffusible pigments produced by this organism are important characteristics to diagnose *Ps. aeruginosa* (Macfaddin, 2000).

2.7.7. *Staphylococcus aureus*

The pathogenicity of *staphylococci* is contributed in blood hemolysis, plasma coagulation, and the production of extracellular enzymes and toxins and *Staph aureus* has a polysaccharide capsule with a cell wall composed of peptidoglycan and teichoic acid, which prevents lysis by

osmotic condition and aid the bacteria to attach mucosal surface, helps bacteria adhere to the mucosal surface.

2.7.8. *Staphylococcus epidermides*

Staphylococci are gram positive bacteria containing a series of opportunistic pathogenic species (Otto, 2004). Among these, *Staphylococcus epidermidis* is the causative agent of hospital infections and medical device infections (Vuong and Otto, 2002). Normally, *S. epidermidis* is harmless and is found as normal flora on the oropharynx, skin and vagina (Sastry and Bhat, 2016). *S. epidermidis* forms white colonies on blood agar , coagulase negative, catalase positive and does not ferment mannitol. It tolerates salt, survives drying, and is highly resistant to antibiotics. This bacteria is transmitted through personal contact or contact with infected patients and hospital staff. The ability to produce slime is an important factor in bacterial virulence .*S. epidermidis* causes infection by adhering to the surface of intravenous plastic catheters and prosthetic devices. Slime also inhibits the action of lymphocytes and neutrophils. *S. epidermidis* is an important agent of hospital acquired infection. It causes endocarditis with prosthetic valves, osteomyelitis, sepsis in neonates and wound infections (Parija , 2012).

2.7.9. *Enterococcus faecalis*

Enterococci are gram positive, spherical, oval or coccobacilli arranged in pairs and short chains and most of species are non-capsulated and non-motile (Parija, 2012). There are at least 47 species of enterococci but less than one-third of them are associated with human diseases. *Enterococcus faecalis* is the most common and causes 85-90% of enterococcal infections , *E. faecium* causes 5-10% growth in the temperature range of 35-37°C (Carroll, *et al.*, 2016). Colonies in the blood medium are 1-2 mm in diameter and are alpha-hemolytic in fact ,they are not hemolytic ; the

formation of alpha-hemolysis is due to the activation of the peroxidase enzyme instead of hemolysins .They produce small, magenta colored colonies on MacConkey agar. However, these enterococci showed greater specificity than the streptococci isolated (Parija , 2012). The term "*Enterococcus*" is derived from their presence in the intestinal tract and that many biochemical and cultural features reflect that habitat. These include the ability to grow in the presence of high concentrations of bile salt and sodium chloride (Ryan and Ray, 2014). The *Enterococci* are usually found in gastrointestinal and genital tract of human and animals Enterococci are nonpathogenic but are now emerging as important agent of nosocomial infection. They cause urinary tract infections, especially in hospitalized patients, Indwelling catheter and urinary tract instrumentation are important predisposing factors They are most often isolated from cases of wound infections particularly intra-abdominal. They also cause infection of the bile duct, bacteremia, and endocarditis (Liu *et al.*,2024).

2.8. Antibiotics

Antibiotics are secondary metabolites produced by various organisms active against bacteria and the most important form of antibacterial agent for fighting bacterial infection . Antibiotic medications are broadly used in the treatment and prevention of many infections. They may either kill or inhibit growth of bacteria (Kang and Park, 2015). While several antibiotics can completely kill other bacteria ,these are called bactericidal and some of them able to inhibit bacterial growth, those are called bacteriostatic (Holten and Onusko, 2000). The extreme use of antibiotics has led to development of some resistant bacterial species, as it must be used according to medical instructions or need to take it in order to avoid and decrease the emergence of the antibiotics resistance (Le Page *et al.*, 2019

2.9 The Immunology of Appendicitis

Inflammation is primarily generated in response to pathogens or tissue damage. The inflammatory process increases the response from innate immune cells to contain and attempt to destroy or reduce a pathogen or to repair damaged tissues . It also engages adaptive arm of immunity to prepare it for antigen presentation. pathogen clearance and the tissue regeneration (Gong *et al.*,2020) The classical indication of inflammation are redness , heat, pain , swelling, and loss of function. It is triggered by pathogen associated molecular patterns or damage associated molecular pattern which activate various pattern recognition receptors such as toll like receptors (TLRs), C-type lectin receptors ,nucleotide-binding and oligomerization domain (NOD) like receptors (NLRs), or RIG-I-like receptors (Takeuchi *et al.*,2010) Most of these receptors are found on the innate immune cells such as macrophages, monocytes, neutrophils, and dendritic cells (Jang *et al.*,2015). These receptors do not have incredible target recognition adaptability of the adaptive immune system but do recognize highly conserved components of pathogen and markers of cellular damage (Akira *et al.*,2001). When activated these cells receptors initiate a cascade of signals that can trigger several responses, one of these is the production of inflammatory mediator which further drive the inflammatory response, Macrophages and T helper cells (Th) are widely recognized as the major producers of many cytokines (Zhang *et al.*,2007). Macrophages are particularly well suited to be the first line of cytokine production due to their prevalence in almost every tissue in the body (Kakar *et al.*,2020). The diagnostic role of some inflammatory variables depend on the etiology and pathogenesis of AA (Yoon *et al.*,2002). AA arises from initial luminal obstruction of appendix (Hayes *et al.*,2004). This results in local edema secondary to impaired blood and

lymphatic flow. Soon the bacterial barrier function of the appendicular epithelium fail and bacterial invasion into the sub mucosal layers occur (Lamps *et al*,.2008). The presence of bacteria in these areas result in the activation of immune defenses and local infiltration by T cells, monocytes and natural killer cells. Locally interleukins and chemokine's are released to recruit these cells (Saliakellis *et al.*,2013). Cytokines are soluble glycoprotein molecules with a small molecular weight (6 – 70 kilodaltons) produced by a wide variety of cell types (mast cells, macrophages, natural killer (NK) cells, lymphocytes, stromal cells, and many others).They are mainly contributed in the immunological response and serve as essential mediators in the cellular communication network (Kulbe *et al.,* 2012). Furthermore, cytokines are essential health determinants since they influence the maturation, proliferation, and responsiveness of immune and non-immune cells (Neurath,2014).Variability in the levels of cytokines in biological fluids such as serum, feces, sweat, and saliva is considered an important indicator of disease progression and diagnosis. Excessive or abnormal cytokine secretion, such as during a cytokine storm, can result in the failure of organs and death. As a result, cytokine levels are now considered a critical indicator for diagnosing clinical disorders .Cytokines include a wide group such us chemokine's, pro-inflammatory, and anti-inflammatory mediators. The largest producers of pro-inflammatory cytokines are activated macrophages (Rivera *et al.*,2003). some cytokines are being investigated for their potential use as precision medicine marker . interleukin 8 (IL-8), interleukin 10 (IL-10), and monocyte chemo attractant protein 1 (MCP-1), also known as CCL2, were all found to be predictive of pediatric appendicitis and/or appendicitis severity.

2.10 Inflammatory markers

2.10.1 White blood cell

White blood cells (WBC) or leucocytes ,involved in the innate and adaptive immune response consequently in appendicitis, an increase in WBC is seen in children and adults (Anderson *et al.*,2004). In experimental models as well as in the observational studies, a local and systemic increase in the number of WBC is seen in early stage of disease .

2.10.2 Procalcitonin

Procalcitonin (PCT) is a type of hormone that is produced by the Para follicular thyroid cells (C cells) ,This hormone then cleaved to produce calcitonin, which is a hormone that primarily work to lower the body's calcium levels (Lohar *et al.*,2014). Procalcitonin (PCT) is an established lab markers for disease severity in patients with infection and sepsis. Concentration of PCT selectively increases in the case of bacterial infections, while in the case of viral infections the concentration remains normal (Lopez *et al.*,2003).It has been seen that the level of Procalcitonin correlates with severity of the inflammation (Kouame *et al.*,2005). It is a better markers than C-reactive protein (CRP) which also rise in inflammatory states(Delèvaux *et al.*,2003).PCT can help in making the diagnosis of acute appendicitis and can prevent unnecessary appendectomies being performed.

2.10.3 Interleukin 8

IL-8 Interleukin 8 or neutrophil activating protein is a cytokine that functions as a lymphocyte chemo attractant and as a neutrophil chemo attractant / activator. It is produced in response to LPS-stimulated monocytes and in a variety of cell types (i.e. endothelial cells ,keratinocytes, fibroblasts, lymphocytes), in response to inflammatory

stimuli such as TNF-α and IL-1β. IL-8 induces shape change, chemo taxis, release of granule contents, up regulation of adhesion proteins, formation of bioactive lipids, and respiratory burst. This chemokine has gained considerable attention because of its ability to attract and activate leukocytes and its undisputed role as mediator of inflammation (Dechkhajorn *et al.*,2020).

2.10.4 Interleukin-10

IL-10 is an immune regulatory cytokine; many cell types secrete IL-10. Its function is inflammatory responses limitation, termination, and the regulation of differentiation and proliferation of T cells, B cells, natural killer cells, antigen-presenting cells (APCs), mast cells, and granulocytes.(Iyer *et al.*, 2012). IL10 is a protein of 160 amino acids. It exists in non-covalent homodimer form. (Rasquinha *et al.*,2021).Its molecular weight of approximately 18.5KD.IL1immunomodulatory functions are various, like supporting B cell differentiation and Ig secretion in order to inducing a strong anti-inflammatory response (Kotiranta-Ainamo, 2006). It was originally described as a murine Th2 cytokine, inhibiting Th1 cytokines. As later studies showed that in addition to the Th2 cells, there are different cell types (Th0 and Th1 cells, B cells and macrophages) that produce IL-10. However many of IL-10 effects of are similar to, or overlap with, those of Th2 cytokines and that due apparently to the close correlation between IL-10 expression and the induction of Th2-like responses (Rasquinha *et al.*, 2021). While its key features are associated with strong immunosuppressive effects, IL-10 has immunostimulatory properties as well, It play a crucial role in preventing overshooting inflammatory response and autoimmune pathologies. The IL-10 family, together with interferon's, forms the class II cytokine family (Ouyang *et al.*, 2011).

2.10.5. Monocyte chemotactic protein- 1

MCP-1 is a CC chemokine essential for monocyte recruitment in in vivo models of inflammation, MCP-1 induce the recruitment of other leukocytes such as T lymphocytes, basophils and eosinophil's. It is mainly expressed by macrophages in response to cytokine such as TNF-α , IL-6, and IL-1β. Upon stimulation, it can also be produced by a variety of cells and tissues such as fibroblasts, endothelial cells and certain tumor cells. Adhesion of human monocytes to P-selectin, the most rapidly expressed endothelial tethering factor, increased the secretion of MCP-1 and TNF-α forms leukocytes stimulated with PAF (Mulholland *et al.*,2019).

2.10.6. Interleukin-1 receptor antagonist (IL-1RA)

IL-1RA is a protein that in human is encoded by the *IL1RN* gene (Chakrabarti *et al* .,2021). IL-1RA was initially called the IL-1 inhibitor and discovered separately in 1984 by two independent laboratories . IL-1RA is an agent that binds non-productively with the cell surface interleukin-1 receptor (IL-1R), the same receptor that binds interleukin_1 family (IL-1), preventing IL-1 from sending a signal to that cell,IL-1RA is a member of the interleukin 1 cytokine family. IL-1RA is secreted by various types of cells including immune cells, epithelial cells, and adipocytes, and is a natural inhibitor of the pro-inflammatory effect of IL1β (Dinarello *et al.*,2018) This protein inhibits the activities of interleukin 1, alpha (IL1A) and interleukin 1, beta (IL1B), and modulates a variety of interleukin 1 related immune and inflammatory responses.

2.10.7. Macrophage inflammatory protein (MIP)-1α

Macrophage inflammatory protein (MIP)-1α/CCL3 is an inflammatory chemokine produced by cells during infection or inflammation. It belongs to the CC chemokine family, which displays potent chemotactic properties. The MIP-1 protein was first identified by Stephen D. Wolpe in 1988. His research group reported the appearance of a new heparin-binding protein from the murine macrophage cell line in response to endotoxin stimulation. This protein was called macrophage inflammatory protein (MIP) because of its biological function of inducing an inflammatory response characterized by neutrophil infiltration (Bhavsar *et al.*,2015). The initial report of the separation of MIP-1 on SDS-PAGE gel was described by (Sherry *et al.*,1988). Their findings suggested that the MIP-1 protein was composed of two peptides. Partial sequencing revealed two proteins: MIP-1α and MIP-1β. Although these proteins exhibited slight variation in the sequence of amino acid residue at their NH_2 terminals, they share approximately 56.7 % sequence homology. The genomic nucleotide sequence of murine MIP1α is highly homologous to human counterparts LD78alpha/GOS19-1/SCYA3 (Suzuki Y *et al.*,2004). Many researchers have independently isolated human and murine MIP-1α and MIP-1β in their laboratories. For this reason, MIP-1α has more than one name in published reports. However, with the introduction of the new nomenclature, human MIP-1α is currently called chemokine (C-C motif) ligand 3 (CCL3), and MIP-1β is called chemokine (C-C motif) ligand 4 (CCL4) (Zlotnik and Yoshie 2000). MIP-1α/CCL3 is synthetized as a precursor protein with 92 amino acids. The premature protein splits at several sites, resulting in the formation of biologically active mature protein. Chemokine exhibit a high affinity toward proteoglycans. An increased tendency of MIP-1α/CCL3 to

bind with heparin (a common proteoglycan) has been demonstrated experimentally by (Wolpe *et al.*, 1988), and this binding can enhance their activity (Ali *et al*., 2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Equipments and Materials

3.1.1. Equipments:

Table 3.1 : Details of the equipment's and the manufacture companies

S	Equipment name	Company manufacture	Country
1	Autoclave	Gallenkamp	England
2	Oven	Memmert	Germany
3	Deep freezer	Ishtar	Iraq
4	Refrigerator	Concord	(France)
5	Light Microscope	Olympus	Japan
6	Sensitive Balance	Boeco	Germany
7	Vortex Mixer	Elektro.mag	Turkey
8	VITEK 2 Compact system	Biomerieux	France
9	Centrifuge	Hitachi	(Japan)
10	ELISA reader	Bio tech	USA
11	Hood	Biotek Instruments	Germany
12	Water Bath	Memmert	Germany
13	Water distiller	Gallenkump	England
14	Microtome	Letz	Germany

15	Bunsen burner	Locally	Iraq

3.1.2. Laboratory Supplies

Table 3.2 : Details of the laboratory supplies

S	Laboratory Supplies	Manufacture company	Country
1	Cotton swab	Afco	Jordan
2	Graduated Cylinder	Bomex	Germany
3	Disposable syringes	Medjecte	Emirate
4	Cover slides	Mehecho	China
5	Forceps	Locally	Iraq
6	Lancet	Greetmed	China
7	Plastic dropper	Locally	Iraq
8	Petri dishes (Plastic)	Afco	Jordan
9	Rack	Locally	Iraq
10	Stick	Labtech	China
11	Sterile cotton	Locally	Iraq
12	Sterile cup	Afco	Jordan
13	Screw cups (glasses)	Locally	Iraq
14	Slides	Mehecho	China
15	Volumetric Flask	Jiassco	India
16	Disposable Loop	Locally	Iraq

17	EDTA Tubes	Alrawan	China
18	Gel Tube	Alrawan	China
19	Micropipette	Biobasic	Canada
20	Pipette tip	Alrawan	China

3.1.3 Culture Media

Table 3.3 : Details of the cultural media used

S	Media	Manufacture company	Country
1	Brain heart infusion broth	LAB-Neogen	UK
2	MacConkey agar	LAB-Neogen	UK
3	Blood agar	LAB-Neogen	UK
4	Mannitol Salt agar	Oxoid	UK
5	Cary-Blair broth	Becton dickinson	USA

3.1.4. The Chemicals and stains

Table 3.4 : Chemical material and stains used in this study

S	Chemical Name	Manufactured Company	Country
1	Formalin	BDH	England

2	Normal Saline	Adwic	Egypt
3	Xylole	BDH	England
4	Oil Immersion	BDH	England
5	Glycerol	LAB-Neogen	UK
7	Hematoxylin	Merck	Germany
8	Eosin stain	Merck	Germany
9	Ethanol 99%	Biosolve	USA

3.1.5. Diagnostic kits

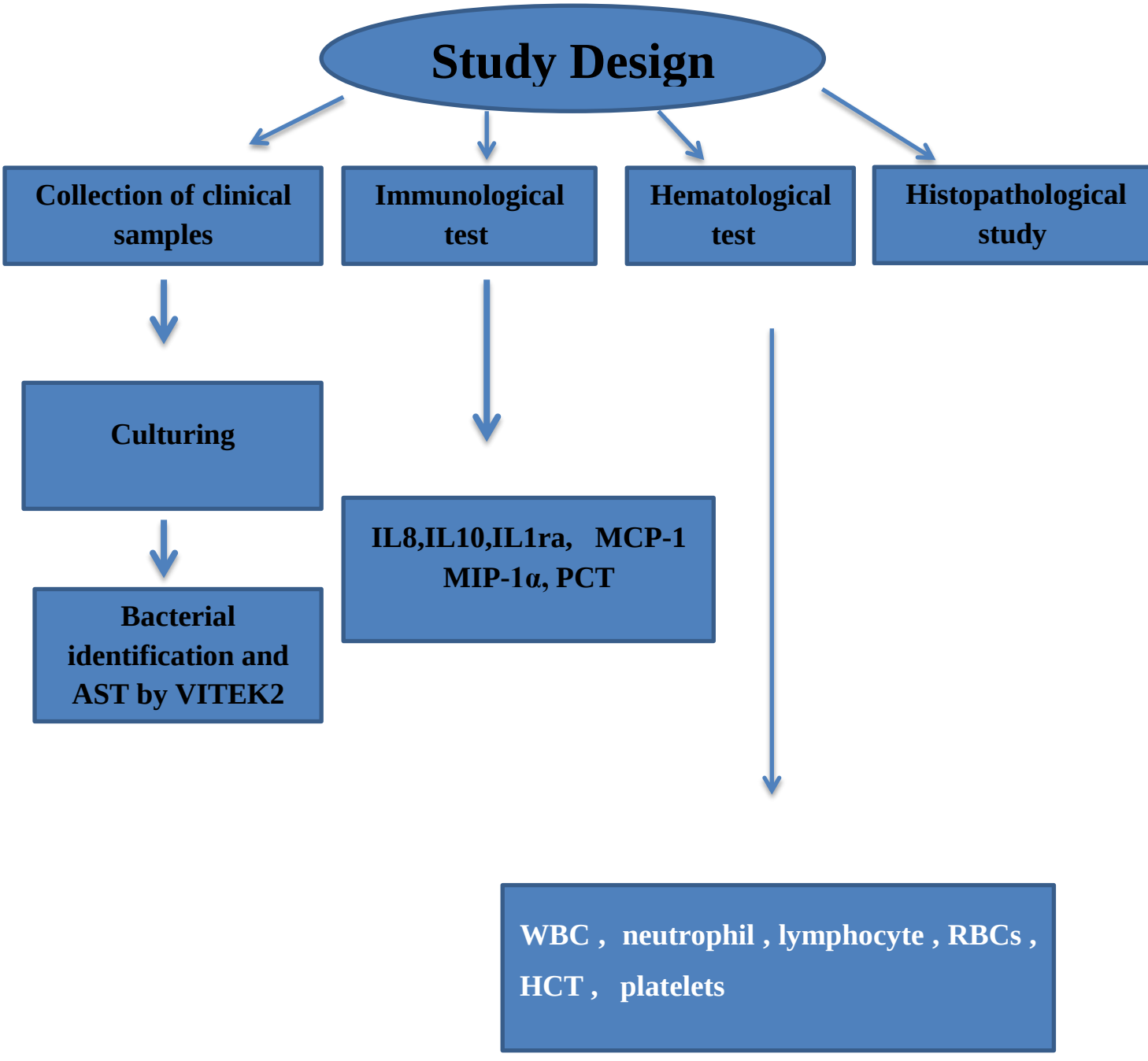
Table 3.5 : Details of the diagnostic kits

S	Kit	Manufactured company	Country
1	Human Procalcitonine (PCT)	MORNMED	China
2	Macrophage Inflammatory Protein-1 Alpha (MIP-1α)	MORNMED	China
3	Monocyte Chemotactic Protein 1 (MCP-1)	MORNMED	China
4	interleukin-1 receptor antagonist (IL-1RA)	MORNMED	China

5	IL-8	MORNMED	China
6	IL-10	MORNMED	China

3.2.Methods

3.2.1.Study design



Schema (3.1):Study design

3.2.2. Questionnaire Sheets

The questionnaire sheet was filled out by the patient participating in our study and included knowledge of their age, sex and other clinical feature approved by them

3.2.3. Ethical Approval

Before the specimen was collected, written permission was obtained from each study patients, and all subjects involved in this experiment were informed. The university of Kerbala ,College of Education for Pure Sciences Ethics Committee gave its approval to this study, under No. 3435, in 25/12/ 2023.

3.3. Bacteriological methods

3.3.1. Culture Media Preparation

The culture media was prepared according to the instructions of manufacturing company and sterilized using an autoclave at a temperature of 121°C and a pressure of at least 15 psi for 15 minutes. After sterilization, the media were cooled to 45°C before use. The details of culture media utilization are presented in Table (3.8).

Table (3.6) : The details of Culture Media utilization

No	Culture Media	Utilization	references
1	MacConkey Agar	Used for the purpose of preliminary diagnosis, and to detect its ability to lactose fermentation	(Luis *et al.*, 2004)
2	Blood Agar	used to cultivate of micro-organism and determinehemolytic reaction	(Choi *et al.*,2024)

3	Brain Heart Infusion Broth (BHI)	Use for to preserve the isolates of bacteria at - 20 °C for long time	(Choi *et al*.,2024)
4	Mannitol Salt Agar	used for identify of *Staphylococcus* and its species.	(Collee *et al*., 1996)

3.3. 2. Preparation of Solutions and stains

3.3.2.1. Preparation of 10% formalin

One quarter (¼)volume of liquid formalin of 40% was added to ¾ volume of normal saline to prepared 10% of formalin to fix the appendix samples for 24- 48 hours at room temperature.

3.3.2.2. Eosin stain

It was prepared by dissolving 1 gram of eosin powder in 99 ml of ethyl alcohol at a concentration of 75% and then stored in tightly capped bottles at room temperature and the dye was used to stain sections .

3.3. 2.3. Hematoxylin stain

It was prepared by mixing 20 grams of potassium alum with 1 gram of hematoxylin dye and 0.5 grams of mercury oxide. Dissolve in 200 ml of distilled water, then add to 10 ml of pure alcohol. and dyes were used to stain sections Histology .

3. 4. Collection of samples

Samples of appendix were collected from patients with acute appendicitis admitted to the Imam Hussein medical city in Holy Karbala. After the operation the samples were placed in 1 mL of normal saline 0.9% within a sterile screw-capped container, then transported to the laboratory .The remain of the samples was placed in 10% formalin fixative for histological examination .

3.5. Collection of Blood samples

About 5 ml of venous blood collected from a suitable vein is withdrawn from the participant's healthy and patients before operation and using 5 ml disposable syringes. The blood sample was divided into two aliquots, the first aliquot blood (2ml) collected in the tube containing EDTA as anticoagulant with a slow mix for hematological study, while the second aliquot blood (3 ml) is dispersal in a gel tube for immunological study, left about 40 min in room temperature then centrifuged for 5 min at 3000 rpm to separate serum and stored in eppendorf tubes at -20C° until assayed.

3.6. Culturing of the samples

One gram of sample was taken and crushed in sterile Petri dish and cultured on MacConkey Agar , Blood Agar and Mannitol Salt Agar and incubated at 37°C for 24 hrs., for storage of bacteria the remain of specimen placed in screw capped (glass tube) contain brain heart infusion broth (BHI) and stored at -20°C , and after incubation period, the growth was examined. If no growth were detected, then the plates were re-incubated for a further 24 hours before discarding as negative result.

3.7. Bacterial Identification using Vitek system

VITEK 2 system (figure 3-1) is used for identification of bacterial isolates after overnight incubation the colonies grown on the agar plates that were used for ID and antibiotic susceptibility testing (AST) using the commercial automated Vitek2 system . As the protocol for institution, the ID and AST results obtained using this traditional workflow were used as the standard for comparison. (Ha *et al*., 2018).

1. After primary organism isolation, there is minimal handling with a simple standardized inoculum

2. Place the inoculum into the VITEK 2 Cassette at the Smart Carrier Station .
3. The VITEK 2 Card and sample are linked via barcode
4. Once the Cassette is loaded, the instrument handles all subsequent steps for incubation and reading the results.

VITEK 2 Compact is an automated biochemical-based tool that includes 48 biochemical features and is widely used in clinical laboratories for microbial detection (Książczyk *et al*., 2016). Microorganisms can be identified for up to 4 hours using VITEK 2 Compact. Each well assesses a strain's metabolic function, including its ability to acidify, alkalize, and enzymatically hydrolyze substrates, as well as bacterial growth in the presence of inhibitors. The instrument detects bacterial growth and metabolic changes in the microwells using fluorescence-based instruments. The findings of the biotyping and biochemical-based methods was influenced by the conditions of bacterial incubation, such as media composition or pH (Książczyk *et al*., 2016). A sterile microloop was used to collect a few colonies of a pure culture that had been grown on blood or macconkey agar for 18 to 24 hours. A bacterial suspension was calibrated to McFarland Turbidity Standard of 0.5–0.63 in 3 mL of a 0.45 percent normal saline using a VITEK 2 DensiChek . The GN card was placed on the cassette and placed in the instrument if the gram stain was negative, and the GP card was placed on the cassette and placed in the instrument if the gram stain was positive. The time between suspension preparation and card filling was less than 30 minutes to prevent turbidity modifications. The cards were incubated at 35.5 1 °C. Colorimetric measurements were taken automatically every 15 minutes when each card was taken out of the incubator. The results were read after 10 to 18 hours incubation (Morka et al., 2018).

3.8. Determination of Antibiotic Susceptibility

Antibiotic susceptibility testing determines a bacterial isolates susceptibility to a set of antibiotics. The cards were loaded into the VITEK 2 automatic reader-incubator after being inoculated. Colony counts were used to make sure the number and density of microorganisms inoculated into the VITEK 2 cards were right (Bazzi *et al*., 2017).

- The microorganism was exposed to antibiotics and the examination determines whether or not the microorganism can grow in the presence of the antibiotics.
- The Minimum Inhibitory Concentration (MIC) an indicator of a microorganism's sensitivity or resistance to an antibiotic is reported to the clinician. Antibiotic susceptibility testing was used to detect antibiotic resistance processes in bacteria. Antibiotic resistance examination findings are used for clinicians to better assess the best care for the infection and the specific patient.

3.9. Hematological test

3.9.1. Complete Blood Count (CBC)

In this test 2 ml of the non-hemolyzed blood is anti-coagulated with EDTA at collection that was examined by using automated system Sysmex XP300 hematology analyzer which is a computerized, highly specialized machine that counts the number of total WBCs and different types of cells such as neutrophil,
lymphocyte, RBCs, HCT, and platelets in a blood sample.

3.10. Histological examination

Tissue samples from the appendix after appendectomy were prepared for histopathological study using to the methods of **(Suvara *et al.*, 2019)**, which
included:

1) Fixation

Each tissue sample (Appendix) was usually cut into small fragments about 2- 3 cm long before fixation to facilitate the penetration of the fixative and preservation of the tissue. One of the best fixatives for routine light microscopy is a buffered isotonic solution of 10% formaldehyde for 48 hours**.**

2) Washing

The tissues were washed with tap water for three minutes before the dehydration process.

3) Dehydration

The tissues were dehydrated by passing them through progressive concentrations of ethanol alcohol 60%, 70%, 80%, 90% (for one hour per each concentration), and 100% (overnight).

4) Clearing

The tissue was cleared by passing them through two steps of xylene for 30 minutes per each step.

5) Infiltration

The tissues were kept in a mixture of xylene and melted paraffin wax at (58-60C^{o}) with a ratio of 1:1 for 30 minutes in an electric oven at 60-65 C^{o} temperature, then the organs were left inside pure melted Paraffin wax through two steps s for 30 minutes per each step.

6) Embedding

Pure melted paraffin wax (60-62 ^{o}C) was poured out gently in a metal template, then each sample was transferred to a template and a hot needle was passed nearby the sample to remove the foam and bubbles around the

sample and left to solidify. The paraffin blocks were kept in the refrigerator at 4-8 ºC until sectioning them.

7) Sectioning

The paraffin blocks were cut by rotary microtome into 5 µ thickness sections and the ribbons of these sections were floated on distilled water in a water bath at 40-45 and allowing the section to flatten out with greater ease. The section was picked up with a clean slide after smeared with a drop of Mayer's egg albumin.

8) Staining and Mounting

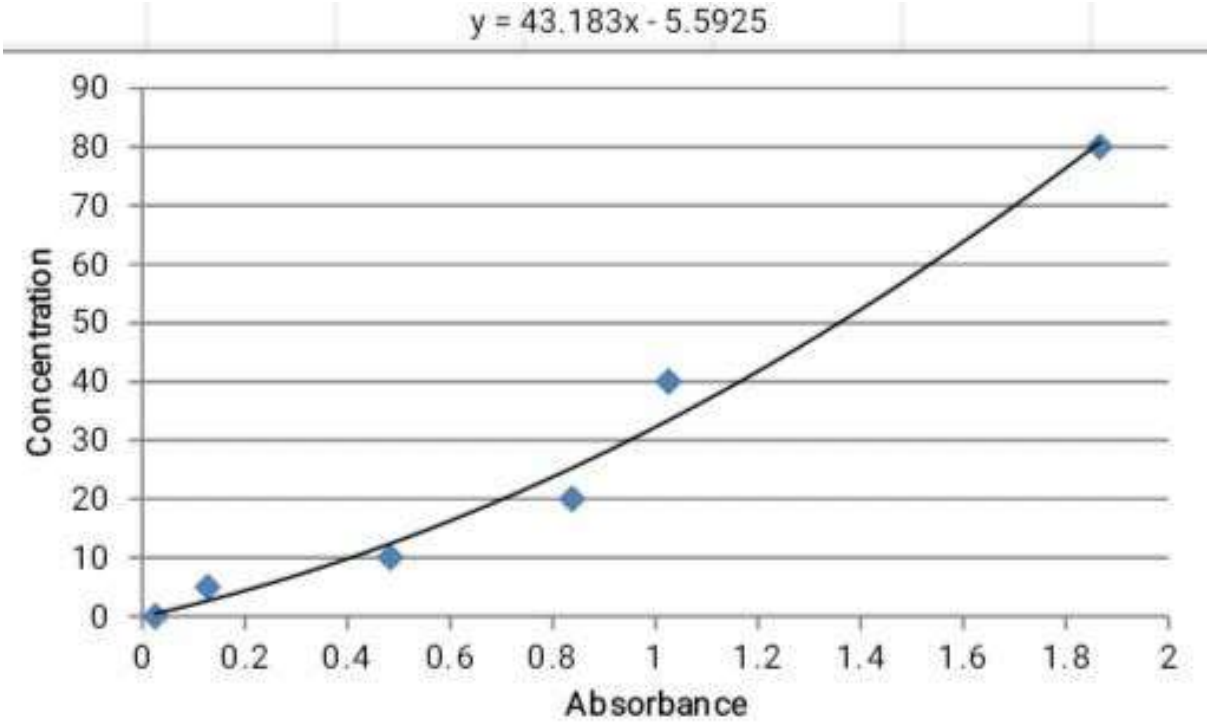
The slides were stained with Hematoxylin and Eosin stain , it was performed via the following steps:

- Deparaffinization by passing the slides basket in two changes of xylene each time for 15 minutes.
- Rehydration by passing through regressive concentrations of ethanol alcohol 100% for five minutes, 90%, 80%, 70%, and 60% for 30 seconds per each concentration, followed by rinsing in tap water for two minutes.
- Staining with Ehrlich's Hematoxylin stain for 3-5 minutes.
- Rinsing in running tap water for 2-3 minutes.
- Staining with Eosin stain for 30 seconds and dipping in tap water for two minutes.
- Dehydration by passing through progressive concentrations of ethanol alcohol 60%, 70%, 80%, 90% for 30 seconds per each concentration and 100% for five minutes.
- Clearing by passing via two changes of xylene for 15 minutes per each change.

- Sections were mounted by DPX then covered with cover slides and left at room temperature to dry. The histological sections were photographed using an Olympus light microscope, an Olympus high-resolution digital camera
  equipped with a microscope digital camera.

3.11. Principle of Immunological test

The principle of ELISA kit where based on Sandwich-ELISA principle, the micro ELISA plate provided in these kits have been pre-coated with one of an antibody specific to Human interleukin .Standards or samples were added to the micro ELISA plate wells and combined with the specific antibody ,then Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated, after that free components were washed away , the substrate solution was added to each wells. Only those wells that contain Human antibody and HRP conjugate would appear blue in color. Finally the enzyme-substrate reaction were terminated by the addition of stop solution which can be noted by changing the blue



color in to yellow,

Figure 3.2 : The standard curve of MIP-1

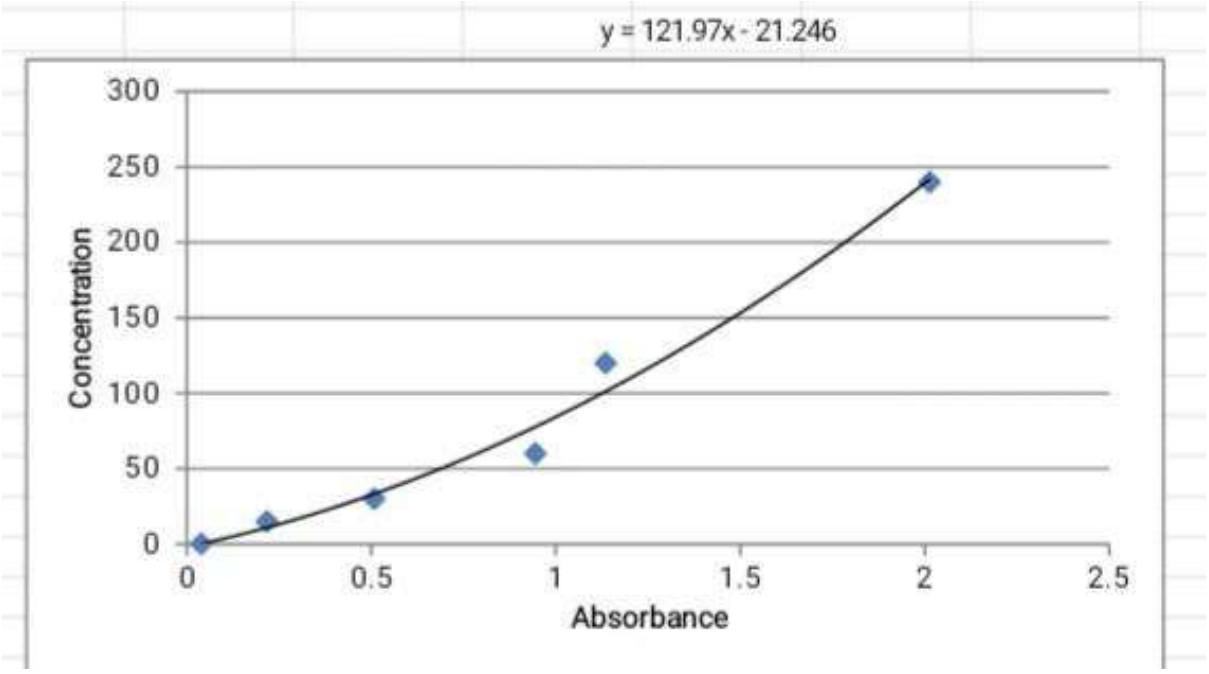


Figure 3.3 : The standard curve of MCP-1

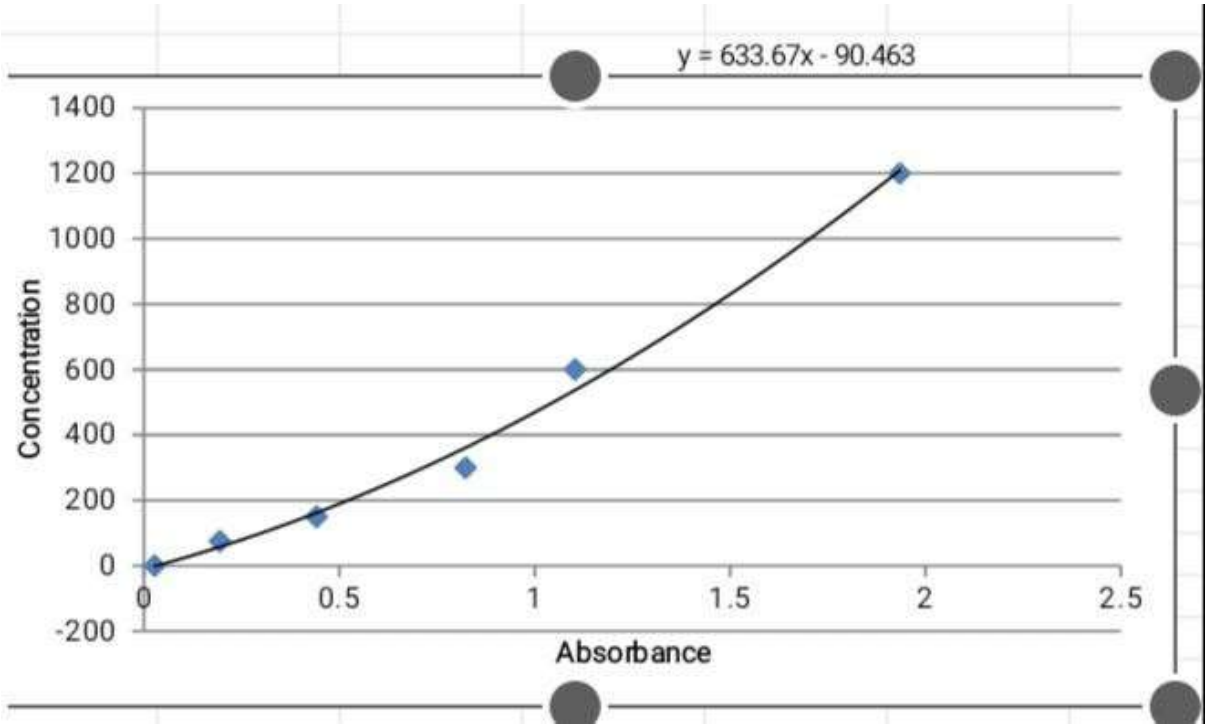


Figure 3.4 : The standard curve of IL-8

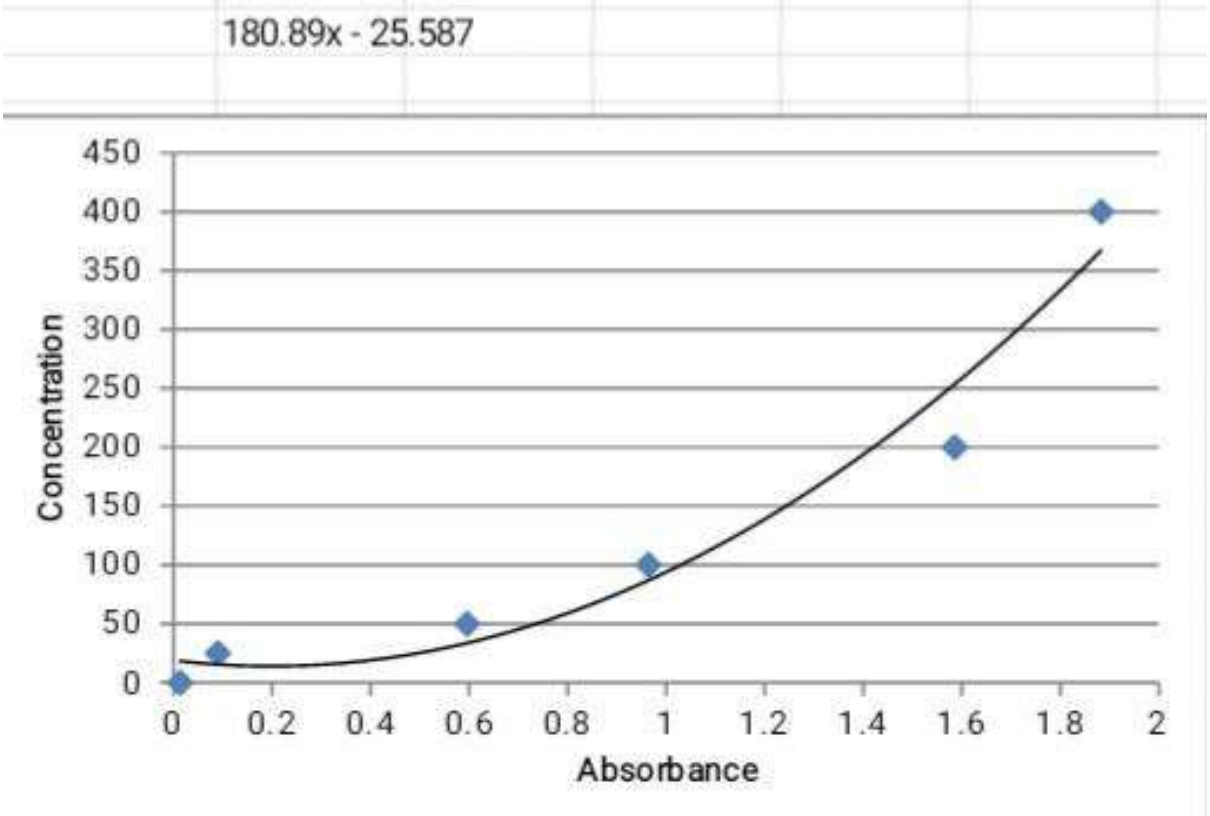


Figure 3.5 : The standard curve of IL-10

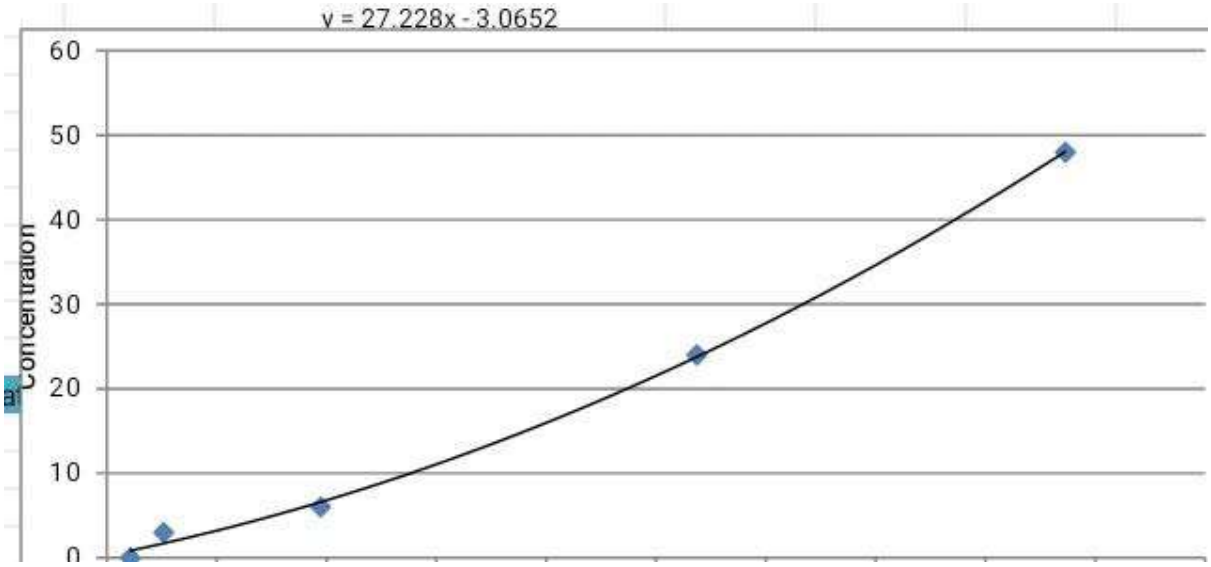


Figure 3.6 : The standard curve of IL-1Ra

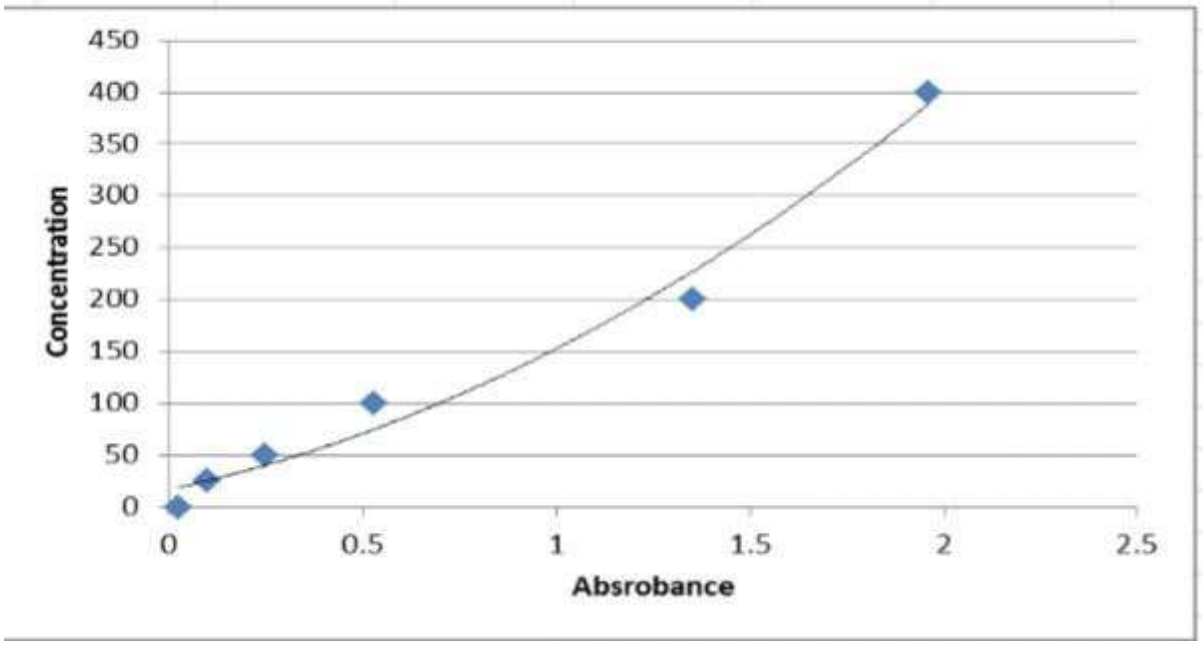


Figure 3.7 : The standard curve of Procalcitonin

3.12. Statistical Analyses

The results were analyzed statistically in SPSS version 23 to find out Chi-square , ANOVA (One away) at significance level (α) in (0.01and 0.05) and Correlation (r).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Samples

This study involved 85 patients excluded only 35 patients with abdominal pain at any location and with no particular suspicion of appendicitis, other patients excluded were those that had peritonitis from ruptured appendix and those that had incidental appendectomy whereby appendix was removed during laparotomy for indication other than acute appendicitis, 50 patients diagnosed with acute appendicitis , (27 males and 23 females) subjected to appendectomy and collected directly from operation theatre at Imam Hussein medical city in Holy Kerbala during the period of January 2024 - June 2024 .

4.2 Characters of the patients

4.2.1. Distribution of Patients with Appendicitis According to sex

The results of the current study revealed that the incidence of appendicitis in males were 27(54%) whereas in females it was 23 (46 %) in figure (4.1) , there were no significant differences (*P value* = 0. 4237) between both sex . The results indicate that the majority of cases were be in males patients then females , this result agree with study reported by Abdulla *et al*(2023) it has been demonstrated incidence of appendicitis in males were 77.2% whereas in females 27.8% which is in accordance with current study.

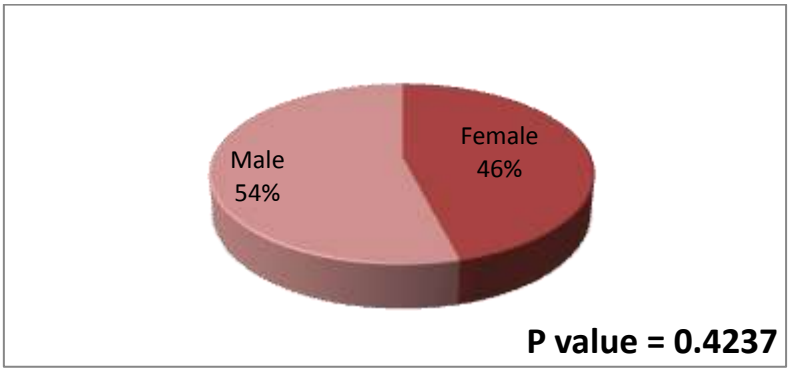


Figure 4.1: Distribution of appendicitis patients according to sex

The differences of distribution of appendicitis between both gender may be due to different in samples size that included in each research or exclusion of some patients cases , or may be due to misdiagnosis of appendicitis cases in women which might have overlapped with other diseases i.e. ovarian cystitis and other inflammation with different causes (Naher and Ktab,2013).

4.2.2.Distribution of Patients with Appendicitis According to Age

There was a total of 100 people who took part in this research ;fifty of them had acute appendicitis while the other fifty in good condition. The ages of the people who took part in the study varied from 7 to 52 years old in the healthyhealthygroups and 8 to 60 in the patient groups. The age means of the patient groups were 21.12±10.86 years ,while the age mean of the healthyhealthygroups were21.76±11.79 years .Furthermore, there was no significant difference between the two groups according to age (*P value* = 0.7783). As shown, respectively in the table (4.1) .

Table 4 .1: Distribution of study groups according age

Groups	N	Mean (year)	SD	Median	Range
Healthy	50	21.76	11.79	19	7 – 52
Patients	50	21.12	10.86	19	8 – 60
P value	0.7783				

In the present study the majority rate of appendicitis was between (16-30) ages with a percentage 44% from the total cases ,and then (1-15) with a percentage 40% , and then (31 - 45) with a percentage 14 % ,the minority rate was 2 %which belongs to age (46-60) ,as in figure (4.2). Current results agree with Abdulla *et al*(2023) and agree with Albahadili (2016) and agree with Mathkoor (2015) as well as Mohammed (2010) &AL-Shahwany *et al*(2009) and agree with a study in Taiwan; where the highest incidence in patients of appendicitis was between 15 - 29 years (Lin, *et al.*, 2015). Another study by Shaker *et al* (2024) recorded that the age group ranging from 10 to 25 years was the most group affected by appendicitis (63%).

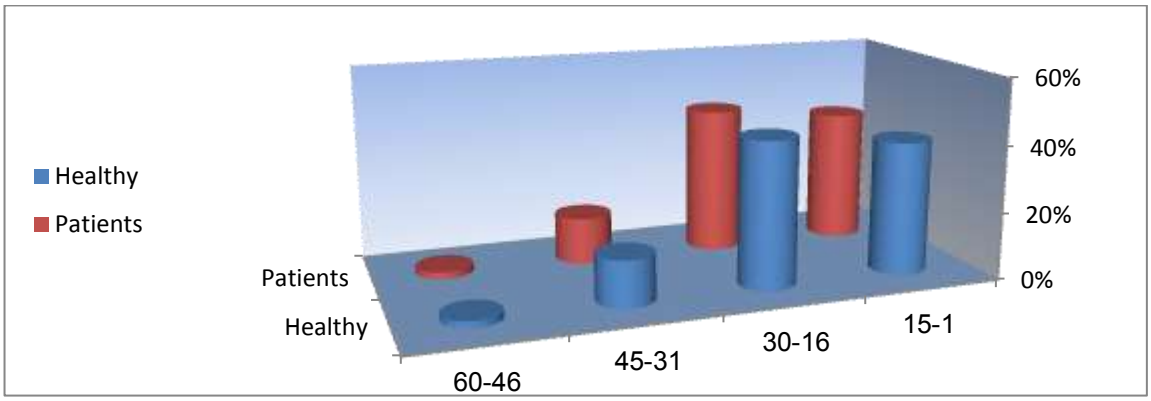


Figure 4.2: Age groups of the study sample

In explanation of our results Almaramhy (2017 **)** concluded that the increase in the incidence of Appendicitis during the age (15-25) might be because appendicitis occur due to obstruction of the appendix as a result of lymphoid hyperplasia as the appendix contains excessive amount of the lymphoid tissue in the sub mucosa which increases in the size and number with growing age, reaching maximum the number and size during teenager with a higher possibility of developing Appendicitis, or may be because an increased number of people in this group are exposed to the pathogens, which is transmitted through the digestive tract as a result of various foods .Differences in the prevalence of appendicitis between age groups may be related to family history and genetics, as a family member is more likely to become infected (if previously infected) than in families that don't have infectious diseases before (Itsbkowitz and Jones, 2004). Appendicitis is most common between 10-20 years, yet, can occur at any age therefore, it is feasible to refer to the fact that appendicitis is more common in younger than in elder people. Differences in prevalence of appendicitis between age groups may be related to family history and genetics , as a family member is more likely to become infected than in families that don't have infectious diseases before(Shaker *et al.*,2024).

4.3 Clinical Features Related to Acute Appendicitis

The clinical features related to appendicitis are shown in figure (4.3) and table (4.2). 50 (100%) showed right iliac fossa pain increased, vomiting was seen in 38(76%) patients , while 37(74%) patients were with nausea, other features were fever 13(26%) and diarrhea 5(10%) , there were highly significant differences between the symptoms and patients. From the results described above one can conclude that the right iliac fossa pain is the commonest symptoms which may draw attention to the case as

suspected appendicitis ,those results were in accordance with related study Kosloske (2004) who pointed out that the right iliac fossa pain was the common feature of appendicitis.

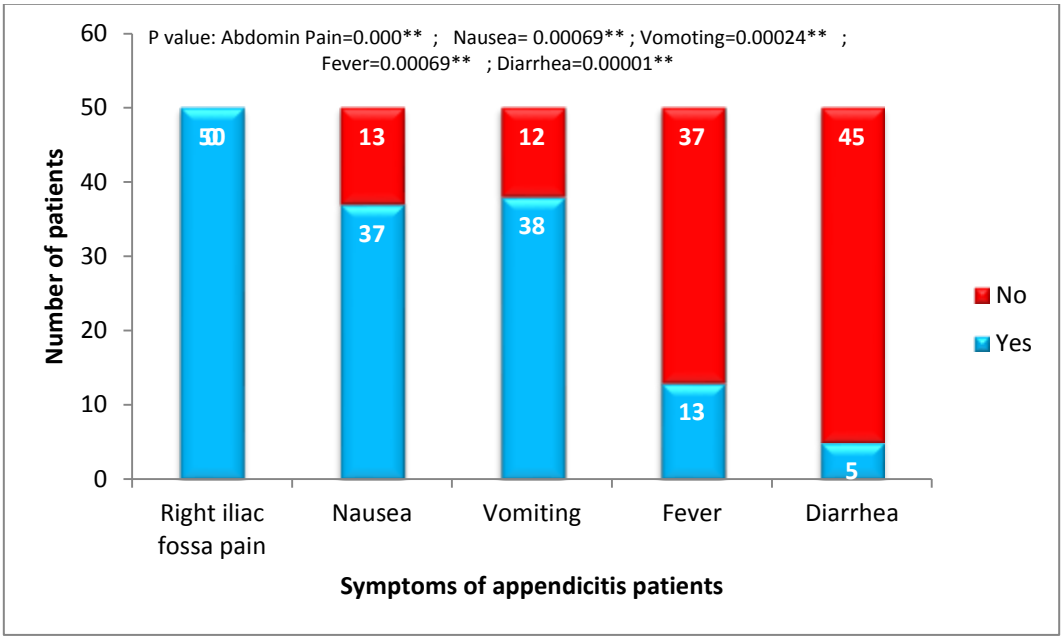


Figure 4.3: Symptoms of appendicitis patients

Table 4.2: Distribution of the acute appendicitis according to the clinical features (n=50)

Clinical feature	No. (%)
Right iliac fossa pain	50 (100%)
Vomiting	38 (76%)
Nausea	37 (74%)
Fever	13 (26%)
Diarrhea	5 (10%)

4.4. Isolation of bacteria from acute appendicitis

In this study all samples yielded positive results for bacterial growth , gram- negative bacteria were the common causes of appendicitis compared with that of gram positive, they were 45(90%) isolates and 5(10%) isolates respectively as shown in table (4.3) , ,there where highly

significant difference between gram positive bacteria and gram negative bacteria (*p-value*= 0.00001**) shown in figure (4.4). Moreover the most frequent pathogen was *E. coli* which accounted for 29(58%) isolates, followed by *Klebsiella pneumoniae* isolates 8(16%) , 5(10%) isolates of *Psedomonas aeruginosa*, 1(2%) isolates of *Enterobacter aerogenes*, 1(2%) isolates of *Salmonella typhi* and 1(2%) of *Proteus mirabilis* . *Staphylococcus aureus* and *Enterococcus faecalis* recorded 2(4%). Whereas *Staphylococcus epidermmidis* was accounted as 1(2%) , as presented in table (4.3).

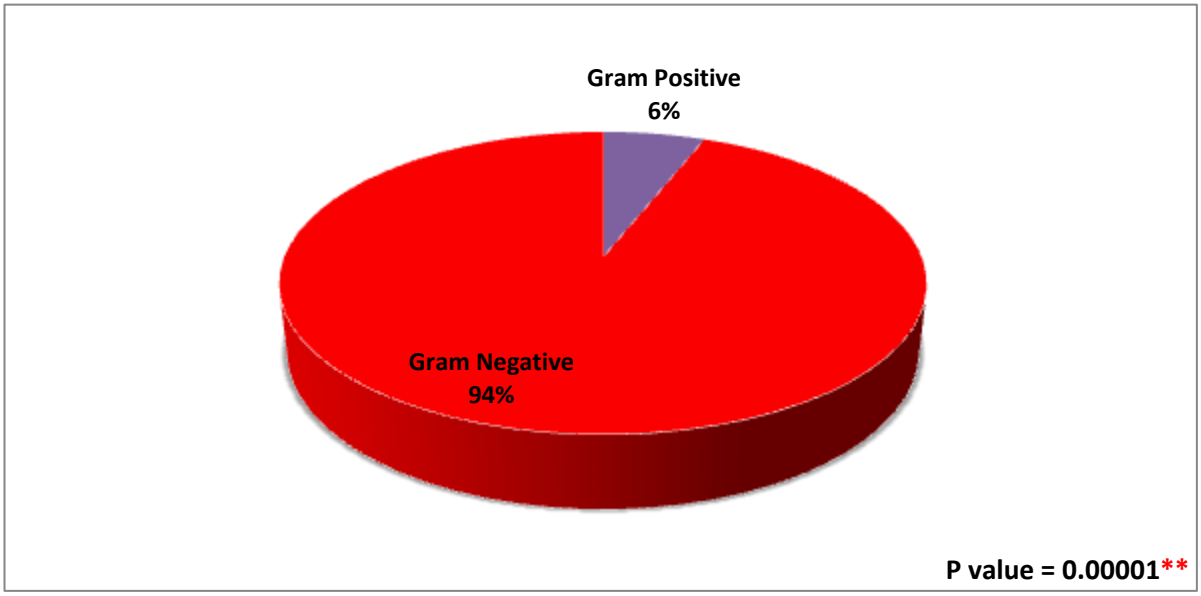


Figure 4.4: Distribution of bacteria isolated from appendicitis patients

Table 4.3 : The type of bacteria isolated from appendicitis patients

Bacteria	N	Percentage (%)
Gram Positive		
Staphylococcus aureus	2	4 %
Staphylococcus epidermmidis	1	2 %
Enterococcus faecalis	2	4 %

Total	**5**	**10%**
Gram Negative		
Escherichia coli	29	58 %
Enterobacter aerogenes	1	2 %
Klebsiella pneumoniae	8	16 %
Proteus mirabilis	1	2 %
Psedomonas aeruginosa	5	10 %
Salamonella typhi	1	2 %
Total	**45**	**90 %**

E. coli is the common organism present in the intestine multiplying rapidly and quickly adhered to the tissue surfaces (Jawad *et al.*, 2022).The adhesion of microorganism to the epithelial cells of the tissue is the first stage of the infection followed by the invasive stage.

Klebsiella pneumonia isolates were 8(16%), this organism has the capsule that plays an important role during the initial steps of the pathogenesis by interacting with the mucus producing cells , several pilli involved in adhesion to the epithelial cells of intestine (Brissac *et al.,* 2021).The explanation for the detection of *Pseudomonas aeruginosa* in appendix as causative agent of appendicitis may be attributed to the abilty of this organism to adhere and colonize epithelial tissue probably by pili and by the algene (slime) layer surrounding the cells of this bacteria, also *Pseudomonas aeruginosa* possesses other virulence factors (enzymes and toxins) , enables it to cause infection (Theodorou *et al.*,2021). *Enterococcus faecalis, Salmonella typhi,, Proteus mirabilis* and *Enterobacter aerogenes* were also detected in appendicitis although in low frequencies compared with the other gram negative. However the implication of these bacteria in appendicitis are suspected, since they belong to the enteric group and frequently present in the intestine and all

these bacteria have virulence factor enabling them to cause disease (Tamura *et al.*, 2022). In this study ,some of gram positive bacteria were also represented by *Staphylococcus aureus*, *Staphylococcus epidermmidis* were also isolated and identified from appendicitis cases but in low frequencies in relative with gram negative. Gram positive bacteria are rarely reported ,this may due to adhesive and colonizer factor being less in gram positive compared with gram negative moreover most of gram positive bacteria are fastidious to require special growth factors (vitamin, amino acids, etc) and growth condition (O2, CO2 …etc) (Burke *et al.*,2024). However qualitatively. The correlation between bacterial infection and appendicitis is characterized by an increase in bacterial presence leading to a higher incidence of appendicitis, studies have shown that specific bacteria, such as *Escherichia coli and Streptococcus* spp. are commonly found in patients with appendicitis, particularly in complicated cases(Zachos *et al.*,2023).

4.5. Sensitivity patterns to antibiotics agents

This is an academic and practical study that determines the effect of different types of antibiotics on microorganisms isolated from samples of acute appendicitis. Also taken into consideration is the extent to which bacteria respond to these antibiotics, and to determine what alternatives are available for Iraqi surgeon to use in cases like these.

4.5.1. Antibiotics susceptibility for gram positive bacteria

From observation , the results of antibiotics susceptibility profile for gram positive bacteria in patients with acute appendicitis found that all species had been isolated were resistant to the Benzyl penicillin and Oxacillin , whereas all species that had been isolated were sensitive to the Rifampicin, Ticarcillin, Vancomycin and Pencillin. Isolates of gram-positive aerobic cocci were resistant to Clindamycin, Fusidic acid,

Erythromycin and Tetracycline (66.6%), and resistant to Gentamicin and Ciprofloxacin 33.3%.figure (4.5).

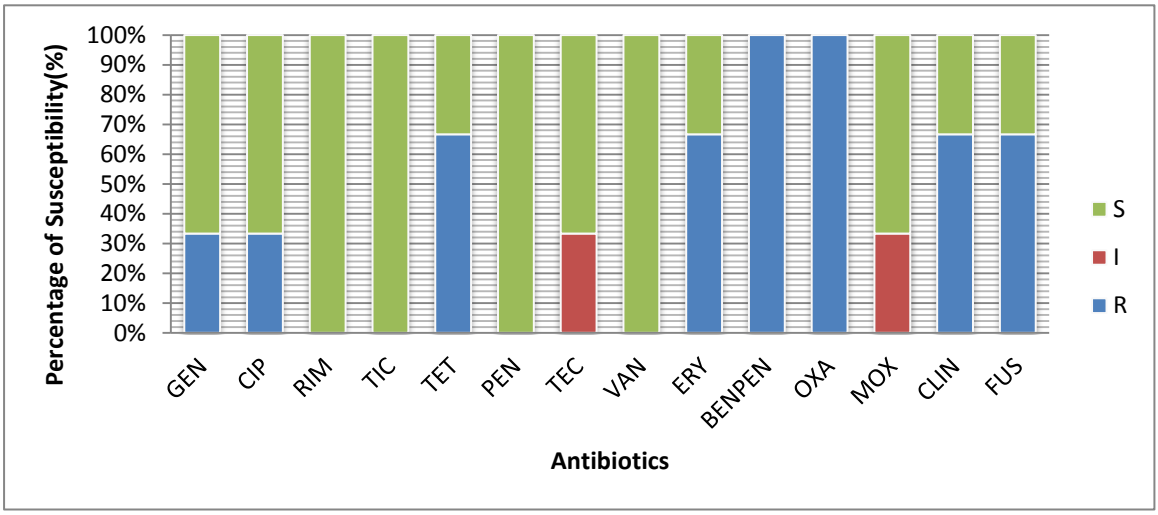


Figure 4.5 : Antibiotic Susceptibility profile for 3 isolates of Gram positive bacteria by Vitek 2 system.

Gram positive bacteria in our study showed sensitivity to Penicillin, Vancomycin, Rifampicin and Ticarcillin which agree with Morfin-Otero (2015). Gram positive bacteria which have been characterized to be sensitive to most common antibiotics in comparison with gram negative bacteria due to the difference in the outer membrane structure which looks to be permeable to most antibiotics in gram positive bacteria than gram negative bacteria (Leus *et al.*,2023). Our study found that all species of gram positive bacteria that had been isolated were resistance to Benzyl penicillin and Oxacillin.

4.5.2. Antibiotics susceptibility for gram negative bacteria

From the observation of the results the antibiotics susceptibility of gram negative bacteria showed all species that had been isolated were sensitive to Amikacin and Imipenem ,while most isolates were resistance to Ticarcillin and Piperacillin92.8%,Aztreonam78.5%,Ciprofloxacin72.7%,Ticarcillin/Clav

ulanicacid71.4%,Cefepime60%,Tobramycin57.1% ,Trimethoprim/Sulfamethoxazole 55.5%,Piperacillin/Tazobactam55% ,Gentamicin50% . figure (4.6).

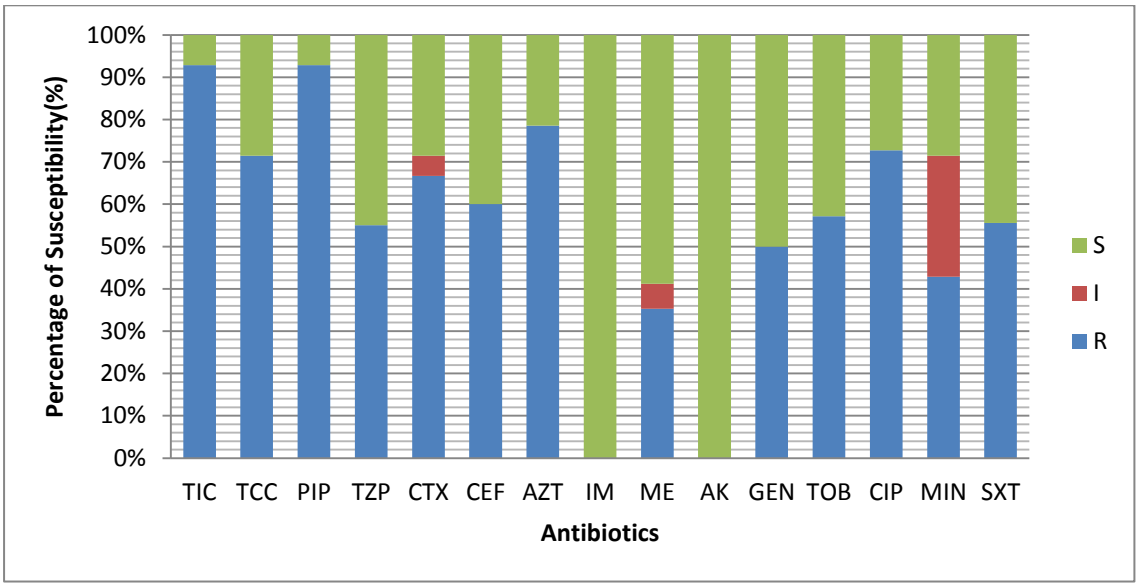


Figure 4.6 : Antibiotic Susceptibility profile for 47 isolates of Gram negative bacteria by Vitek 2 system.

Resistance may be attributed to the continuous and excessive intake of the antibiotics by the patient that results in the development of the bacterial resistance. Iraqi patients are well known for taking antibiotics for everything without medical consultation so this is a very strong reasons for this resistance. In some time use of lower dose of antibiotics gives the appearance that bacteria are resistant whereas in actual fact they are not affected by little doses of antibiotics given or use large doses of antibiotic to patient with low immunity. Generally, combination of antibiotics can lead to declined efficiency of drugs or sometimes increased effect on bacteria (Basavegowda *et al.*,2022). Also problems of resistance occur in patients susceptible to colonization as in hospital which associated to presence of drug resistant bacteria that may originate in hospital. Abdulkarem *et al* (2024) observed that acquired drug resistant

can also be the result of therapy failure. The importance of the use of prophylactic antibiotic before operation has been demonstrated in a number of studies as it was mentioned by Colleran *et al* (2005) who founded the aim of this treatment line is to prevent post-surgical infections following open appendectomy .The optimum type and dose of antibiotics are unknown, so this carries the possibility of either under treatment with increased risk of post-operative infection or over treatment which could result in the microbial resistance. Swab taken during appendectomy is used to direct antibiotic therapy according to the microorganisms identified. It is of paramount importance that the cultural technique used is optimal, microbiological reporting correct and the clinician must use therapy that is indicated by the result. Gladman *et al* (2004) who explained the culture findings should be specific to clinical and operative findings and finally, the therapy should be of benefit in reducing the possibility of early post-operative complication. There are number of reasons to which the differences in the antibiotics sensitivity reported in our study can be attributed. The unnecessary prophylactic use of antibiotics should be discouraged since this may result in increased selection of resistant variants or super infection with resistant flora (Lalitha,2008).However, the poor quality of the antibiotic source, absence qualityhealthyfor imported antibiotics and bad storage conditions for imported antibiotic , all these participate in difference of results. Antibiotic resistance significantly affects the management of bacterial infections in appendicitis, leading to increased complications and necessitating careful selection of empirical antibiotic therapy .Ongoing surveillance of resistance patterns and adaptation of treatment protocols are crucial to improving patient outcomes (Salam *et al.*,2023).

4.6 Studied markers

As shown from the statistical analysis of Table (4.4) the results were significantly increased (*p-value*= 0.0001**) in the mean of PCT patients of appendicitis comparison withhealthy, as the mean of PCT for patients and the healthy(231.10 and 64.11) ng/ml, respectively. Numerous studies have shown that Procalcitonin level was high in the ideology of bacterial origin especially in infected people as in our study patients . Other studies showed that Procalcitonine can be used for freedom from bacterial pathogens and that there is a positive significant correlation between the increase in their level in patients with appendicitis (Marc *et al*.,2002). Also Kaya *et al* (2012) demonstrated in similar work that Procalcitonin accompanied by total WBC count were good predictor for diagnosis of acute appendicitis. Yu *et al* (2013) used Procalcitonin and WBC count for suspected acute appendicitis and found positive correlation for mentioned parameters in patients and recommended for using in the diagnosis of disease. Results agree with Raoof *et al* (2019) who show that the maximum mean of PCT was observed in patients with acute appendicitis as compared with healthy persons (17.31±0.51 versus 6.22±0.34 ng/ml) with highly significant difference between the two groups.The results were significantly increased (*p-value*=0001**) in the mean of IL-8 patients of appendicitis comparison with control, as the mean of IL-8 for patients and thehealthy(624.19 and 144.96) ng/ ml , respectively. IL-8 is a chemokine produced by macrophages and keratinocytes. In appendicitis, the obstruction of the appendiceal lumen leads to bacterial overgrowth and inflammation. These triggers immune cells to release pro-inflammatory cytokines, including IL-8, IL-8 is a potent chemokine that attracts neutrophils to the site of inflammation and promoting a robust immune response. Also studies have shown that people suffered from acute perforated appendicitis increase in IL-8

levels in their serum, indicating the effectiveness of IL-8 as a diagnostic marker for advanced appendicitis (Daneshmandi *et al*., 2009) . This result agreed with Naqvi *et al* (2019) who reported that there were significant differences in IL-8 ($P \leq 0.001$) concentration between patient and healthy, when measured children with appendicitis and healthyhealthy, IL-8 elevated in patients with acute appendicitis in the study of Yoon *et al* (2002). Whereas Stankovic *et al* (2019) , Zviedre *et al* (2016) & Dalal *et al* (2005) disagreed with us, also Peeters *et al*(2020) found low serum levels of IL-8 in patients compared to control.

Results were significantly increased (*p-value*=0.0001^{**}) in the mean of IL-10 patients of appendicitis comparison withhealthy, as the mean of IL-10 for patients and thehealthy(189.06 and 11.49) ng/ml, respectively. We have identified elevated levels of IL-10 in the severe group compared tohealthygroup. IL-10 is an anti-inflammatory cytokine that plays a crucial role in the regulating immune responses. In the context of acute appendicitis, research demonstrated that levels of IL-10 are significantly elevated in the patients compared to healthy individuals, response to the inflammatory process in appendicitis , as it helps to counterbalance pro-inflammatory cytokines and prevent excessive tissue damage. Elevated IL-10 levels can indicate an ongoing attempt by the immune system to regulate inflammation and promote healing (Buchanan *et al.*, 2019). Our results agree with (Naqvi *et al*.,2019) who concluded that there were significant differences in IL-10 concentration ($P \leq 0.01$) between patient and healthy , as well as Zviedre *et al* (2016) & Rivera-chavez *et al* (2003) found that there was elevated level of IL-10 in the serum of patients with AA compared with the healthyhealthyand agreement with Stankovic *et al* (2019) who showed statistically different concentrations

between pathohistological groups and healthy groups . They were also agree with Peeters *et al*(2020) who found high serum levels of IL-10 in patients compared to healthy controls. IL-10 has also been found in other studies to be increased during severe appendicitis (Ruber *et al.*,2006). IL-10 was elevated in patients with acute appendicitis in the study of (Yoon *et al.*,2002).

Results significantly increased (*p-value*= 0.0001**) in the mean of IL-1ra in patients of appendicitis compared with control, as the mean of IL-1ra for patients and thehealthy(21.00 and 7.83) ng/ml, respectively. Interleukin-1 receptor antagonist (IL-1ra) is an important cytokine that plays a role in modulating the inflammatory response. .This results in agreement with Rivera *et al* (2003) who concluded high levels of IL-1ra in the plasma and peritoneal fluids of patients with appendicitis. It's also in agreement with Peeters *et al*(2020) who found elevation of IL-1ra in the serum of patients with appendicitis compared to healthy controls.

The results significantly increased (*P value = 0.0001***) in the mean of MCP-1 in patients of appendicitis comparison with control, as the mean of MCP-1 for patients and thehealthy(151.21 and 34.53) ng/ml, respectively. Monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) are members of the Cysteine-Cysteine (C-C) chemokine family, which has been shown to play a major role in the migration of monocytes to an inflammatory focus (Deshmane *et al.*,2009). The current results are consistent with other studies that have focused on bacteria and their products especially polysaccharides and endotoxin is one of the most important factor that causes an increase in the secretion of a number of cytokines during infection such as MCP-1to increase the speed of the immune response, both local and systemic (Paajanen *et al*.,2002). Local MCP-1 synthesis of endothelial cells in the peritoneal abdominal fluid respond to the activity

of microorganisms and promoting the formation of operative septic complications (Riese *et al.*, 2002; Riese *et al.*, 2004).This is the explanation to the statistically significant increase MCP-1 concentrations in cases of AA in our study. Also (Naqvi *et al.*,2019) observed plasma levels of MCP-1 were significantly different ($p < 0.001$) in children with appendicitis compared to those with non-appendicitis abdominal pain (361.5and 88.3) respectively .While this results is in disagreement with (Zviedr *et al.*,2016) who concluded that there were no significant differences between MCP-1 in both appendicitis and healthy. Results were significantly increased (*p-value= 0.0001***) in the mean of MIP-1 in patients of appendicitis comparison with controls, as the mean of MIP-1 for patients and thehealthy(45.91 and 2.493) pg/mL, respectively. Studies suggest that increased levels of MIP-1 in appendicitis may correlate with inflammatory response associated with the condition. For instance elevated MIP-1 levels are thought to facilitate recruitment of monocytes and other immune cells to the site of inflammation, contributing to pathological process (Davis *et al.*,2021). Macrophage inflammatory protein 1α (MIP-1α) belongs to a family of chemokine's primarily produced by macrophage cells activated by bacterial endotoxin, and has a crucial role in immune response to infection (Cook,1996). The pro-inflammatory role of this cytokine reflected in the activation of granulocytes and the induction of synthesis of other pro-inflammatory cytokines. This chemokine plays significant role in attraction and activation of granulocytes, and its serum concentration was expectedly higher in acute appendicitis.

Table 4.4: The mean of immune markers in patients and healthy group.

Interleukins	Healthy (N=50) Mean ± SD	Patients(N=50) Mean ± SD	*P value*
Procalcitonin ng/ml	64.11 ± 44.02	231.10 ± 79.14	0.0001 **
IL-8 ng/ml	144.96 ± 123.95	624.19 ± 244.47	0.0001 **
IL-10 ng/ml	11.49 ± 7.62	189.06 ± 65.18	0.0001 **
IL-1Ra ng/ml	7.83 ± 1.83	21.00 ± 3.31	0.0001 **
MCP-1 ng/ml	34.53 ± 11.98	151.21 ± 39.00	0.0001 **
MIP-1 Pg/ml	2.493 ± 2.432	45.91 ± 9.38	0.0001 **

4.7 The Hematological Study

The statistical analysis of the result in Table (4.5) showed that there was a high significant increase (*p-value*=0.0001**) in the mean of WBC in patients of a cute appendicitis comparison to the control, as the mean of WBC for patients and thehealthy(11.12 and 6.97) 10^3/µl respectively, there was insignificances decrease (*p-value*= 0.6231) in the mean of the platelets in patients of a cute appendicitis comparison to the control, as the mean of The platelets (258.96 and 264.84) cells/µl respectively. There was a high significant increase *(p-value*=0. 001**) in the mean of neutrophils in patients of a cute appendicitis comparison to the control, as the mean of neutrophils for patients and thehealthy(12.45 and 4.50) cells/µl respectively, while the lymphocyte, RBCs, and HCT count, the result showed a significant decrease between AA patients group as the mean (1.86 , 4.87 and 37.60) cells/µl respectively, and controls (2.38 , 5.11 and 42.42) cells/µl respectively.

Table 4.5 :Complete blood count (CBC) of appendicitis patients and healthy group

Markers	Healthy (N=50) Mean ± SD	Patients(N=50) Mean ± SD	*P value*
WBC (10^3 /µL)	6.97 ± 1.44	11.12 ± 5.22	0.0001**
Platelets (cells/µL)	264.84 ± 46.16	258.96 ± 70.57	0.6231
Neutrophils (cells/µL)	4.50 ± 0.35	12.45 ± 1.42	0.001**
Lymphocyte (cells/µL)	2.38 ± 0.57	1.86 ± 0.91	0.0009 **
RBC (10^6/µl)	5.11 ± 0.53	4.87 ± 0.46	0.0174 *
HCT %	42.42 ± 4.57	37.60 ± 3.93	0.0001 **

There is a possibility that the symptoms of acute appendicitis throughout many people may be non-specific and will be similar to many other acute abdominal illnesses. The choice of whether or not to have surgery is likely to be crucial, but it will also be challenging due to the fact that there is a significant risk of morbidity and death associated with surgical intervention (Kamran *et al.*, 2008). Due to the ease of interpretation, the white blood cell count is test that frequently employed . Our result agrees with the studies of Sucu *et al* (2018) , Kostakis *et al* (2018), Nia and Zareifar (2018) and Oktay *et al* (2020) which showed there is an increase in the total WBC count of the patients group who undergoes an appendectomy. While the study of Kim *et al* (2016) showed a non-significant difference in WBC count complicated appendicitis with the uncomplicated appendicitis patients.

Platelets may be sequestered and destroyed to introduce new platelets into circulation when IL-6 the inflammatory cytokine excreted as a result

of intra-abdominal inflammation in the conditions including acute appendicitis and ovarian torsion, therefore ,early inflammatory phase ought to be the period when big platelets are discovered followed by the development into the late stage of the sepsis. In the meantime full blood count examination should reveal the presence of the platelets of a small size , which is indicative of serious intra-abdominal infections . Result agree with the studies of Oktay *et al* (2020) who showed no significant difference in the platelet counts between patients with AA and those without AA. Also Yılmaz *et al* (2015) founded there is no significant difference in the platelet counts between the children with appendicitis and normal , and that means the platelet indices have no diagnostic value in acute appendicitis when most of the estimations from the studies were combined, it was shown that there had been no significant connection between platelets level with acute appendicitis (Shen *et al.,* 2021). The current result showed a significant increase in the neutrophil count of AA patients compared withhealthyand this result is in agreement with the studies of Ulukent *et al* (2016) ,Yang *et al* (2019) and Tzortzopoulou *et al* (2019) they have reported an elevation in the number of neutrophil in AA patients. While Chen *et al* (2017) have reported non-significantly values in the number of neutrophils between patients with prolonged hospitalization and those who left the hospital within 15 days, the significant increase in neutrophils may be related to belief that neutrophils are the first line of the protection of the body against microorganisms and the primary function of which to identify and destroy invading microbe (Abbas *et al*., 2018). These phagocytes secrete lytic enzymes and produce free oxygen radicals and high antibioticspotential. Their activation is triggered by the bacteria , and by secreted cytokines and chemokines (Stankovic *et al*., 2019). It rapidly responds to the chemokine and predominates in the inflammatory

infiltrate for 6 to 24 hours (Finn, 2015). However, its possibly mediates monocyte/macrophage function (Soehnlein *et al*., 2009). After the injury neutrophils, macrophages and mast cells are recruited to involved area and can produce a variety of pro-inflammatory cytokines (Marchand *et al*., 2005).

4.8 The correlation between age and other parameters

There was no significant difference correlation between all parameters in appendicitis with age , except WBC in patients were negative correlation and significant differences as shown in table (4.6) . This can be explained by the occurrence of physiological events at these ages that affect the level and height of the WBC compared to adults in the normal state (Lewis *et al.*, 2001).Also the body's immune defenses play a role in this increase due to the incomplete specialized immune system, especially in children. Therefore, the process of protecting the body from infections is based on the shoulders of white blood cells. This agreement with Wu *et al*(2003) which showed the adoption of the level of WBC in the early diagnosis of infection in children and many studies have shown that older patients often have lower WBC counts compared with younger patients diagnosed with acute appendicitis, which can complicate the diagnosis and management (Rafiei *et al.*,2019).Our result are in agreement with Bhatti *et al* (2009) who found a significant difference between mean WBC counts and different age groups (*P value* =0.004) and agreement with AL-Hindi (2006) who showed a significant difference and negative correlations between mean WBC counts and different age groups , increase in the total number of white blood cells in the first age groups and decreased in adults and the elderly.

Table 4.6: The correlation between age and other parameters

Parameters	Healthy group r (*P value*)	Patients group r (*P value*)
Procalcitonin	- 0.269 (0.058)	- 0.056 (0.697)
IL-8	- 0.204 (0.156)	0.021 (0.887)
IL-10	0.270 (0.058)	0.023 (0.874)
IL-1Ra	0.061 (0.675)	0.077 (0.593)
MCP-1	0.080 (0.583)	0.102 (0.479)
MIP-1	- 0.192 (0.181)	- 0.038 (0.794)
WBC	0.166 (0.248)	- 0.292 * (0.039)

***. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).**

4.9 The correlation between IL-8 and other parameters

There was no significant difference correlation between all parameters in patients with appendicitis and IL-8 except with procalcitonin there were positive correlation and significant differences between IL-8 and Procalcitonin (*p-value*= 0.022*) .There were negative correlation and significant differences with IL10, positive correlation and significant differences with MCP-1 in healthy group as shown in table (4.7).

Table 4.7: The correlation between IL-8 and other parameters.

Parameters	Healthy group r (*P value*)	Patients group r (*P value*)
Age	- 0.204 (0.156)	0.021 (0.887)
Procalcitonin	0.095 (0.511)	0.323 * (0.022)
IL-10	- 0.294 * (0.038)	0.075 (0.606)

IL-1Ra	0.170 (0.238)	0.110 (0.448)
MCP-1	0.288 * (0.043)	- 0.117 (0.418)
MIP-1	- 0.096 (0.509)	0.263 (0.065)
WBC	- 0.259 (0.069)	- 0.262 (0.066)
***. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).**		

The positive correlation between IL-8 and procalcitonin in patients with acute appendicitis underlines their balance roles in the immune response toward bacterial infection , both markers were elevated in response to infection . In acute appendicitis , IL-8 levels increase as a result of the infection, mostly when appendix becomes infected with bacteria and inflamed , high levels of IL-8 are frequently correlated with the severity of infection .As the infection develops, immune system releases IL-8 to recruit neutrophils and stimulate a local inflammatory response. Therefore, IL-8 serves as indicator of the inflammation and the presence of bacterial infection in appendicitis (Zeillemaker *et al.*,1996). While procalcitonin hormone that is released in response for bacterial infection , PCT levels increase with severity of infection, high PCT levels certainly seen in cases where the infection spread outside the appendix (Acharia *et al.*,2017). Both markers increase proportionally causing positive correlation in appendicitis .Whereas in healthy individuals show positive correlation with no significant difference in the levels of IL-8 and PCT , this indicates that both markers can be present at similar levels in a healthy state, while IL-8 is recruitment neutrophils during inflammatory responses and procalcitonin is released in response for bacterial infection, In the absence of infection both markers stay low, which contribute to the lack of correlation (Stankovic *et al.*,2019).Negative correlation with significant difference in healthy individuals between (IL-8) and (IL-10) is due to the immune regulation, in healthy individual IL-8 and IL-10

demonstration a negative correlation , this suggests that as levels of IL-8 rise, levels of IL-10 tend to decline, and vice versa. This correlation reflects balanced immune response where IL-10 an anti-inflammatory cytokine, acts to inhibition pro-inflammatory effects of IL-8 , this balance is essential for maintain homeostasis and preventing extreme inflammation, which may lead to damage in the tissue (Peeters *et al.*,2020). or due to the physiological homeostasis . The negative correlation point to regulatory mechanism in healthy immune system.IL-10 assists to suppress inflammatory response started by IL-8, which is produced in response to different stimuli, includes infection and injury , this interaction assists to ensure that inflammation is properly controlled , avoiding chronic inflammatory conditions (Kumar *et al* .,2015) . Many studies agree with our study Rivera *et al* (2003) show that there is no significant correlation between IL-8 and IL-10 levels in patients with appendicitis. This lack of correlation may be attributed to acute inflammatory environment current in appendicitis, where the level of IL-8 are markedly raised due to recruitment of neutrophils to the place of inflammation. Nevertheless, IL-10 may not be produced in enough quantities to counterbalance this response, leading to the dissociation between two cytokine. Positive correlation and significant differences between IL8 and MCP-1 in healthy group agree with other studies that show healthy individuals display significantly different levels of IL-8 and MCP-1 compared to patients. In a study including healthy adults, levels of these cytokines were measured, showed that IL-8 levels were positively correlated with MCP-1 levels. This suggests that as the body encounters inflammatory stimuli, both cytokines are up regulated in coordinated way(Lagzdina *et al.*,2023).Negative correlation with no significant differences in IL-8 and MCP-1 levels in patients with acute appendicitis may be due to the variability in immune responses. Many

factors such as stage of appendicitis (uncomplicated vs. complicated) and the time of specimen collection can effect in cytokine levels, leading to a consequence where differences not statistically significant (*Naqvi et al.*, 2019).

4.10 The correlation between MIP-1 and other parameters

There was no significant difference correlation between all parameters with MIP-1 in patients and healthy group, as shown in table (4.8**) .**

Results indicate that MIP-1 levels don't correlate significantly with other inflammatory markers or clinical parameters. This supports conclusions from other studies that have explored cytokine profiles in patients with acute appendicitis. For example, a study found that while certain cytokines like IL-10 showed significant differences between groups, MIP-1α did not demonstrate a strong correlation with clinical results or inflammatory status (Stankovic *et al.*,2019). The search for active biomarkers in acute appendicitis continues, with researchers focusing on additional inflammatory markers such as IL-6, procalcitonin , and C-reactive protein These markers showed more ability in the correlating with the disease severity and inflammatory response, suggesting that they may be more useful than MIP-1 in clinical settings (Akbulut and Sahin,2020). The lack of significant correlation between MIP-1 and the measured parameters points to that clinicians should be wary depending on MIP-1 as a diagnostic tool for acute appendicitis. A comprehensive approach that excludes clinical evaluation, imaging studies, and a panel of inflammatory markers may provide other accurate diagnoses and treatment assessments.

Table 4.8: The correlation between MIP-1 and other parameters

Parameters	Healthy group r (*P value*)	Patients group r (*P value*)
Age	- 0.192 (0.181)	- 0.038 (0.794)
Procalcitonine	- 0.063 (0.663)	- 0.060 (0.679)
IL-8	- 0.096 (0.509)	0.263 (0.065)
IL-10	0.004 (0.980)	- 0.148 (0.306)
IL-1Ra	0.163 (0.259)	0.059 (0.685)
MCP-1	- 0.029 (0.841)	- 0.120 (0.406)
WBC	- 0.138 (0.340)	0.056 (0.699)
***. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).**		

4.11 The correlation between IL-1Ra and other parameters

There was no significant difference correlation between all parameters in patients with Appendicitis and IL-1Ra , where's there were negative correlations and high significant differences between IL-1Ra and MCP-1 in healthy group, as shown in table (4.9).

Table 4.9: The correlation between IL-1Ra and other parameters.

Parameters	Healthy group r (*P value*)	Patients group r (*P value*)
Age	0.061 (0.675)	0.077 (0.593)
Procalcitonine	- 0.111 (0.443)	0.259 (0.070)
IL-8	0.170 (0.238)	0.110 (0.448)
IL-10	0.256 (0.073)	- 0.196 (0.171)
MCP-1	- 0.464 ** (0.001)	0.027 (0.851)

MIP-1	0.163 (0.259)	0.059 (0.685)
WBC	- 0.264 (0.064)	- 0.083 (0.569)
***. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).**		

Negative correlations and high significant difference between IL-1Ra with MCP-1 in healthy point to that when one is elevated the other is probable suppressed to avoid overexcited inflammation, which could lead to a chronic inflammatory conditions (Peeters *et al.*,2020).

While positive correlation with no significant difference between IL-1Ra and MCP-1 in patients with appendicitis due to the body experiences an acute inflammatory response characterized by raised levels of several cytokines and chemokine's , such as MCP-1 and IL-1Ra. Nevertheless, the immune response may not reflect the similar regulatory dynamics seen in the healthy individuals. Instead both MCP-1 and IL-1Ra may be inflammatory stimuli , leading to a raised at the same time due to the condition where their levels not show significant differences(Peeters *et al.*,2020).

4.12 The correlation between IL-10 and other parameters

There was no significant difference correlation between all parameters in patients with acute appendicitis and IL-10, where's there were negative correlations and significant differences between IL-10 with IL-8 and Procalcitonin $(0.038)^{*}$, $(0.017)^{*}$ respectively , and there were negative correlations and high significant differences between IL-10 with MCP-1 (0.000^{**}) in healthy group, as shown in table (4.10).

Table 4.10: The correlation between IL-10 and other parameters.

Parameters	Healthy group r (*P value*)	Patients group r (*P value*)
Age	0.270 (0.58)	0.023 (0.874)
Procalcitonin	- 0.338 * (0.017)	0.098 (0.501)
IL-8	- 0.294 * (0.038)	0.075 (0.606)
IL-1Ra	0.256 (0.073)	- 0.196 (0.171)
MCP-1	- 0.475 ** (0.000)	0.099 (0.495)
MIP-1	0.004 (0.980)	- 0.148 (0.306)
WBC	- 0.165 (0.253)	- 0.077 (0.595)

***. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).**

The negative correlation and significant differences between procalcitonin (PCT) and interleukin-10 (IL-10) in healthy people were due to maintains a balance between pro-inflammatory and anti-inflammatory signals. When PCT levels increase due to an inflammatory stimulus, IL-10 levels might decrease as the body changes towards a pro-inflammatory state to combat possible infections, both PCT and IL-10 are mostly present in low levels. Nevertheless, presence of a significant difference in their levels can point readiness of the body to respond to any infections or inflammation , a higher PCT level compared to IL-10 may suggest an acute inflammatory response, even in the absence of evident infection (Yang *et al.*,2023). The absence of correlation between PCT and IL-10 in appendicitis agrees with Dale *et al* (2022) who show there is no significant correlation between PCT and IL-10 levels in patients with acute appendicitis whereas PCT level were raised in complicated appendicitis, IL-10 levels didn't show corresponding

increase, suggest a lack of correlation between these markers. The lack correlation may be due to different roles these biomarkers plays in the immune response. PCT is mainly a marker of infection, whereas IL-10 assists to modulate inflammation. In the cases of appendicitis, the body may show high levels of PCT due to the bacterial infection, whereas IL-10 levels may not increase if the regulatory mechanisms are overwhelmed or else if the inflammatory response is skewed in the direction of a pro-inflammatory state Yamashita *et al.*,2016). Negative correlation and high significant difference between (MCP-1) and (IL-10) in the healthy individuals suggest a balance between pro inflammatory and anti-inflammatory signals. When MCP-1 level increase, indicate increasing monocyte recruitment . whereas both MCP-1 and IL-10 are essential in the inflammatory response related with appendicitis, their weak correlation point to that they may function independent in disease process. Studies have showed that while both MCP-1 and IL-10 are raised through appendicitis, their correlation not strong. For instance one study noted although IL-10 levels were significantly higher in complicated appendicitis , the connection with MCP-1 was not strong, suggestive of that other factors may effect on their levels independently (Peeters *et al.*,2020). Another study into pediatric appendicitis found significant differences in IL-10 level across diverse stages of appendicitis but did not found a strong relationship with MCP-1 levels, indicating that whereas both cytokines are involved in the inflammatory response, their connections may be indirectly linked (Stankovic *et al.*,2019).

4.13 Histopathological Study

4.13.1 Morphology of appendix

The macroscopic examination of the surgically removed appendices showed inflamed appendices appear swollen and bigger than normal appendices, as well as change color to pale and blood vessels clarity on their surfaces , a change in color of the inflamed appendix was also observed to blackish-red in color, it may be greenish-gray in color figure 4 -7 (a) , It was also observed on the surfaces of some of the affected appendices that fatty vesicles extended along the length of the appendix, distorting their shape, leading to difficulty distinguishing the direction of the appendix figure 4-7 (b) , it was also noted that some inflamed appendices are rough and ulcerated with part or all of the tissue becoming fibrous, difficult to manage figure 4 -7 (c) ,the spread and abundance of ulcers and fibrosis on the outer surface of the appendix is clear evidence of the severity of the inflammation(Redmond *et al.*,2002).Macroscopic examination of this study revealed that there was fecalith inside the appendix and it recurred , the fecalith found in six samples, and the fecalith were of different sizes , causing the obstruction of the appendix cavity , which provides suitable conditions for bacteria to multiply and attack the appendix tissue it causes inflammation and becomes infected with pus figure 4-7 (d) , the presence of fecalith inside the appendix cavity raises controversy the reason for their existence may be the result of calcification of food waste with secretions from the digestive system , the pus has accumulated as a result of secretions from the mucous layer in the cavity of the appendix due to obstruction of the appendix partially or completely, then the pressure on the appendix wall increases and makes the inflammation acute , as well as the nature of the diet for those affected, as low-fiber foods encourage the formation of fecolith more

than foods containing large percentage of them, thus increasing the possibility obstruction appendicle cavity (Robbins *et al.*,1996).

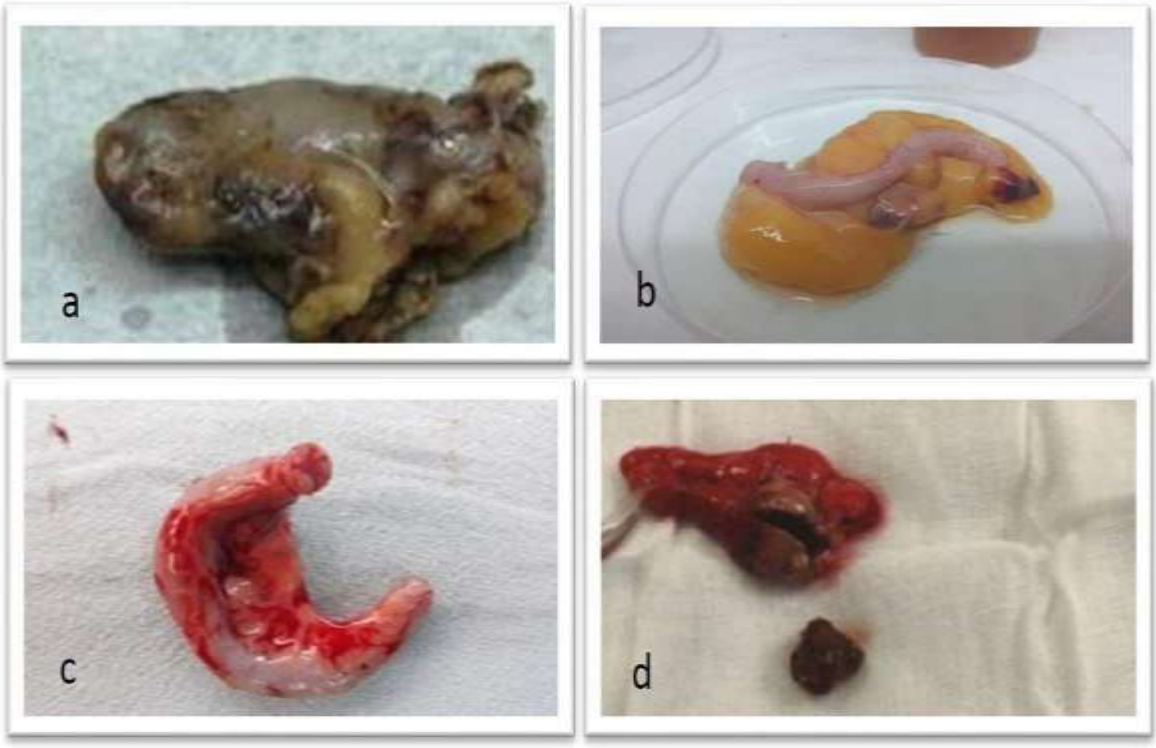


Figure 4.7: The macroscopic appendix feature from patients with acute appendicitis.

(a) A severely inflamed appendix turns discoloured blackish red (b). An inflamed appendix with fatty vesicles, distorted shape, and diameter (c) An inflamed appendix with a rough and ulcerated wall . (d) The presence of fecalith inside the appendix

4.13.2 Histopathological features of acute appendicitis

The histological examination of resected appendices aims to achieve two purposes: to enhance confirming the diagnosis of the inflammatory condition, especially if no macroscopic changes are observed during the surgical operation and to detect additional causes that may be discovered during the examinations (Jones *et al.*, 2007). Pathological evaluation was the gold standard method for diagnoses of appendicitis by doing the

routine histopathological evaluation which performed to confirm the diagnosis of appendicitis and it might reveal other important pathological details (Lal *et al*.,2014).

All samples in our study showed changes in the histological structure of appendix. The histological examination in figure 4.8 (a) showed that there is destruction of the epithelial wall lining the appendix resulting from its obstruction by a fecalith . Most of the destruction occurs in the mucosal and sub mucosal layer, and the expansion and swelling of the appendix makes the wall thin, and this result is similar to a number of studies (Yilmaz *et al.*, 2013).

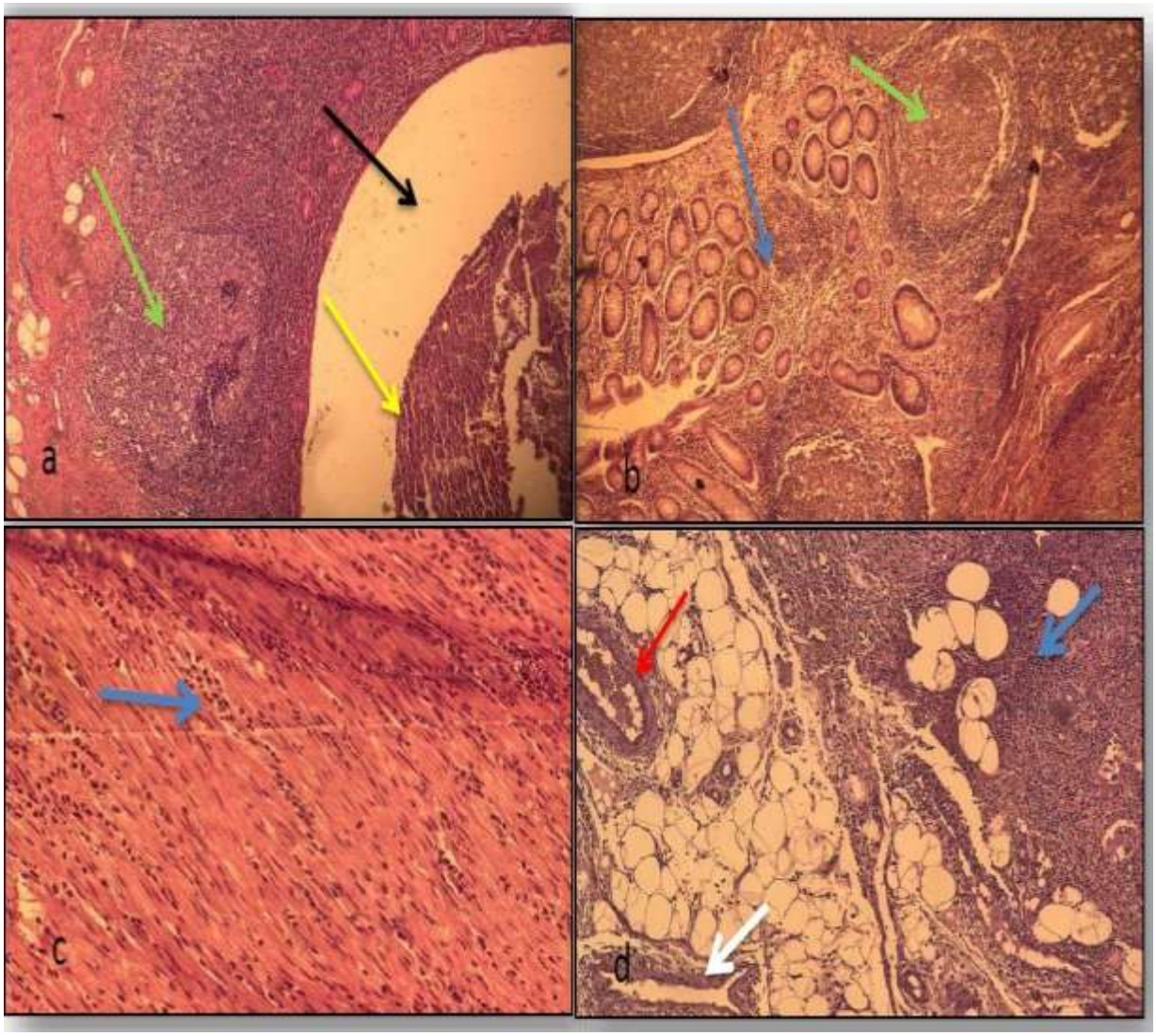
An inflammation of appendix which were noted by hyperplasia increasing in the number of lymphoid follicle noted in figure 4.8 (b) which were appeared to be agreed with Bokhary & Riddell (2009) and Singhal & Jadhav (2007). This may be regarded as a result of viral gastroenteritis or mesenteric adenitis (XU *et al*.,2016).While Torigian *et al* (2001) demonstrate that lymphoid hyperplasia might occur as a result of bacterial infection. Lymphoid hyperplasia is a physiologic response to inflammation particularly in gastrointestinal infections can regress if the accompanying gastrointestinal inflammation is resolved, it is most commonly identified in pediatric patients (Xu *et al*., 2016). Appendical lymphoid hyperplasia may be regarded as secondary to stimulation, especially for viral or bacterial infections, giardiasis, cow milk protein allergy, or IgA deficiency (Mansueto *et al*., 2012). Lymphoid hyperplasia of appendix is typically associated with inflammatory conditions such as viral gastroenteritis and mesenteric adenitis, it is may be obstructing the appendicular lumen, cause AA, which can progress to peritonitis, and resolves spontaneously only in a few cases (Whittle *et al*., 2020).

It was also observed increasing in numbers of neutrophil, as in figure 4.8(c). As a result for an immune response to infection to occur , appendix is one of the lymphatic organs involved in defense against infection with pathogenic agents , including bacteria , also that would explain increasing in numbers of different types of leukocyte such as neutrophil (Carvalho *et al.,* 2021).

It was also observed in some cases the appendical cavity expanded significantly, and vascular changes were observed, represented by congestion blood vessels and their cavities expand in the serosa and sub mucosal layers figure4.8(d) which was noted to be agreed with Bokhary and Riddell (2009) and agreement with Carr (2000) who founded dilatation in the blood vessels of the serosal layer which might belong to explain our finding of hyperemia and congestion. In most cases, gases produced by bacterial species that cause inflammation as metabolites contribute to increased pressure on the walls of appendix (Robbins *et al.,*1996) .

The expansion of appendix cavity causes pressure on the arteries and blood vessels supplying the appendix which leads to interruption of blood supply, so it appears dark in color and blood clotting is observed in the blood vessels with exudation of red blood cells within the tissues of the appendix wall and its lumen . When the luminal pressure exceeds the venous pressure, ischemia, or vasospasm, occurs, it may cause clotting of the veins and capillaries, and when there is continued arteriolar inflow and impaired lymphatic and venous drainage, mucosal ischemia develops, and inflammation extends to the sub mucosal , muscular layers and serosa causing congestion and hemorrhage in blood vessels (Alvarado, 2018).

The histological changes of the excised appendices in this study varied greatly among them , this may be due to the degree of appendicitis in those affected , or it may be due to its physiological and anatomical nature for appendiceal tissue , which varies according to age groups , despite the seriousness of the condition the surgeon's attempt to perform the surgical operation before it reaches advanced stages to avoid perforation of the appendix is noted ,sometimes such cases happen this may be due to the differences in symptoms of appendicitis and its development the inflammatory condition between one patient and another, especially young or old patients, the development of the condition in them is faster than in young people due to their weak immune resistance (Watson *et al.*, 2007).



Figure(4.8) Histological cross sections from patients with acute appendicitis.

(a) Follicular hyperplasia with fecalith note the fecalith (yellow arrow) in lumen (black arrow) , follicular hyperplasia in submucosa (green arrow) (H&E 4 x) (b) lymphoid follicles are more active and larger than normal (reactive hyperplasia) in the submucosal layer (green arrow) and neutrophil infiltration in the mucosa (blue arrow) (H&E 10x) (c) neutrophil infiltration (green arrow) in the muscular externa (H&E 20x) (d) periappendicitis the congested blood vessels (red arrow),neutrophil infiltration ,(blue arrow), periappendicitis (white arrow) (H&E 10 x)

Conclusions

1- Acute appendicitis can be resulted due to the bacterial infection of appendix, gram positive and negative were implicated in acute appendicitis , gram negative was more common acute appendicitis than gram positive and *E. coli* constitutes the predominant bacteria in acute appendicitis.

2- This study showed that acute appendicitis occurs at all stages of life and the age group ranges from (16 - 30) years are more susceptible for acute appendicitis and males were affected more than females and there were significant variations between symptoms and patients with acute appendicitis.

3- Both Imipenem and Amikacin were effective antibiotics against gram negative bacteria; meanwhile, Vancomycin , Penicillin , Rifampicin and Ticarcillin were effective against gram positive bacteria, all species of gram positive bacteria had be isolated were resistance to Benzylpenicillin and Oxacillin, most gram negative isolates were resistance to Ticarcillin and Piperacillin 92.8%. Gram negative isolates showed to be more resistance to the antibiotics than gram positive isolates.

4 - We can depend on the levels of Procalcitonine , IL-10 , IL8 , MCP-1 , MIP -1α and IL-1Ra from the serum of patients as predicator for acute appendicitis.

5 - The hematological result showed high significant increase in White blood cells (WBC) and Neutrophils in patients with acute appendicitis compare to the control, that due to the presence of an inflammatory response.

6- The histopathological study of all appendices specimens showed inflammation in all layers of the tissues characterized by dilatation of blood vessels with increasing amount of lymphatic tissue spread through layers of appendix, lymphoid follicular hyperplasia , large amount of adipose tissue with fatty necrosis , infiltration of inflammatory cells spread through the layers of appendix.

Recommendations

1. A broader spectrum study may be conducted to include a larger number of patients and healthy individuals of different age groups to determine the exact incidence of acute appendicitis and to avoid bias.

2. Study the possibility of the present of genetic finger human leukocyte antigen (HLA) that may determine susceptibility of persons for acute appendicitis.

3. Conduct an extensive study on pathogens from other microorganisms and determine their proportion in causing acute appendicitis and its relationship with the elevation of each type of the immune marker.

4. Investigating the anaerobic species present in the inflamed appendix and their coexistence with aerobic bacteria to obtain a complete impression of the bacteria that causes acute appendicitis .

5. Achievement of further studies to detect the role of diet type and nature in acute appendicitis occurrence.

6. Investigating the virulence factors possessed by the bacterial species found in surgically removed appendices, which are important in the development of the condition , such as their possession of cilia and the production of toxins.

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Abbas, P. I., Zamora, I. J., Elder, S. C., Brandt, M. L., Lopez, M. E., Orth, R. C., Bisset, G. S., & Cruz, A. T. (2018). How long does it take to diagnose appendicitis? Time point process mapping in the emergency department. *Pediatric Emergency Care, 34*(6), 381–384.

Abdulkarem, A. T., Hasan, A. A., Khudhur, H. R., & Abed, S. M. (2024). Overview of opportunistic bacteria. *Kerbala Journal of Pharmaceutical Sciences, 12*(23). https://doi.org/10.1032/25345.v.12-23

Abdulla, M. A., Abdulsattar, A. A., Hadi, H. A., Mahamood, S. A., Raheem, H. A., & Ramadan, N. J. (2023). Pattern of acute appendicitis in Basrah, Iraq: A retrospective study. *Basrah Journal of Surgery*, *29*(2), 88–98. https://doi.org/10.33762/bsurg.144641.1063

Acharya, S. R., Markar, M., Ni, G. B., & Hanna, G. B. (2017). Biomarkers of acute appendicitis: Systematic review and cost-benefit trade-off analysis. *Surgical Endoscopy, 31*(3), 1022–1031.

Ahmed, I., Asgeirsson, K. S., Beckingham, I. J., & Lobo, D. N. (2007). The position of the vermiform appendix at laparoscopy. *Surgical Radiology Anatomy, 29*, 165–168.

Ahmed, W., Akhtar, M. S., & Khan, S. (2018). Seasonal variation of acute appendicitis. *Pakistan Journal of Medical Sciences*, *34*(3), 564.

Akbulut, S., & Sahin, T. T. (2020). Comment on the correlation between complete blood count parameters and appendix diameter for the diagnosis of acute appendicitis. Healthcare (Basel), 8(4), 461. https://doi.org/10.3390/healthcare8040461

Akbulut, S., Koç, C., Sarıcı, B., Özcan, M., Şamdancı, E., & Yılmaz, S. (2020). Histopathological features of incidental appendectomy

specimens obtained from living liver donors. *The Turkish Journal of Gastroenterology, 31*(3), 257–263.

Al-gaithy, Z. K. (2012). Clinical value of total white blood cells and neutrophil counts in patients with suspected appendicitis: Retrospective study. *World Journal of Emergency Surgery, 7*, 32.

Al-Hindi, Z. S. K. (2006). Bacteriological, immunological and histological study on patients with appendicitis in Babylon province.

Ali, S., Palmer, A. C., Banerjee, B., Fritchley, S. J., & Kirby, J. A. (2000). Examination of the function of RANTES, MIP-1alpha, and MIP-1beta following interaction with heparin-like glycosaminoglycans. *Journal of Biological Chemistry, 275*(16), 11721–11727.

Almaramhy, H. H. (2017). Acute appendicitis in young children less than 5 years. *Italian Journal of Pediatrics, 43*(15), 1–9.

Alshammari, F. D., Oreiby, H. A., Ahmed, H. G., Alshaghdali, K., Alcantara, J. C., Ahmed, G. M., Seifeldin, S. A., Abboh, E. A., Al Shammari, W. M., Al Tayeeb, F. M., Al Saif, B. S., Al Qahtani, A. A., Alharbi, S. A., and Elnaem, I. S. (2020). The common motives for appendectomy in Hail Region Saudi Arabia. AIMS Public Health, 7(1), 114–122.

Alvarado, A. (2018). Clinical approach in the diagnosis of acute appendicitis. In *Current Issues in the Diagnostics and Treatment of Acute Appendicitis*. Intech Open.

Andersson, R. E. (2004). Meta-analysis of the clinical and laboratory diagnosis of appendicitis. *The British Journal of Surgery, 91*(1), 28–37.

Ansaloni, L., Catena, F., & Pinna, A. (2009). What is the function of the human vermiform appendix? *European Surgical Research, 43*(2), 67.

Arias, S. L., & Brito, I. L. (2021). Biophysical determinants of biofilm formation in the gut. *Current Opinion in Biomedical Engineering*, *18*, 100275. https://doi.org/10.1016/j.cobme.2021.100275

Arjomand Fard, N., Bording-Jorgensen, M., Webb, J., et al. (2023). Biofilms in the appendix and other non-inflamed sections of the colon in pediatric patients with inflammatory bowel diseases. *Journal of the Canadian Association of Gastroenterology*, *6*(Suppl 1), https://doi.org/10.1093/jcag/gwac036

Arredondo-Alonso, S., Blundell-Hunter, G., Fu, Z. (2023). Evolutionary and functional history of the Escherichia coli K1 capsule. *Nature Communications*, *14*(1), 3294. *https://doi.org/10.1038/s41467-023-39052-w*

B

Bachmann, R. A., & Gifford, R. (2021). The role of cytokines in *Salmonella* pathogenesis. *Clinical Microbiology Reviews*, *34*(3), e00022-21.

Bandyopadhyay, A., Majumdar, K., & Mishra, V. (2022). Non-neoplastic lesions of the appendix. In *Surgical Pathology of the Gastrointestinal System* (pp. 481-519). Springer.

Barroso, T. V. V., Sales, P. G. O., & Petroianu, A. (2015). Assessment of the vasoactive intestinal polypeptide (VIP) in morphologically normal appendices removed from patients with clinical diagnosis of acute appendicitis. *Emergency Medicine Journal*, *5*(3), 1-4.

Basavegowda, N., & Baek, K. H. (2022). Combination strategies of different antimicrobials: An efficient and alternative tool for pathogen inactivation. *Biomedicines*, *10*(9), 2219.https://doi.org/10.3390/biomedicines10092219

Bazzi, A. M., Rabban, A. A., Fawarah, M. M., Al-Tawfiq, J. A. J. J. O. I. & Health, P. (2017). Direct identification and susceptibility testing of positive blood cultures using high speed cold centrifugation and Vitek II system. *10*, 299-307.

Benabbas, R., Hanna, M., Shah, J., & Sinert, R. (2017). Diagnostic accuracy of history, physical examination, laboratory tests, and point-of-care ultrasound for pediatric acute appendicitis in the emergency department: A systematic review and meta-analysis. *Academic Emergency Medicine*, *24*(5), 523–551.

Bennion, R. S., Baron, E. J., Thompson, J. E., Downes, J., Summancn, P., Talarand, D. A., & Finegold, S. M. (1990). The bacteriology of gangrenous and perforated appendicitis. *Annals of Surgery*, *211*, 165–167.

Bhangu, A., Søreide, K., Di Saverio, S., Assarsson, J., & Drake, F. (2015). Acute appendicitis: Modern understanding of pathogenesis, diagnosis, and management. *The Lancet*, *386*, 1278–1287.

Bhatti, A. B. H., Dawood, A., & Zaman, J. (2009). Acute appendicitis: Can WBC count, age and duration of symptoms predict severity of disease? *Pakistan Journal of Surgery*, *25*(3).

Bhavsar, I., Miller, C. S., & Al-Sabbagh, M. (2015). Macrophage inflammatory protein-1 alpha (MIP-1 alpha)/CCL3: As a biomarker. In *General Methods in Biomarker Research and Their Applications* (pp. 223-249).

Bokhary, R. Y., & Riddell, R. H. (2009). Appendiceal diverticulitis and epithelialization of the serosal surface. *Medical Science*, *16*(4).

Bollinger, R. R., Everett, M. L., Palestrant, D., Love, S. D., Lin, S. S., & Parker, W. (2003). Human secretory immunoglobulin A may contribute to biofilm formation in the gut. *Immunology*, *109*, 580-587.

Bornali, H., & Sekhar, D. R. (2016). A study on length of human appendix in different ages. *IJHRMLP*, *2*(2), 101-106.

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it. *Molecules*, *25*(6), 1340. https://doi.org/10.3390/molecules25061340

Brissac, T., Martínez, E., Kruckow, K. L., Riegler, A. N., Ganaie, F., Im, H., Bakshi, S., Arroyo-Diaz, N. M., Spencer, B. L., Saad, J. S., & et al. (2021). Capsule promotes intracellular survival and vascular endothelial cell translocation during invasive

pneumococcal disease. *mBio, 12*, e0251621. https://doi.org/10.1128/mBio.02516-21

Brooks, G. F., Carroll, K. C., Butel, J. S., & Morse, S. A. (2007). In: Jawetz, Melnick, and Adelberg's *Medical Microbiology* (24th ed.). Lange Medical Books/MC Graw-Hill.

Buchanan, A. (2019). The role of IL-10 in acute appendicitis: A study of cytokine profiles. *Journal of Surgical Research*, *240*, 208-215.

Burke, Ó., Zeden, M. S., & O'Gara, J. P. (2024). The pathogenicity and virulence of the opportunistic pathogen *Staphylococcus epidermidis. Virulence, 15*(1), 23594 https://doi.org/10.1080/21505594.2024.2359483.

C

Carroll, K. C., Butel, J. S., Morse, S. A., Mietzner, T. A., & Miller, S. (2016). Jawetz, Melnick and Adelberg's *Medical Microbiology* (27th ed.). Lange Medical Books/MC Graw-Hill.

Carvalho, N., Barros, A., Coelho, H., Cóias, A., Botelho, P., Cismasiu, B., Costa, P. (2021). Increased IgE deposition in appendicular tissue specimens is compatible with a type I hypersensitivity reaction in acute appendicitis. *Mediators of inflammation, 2021*.

Chakkour, M., Hammoud, Z., Farhat, S., El Roz, A., Ezzeddine, Z., & Ghssein, G. (2024). Overview of Proteus mirabilis pathogenicity and virulence: Insights into the role of metals. *Frontiers in Microbiology*, *15*, 1383618. https://doi.org/10.3389/fmicb.2024.1383618

Chakrabarti, S., Prorok, T., Roy, A., Patel, D., Dasarathi, S., & Pahan, K. (2021). Upregulation of IL-1 receptor antagonist by aspirin in glial cells via peroxisome proliferator-activated receptor-alpha. *Journal of Alzheimer's Disease Reports*, *5*(1), 647-661. https://doi.org/10.3233/ADR-210026

Chen, C.-L., Chao, H.-C., Kong, M.-S., & Chen, S.-Y. (2017). Risk factors for prolonged hospitalization in pediatric appendicitis patients with medical treatment. *Pediatrics and Neonatology*, *58*(3), 223–228.

Childers, C. P., Dworsky, J. Q., Maggard-Gibbons, M., & Russell, M. M. (2019). The contemporary appendectomy for acute uncomplicated appendicitis in adults. *Surgery*, *165*(3), 593–601.

Collee, J. G., Fraser, A. G., Marmion, B. P., & Simmon, A. (1996). Tests for the identification of bacteria. In *Mackie and McCartney's Practical Medical Microbiology* (14th ed.). Churchill Livingstone.

Colleran, G., Heneghan, H., Sweeney, K. J., & Kerin, M. J. (2005). A comparison of surgical impression, histological findings and microbiological results at open appendicectomy. *International Journal of Medicine*.

Cook, D. N. (1996). The role of MIP-1 alpha in inflammation and hematopoiesis. *Journal of Leukocyte Biology*, *59*(1), 61–66. https://doi.org/10.1002/jlb.59.1.61

D

Dalal, I., Somekh, E., Bilker-Reich, A., Boaz, M., Gorenstein, A., & Serour, F. (2005). Serum and peritoneal inflammatory mediators in children with suspected acute appendicitis. *Archives of Surgery*, *140*(2), 169-173.

Daldal, E., & Dagmura, H. (2020). The correlation between complete blood count parameters and appendix diameter for the diagnosis of acute appendicitis. *Healthcare*, *8*(1), https://doi.org/10.3390/healthcare8010039

Dale, L. (2022). The use of procalcitonin in the diagnosis of acute appendicitis: A systematic review. *Cureus*, *14*(10), e30292.

Daneshmandi, S., Ghasemi, A., & Pourfathollah, A. A. (2009). IL-6 -174G/C promoter polymorphism in acute appendicitis. *Laboratory Medicine*, *40*(10), 600–603.

Davis, A., et al. (2021). Elevated levels of MIP-1 in patients with acute appendicitis: Implications for diagnosis and treatment. *Journal of Surgical Research, 260*, 45-52.

Davis, R. W., et al. (2023). The impact of intracellular pathogens on leukocyte dynamics. *Nature Reviews Immunology, 23*(1), 45-60.

De Coppi, P., Pozzobon, M., Piccoli, M., Gazzola, M. V., Boldrin, L., Slanzi, E., Destro, R., Zanesco, L., Zanon, G. F., & Gamba, P. (2006). Isolation of mesenchymal stem cells from human vermiform appendix. *Journal of Surgical Research, 135*(1), 85-91.

Dechkhajorn, W., Maneerat, Y., Prasongsukarn, K., Kanchanaphum, P., & Kumsiri, R. (2020). Interleukin-8 in hyperlipidemia and coronary heart disease in Thai patients taking statin cholesterol-lowering medication while undergoing coronary artery bypass grafting treatment. *Scientifica (Cairo), 2020*, 5843958. https://doi.org/10.1155/2020/5843958

Delèvaux, I., André, M., Colombier, M., Albuisson, E., Meylheuc, F., & Bègue, R.-J. (2003). Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? *Annals of the Rheumatic Diseases, 62*(4), 337-340.

Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. *J Interferon Cytokine Res*. 2009;29(6):313-326. doi:10.1089/jir.2008.0027

Dinarello, C. A. (2018). Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunological Reviews, 281*(1), 8-27. https://doi.org/10.1111/imr.12621

E

Everett, M., Palestrant, D., Miller, S., Bollinger, R., & Parker, W. (2004). Immune exclusion and immune inclusion: A new model of host-bacterial interactions in the gut. *Clinical and Applied Immunology Reviews, 4*, 321-332.

F

Finn, A. (2015). Biochemical biomarkers at the site of inflammation and in peripheral blood. *Department of Physiology and Pharmacology*.

G

George, B. H., Quick, C. R. G., Reed, J. B., & Deakin, P. J. (2007). *Essential surgery: Problems, diagnosis and management* (4th ed.). Churchill Livingstone Elsevier. Available at: https://trove.nla.gov.au/work/11478772

Gignoux, B., Blanchet, M. C., Lanz, T., Vulliez, A., Saffarini, M., Bothorel, H., ... & Frering, V. (2018). Should ambulatory appendectomy become the standard treatment for acute appendicitis? *World Journal of Emergency Surgery*, *13*(1), 28.

Girard, M. J., Gomez de Agüero, M., Ganal-Vonarburg, S. C., Mooser, C., Belz, G. T., Macpherson, A. J., & Vivier, E. (2018). The immunological functions of the appendix: An example of redundancy? *Seminars in Immunology*, *36*, 31–44.

Gong, T., Liu, L., Jiang, W., & Zhou, R. (2020). DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nature Reviews Immunology*, *20*(2), 95–112. https://doi.org/10.1038/s41577-019-0215-7

Guasco, C., Roncheto, F., Milani, E., Stocchini, A., & Pistono. (1991). Bacteriology of abdominal pus in 43 cases of acute appendicitis and appendiceal abscess at the Ivra-Castellamonate Hospital: Isolation of aerobic and anaerobic bacteria and drug sensitivity. *Journal of Bacteriology, Virology, and Immunology*, *84*(1-12), 77.

H

HA, J., Hong, S. K., Han, G. H., Kim, M., Yong, D., & Lee, K. J. A. O. L. M. (2018). Same-day identification and antimicrobial susceptibility testing of bacteria in positive blood culture broths using short-term incubation on solid medium with the MicroFlex LT, Vitek-MS, and Vitek2 systems. *Journal of Clinical Microbiology*, *38*, 235-241.

Hajibandeh, S., Hajibandeh, S., Hobbs, N., & Mansour, M. (2020). Neutrophil-to-lymphocyte ratio predicts acute appendicitis and distinguishes between complicated and uncomplicated

appendicitis: A systematic review and meta-analysis. *American Journal of Surgery, 219*, 154-163.

Holt, J.G.; Kreig, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). In Bergey's Manual of determinative bacteriology , 9th ed. The Williams and Wilkins Co., Baltimore.

Holten, KB. ; Onusko, E. M**. (2000).** Appropriate prescribing of oral beta-lactam antibiotics. ***Am Fam Physician***. 62(3): 611-620.

I

Itskowitz, M. S., & Jones, S. M. (2004). Appendicitis. *Emergency Medicine*, *36*(10), 10-15.

Iyer, S. S., & Cheng, G. (2012). Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. *Critical Reviews in Immunology, 32*(1), 23 https://doi.org/10.1615/critrevimmunol.v32.i1.30

J

Jagdish, P., & Ashoka, R. (2018). Morphometry of vermiform appendix: A human cadaveric study. *Journal of Dental and Medical Sciences, 17*(8), 72-76.

Jakobsen, J., Andersen, J. C., & Klausen, I. B. (1987). Beta haemolytic streptococci in acute appendicitis. *Acta Chirurgica Scandinavica, 154*, 30.

Jana, S., & Deb, J. K. (2006). Molecular understanding of aminoglycoside action and resistance. *Applied Microbiology and Biotechnology, 70*, 140-150.

Jawad, K. A., Qian, S., Vasileiou, G., Larentzakis, A., Rattan, R., Dodgion, C., Kaafarani, H., Zielinski, M., Namias, N., & Yeh, D. D. (2022). Microbial epidemiology of acute and perforated appendicitis: A post-hoc analysis of an EAST multicenter study. *Journal of Surgical Research, 262*, 69–75. https://doi.org/10.1016/j.jss.2021.07.026

Jones, A. E., Phillips, A. W., Jarvis, J. R., & Sargen, K. (2007). The value of routine histopathological examination of appendicectomy specimens. *BMC Surgery, 7*(17), 1-4.

K

Kahai, P., Mandiga, P., Wehrle, C. J., & Lobo, S. (2020). Anatomy, abdomen and pelvis, large intestine. In *StatPearls*. StatPearls Publishing.

Kamran, H., Naveed, D., Nazir, A., Hameed, M., Ahmed, M., & Khan, U. (2008). Role of total leukocyte count in the diagnosis of acute appendicitis. *Journal of Ayub Medical College Abbottabad, 20*(3), 70-71.

Kang, H. K., and Park Y., (2015). Glycopeptide antibiotics: Structureand mechanism of action. ***J Bacteriol Virol*** 45(2): 67-78.

Kazlow, P. G., Freed, J. R., Rosh, J. R., Reiner, M., Dische, R., Benkov, K., & Leleiko, W. S. (1991). *Salmonella typhimurium* appendicitis. *Journal of Pediatric Gastroenterology and Nutrition, 13*(1), 101.

Khatun, S., Thakur, D., & Shah, D. K. (2019). Prevalence of retrocaecal appendix among patients with appendicitis in a tertiary care hospital of Nepal. *JNMA: Journal of the Nepal Medical Association, 57*(217), 150.

Kim, M., Kim, S.-J., & Cho, H. J. (2016). International normalized ratio and serum C-reactive protein are feasible markers to predict complicated appendicitis. *World Journal of Emergency Surgery, 11*(1), 31.

Klebba, P. E., Newton, S. M. C., Six, D. A., et al. (2021). Iron acquisition systems of Gram-negative bacterial pathogens define TonB-dependent pathways to novel antibiotics. *Chemical Reviews, 121*(9), 5193-5239. https://doi.org/10.1021/acs.chemrev.0c01005

Kooij, I. A., Sahami, S., Meijer, S. L., Buskens, C. J., & Te Velde, A. A. (2016). The immunology of the vermiform appendix: A review of the literature. *Clinical and Experimental Immunology, 186*(1), 1-9. https://doi.org/10.1111/cei.12821

Kosloske, A. (2004). The diagnosis of appendicitis in children: Outcomes of a

strategy based on pediatric surgical evaluation. Ped. Jan. 62-68.

Kostakis, I., Angelidou, M., Kambouri, K., Gardikis, S., Cholidou, K., Gioka, T., & Vaos, G. (2018). Hematological diagnostic markers of acute appendicitis in children. *Hellenic Journal of Surgery, 90*, 127–136.

Kotiranta, A. (2006). Ontogeny of IL-10 secretion and relation to the secretion of IFN, immunoglobulin M, G, and A, and mononuclear cell composition in newborns (Ph.D. thesis). Hospital for Children and Adolescents, University of Helsinki, Finland.

Ksiazczyk, M., Kuczkowski, M., Dudek, B., Korzekwa, K., Tobiasz, A., Korzeniowska-Kowal, A., Paluch, E., Wieliczko, O. A., & Bugla-Polskonska, G. J. C. M. (2016). Application of routine diagnostic procedures, VITEK 2 compact, MALDI-TOF MS, and PCR assays in identification procedures of bacterial strains with ambiguous phenotypes. *72*, 570-582.

Kumar, S. S., Kumari, N., Mittal, R. D., Mohindra, S., & Ghoshal, U. C. (2015). Association between pro-(IL-8) and anti-inflammatory (IL-10) cytokine variants and their serum levels and *H. pylori*-related gastric carcinogenesis in northern India. *Meta Gene, 6*, 9-16. https://doi.org/10.1016/j.mgene.2015.07.008

Kumar, M. S. V., Tiwari, M. K., Singh, J., & Malik, A. (2021). Plasma fibrinogen: An independent predictor of pediatric appendicitis. *Journal of Indian Association of Pediatric Surgeons, 26*(4), 240-245. https://doi.org/10.4103/jiaps.JIAPS_123_20

L

Lagzdina, R., Rumaka, M., Gersone, G., & Tretjakovs, P. (2023). Circulating levels of IL-8 and MCP-1 in healthy adults: Changes after an acute aerobic exercise and association with body composition and energy metabolism. *International Journal of Molecular Sciences, 24*(19), 14725.

Lal, D. P. C. K. A., Samarasekara, N., Sivaganesh, S., & Zoysa, I. D. (2014). What can histopathology say about acute appendicitis? *American Journal of Clinical Pathology, 142*(1), 233.

Lalitha, M. K. (2008). Manual on antimicrobial susceptibility testing. Int.

Lamichhane, B., Mawad, A. M. M., Saleh, M., Kelley, W. G., Harrington, P. J., II, Lovestad, C. W., Amezcua, J., Sarhan, M. M., El Zowalaty, M. E., Ramadan, H. (2024). Salmonellosis: An overview of epidemiology, pathogenesis, and innovative approaches to mitigate the antimicrobial resistantinfections.Antibiotics,*13*(1),76.https://doi.org/10.3390/antibiotics13010076

Lang, K. (2015). Regulation of cytokine responses: Insights into the role of IL-10. *Nature Reviews Immunology*, *15*(7), 468-478.

Le Page, S., Dubourg, G., Baron, S.A., Rolain, J.M. and Raoult, D.(2019). No global increase in resistance to antibiotics: a snapshot ofresistance from 2001 to 2016 in Marseille, France. *European Journalof Clinical Microbiology & Infectious Diseases*, *38*(2):395-407.

Leus, I. V., Adamiak, J., Chandar, B., Bonifay, V., Zhao, S., Walker, S. S., Squadroni, B., Balibar, C. J., Kinarivala, N., Standke, L. C., Voss, H. U., Tan, D. S., Rybenkov, V. V., & Zgurskaya, H. I. (2023). Functional diversity of gram-negative permeability barriers reflected in antibacterial activities and intracellular accumulation of antibiotics. *Antimicrobial Agents and Chemotherapy*, *67*(2), https://doi.org/10.1128/aac.01377-22

Lewis, S. M., Bain, B. J., & Bates, I. (2001). *Dacie and Lewis practical hematology* (9th ed.). Churchill Livingstone.

Li, Z., Li, Z., Zhao, L., Cheng, Y., Cheng, N., & Deng, Y. (2021). Abdominal drainage to prevent intra-peritoneal abscess after appendectomy for complicated appendicitis. *Cochrane Database of Systematic Reviews*, *(8)*.

Lin, K. B., Lai, K. R., Yang, N. P., Chan, C. L., Liu, Y. H., Pan, R. H., & Huang, C. H. (2015). Epidemiology and socioeconomic features of appendicitis in Taiwan: A 12-year population-based study. *World Journal of Emergency Surgery*, *10*, 42.

Liu, J., Hao, X., Shang, X., Chi, R., & Xu, T. (2024). Incidence and risk factors of urinary tract infection in hospitalized patients with spinal cord injury in a hospital of China. *Scientific Reports*, *14*(1), 3579. https://doi.org/10.1038/s41598-024-54234-2

Liu, Y., *et al.* (2021). Secretory IgA promotes the adherence of commensal bacteria to the intestinal epithelium. *Nature Communications*. https://doi.org/10.1038/s41467-021-23116-5

Lob, S., Hackel, M., Kazmierczak, K., Hoban, D., Young, K., Motyl, M., Karlowsky, J., & Sahm, D. (2017). In vitro activity of imipenem-relebactam against gram-negative bacilli isolated from patients with lower respiratory tract infections in the United States in 2015 – Results from the SMART global surveillance program. *Antimicrobial Agents and Chemotherapy, 88*(2), 171-176.

Lohar, H. P., Ali, M., Calcuttawala, A., Nirhale, D. S., Athavale, V. S., & Malhotra, M. (2014). Epidemiological aspects of appendicitis in a rural setup. *Medical Journal of Dr. DY Patil University, 7*(6), 753-757.

López, F., Cubells, C. L., García, J. J. G., & Pou, J. F. (2003). Procalcitonin in pediatric emergency departments for the early diagnosis of invasive bacterial infections in febrile infants: Results of a multicenter study and utility of a rapid qualitative test for this marker. *Pediatric Infectious Disease Journal, 22*(10), 895-904.

López, M., & Huerta, M. (2022). *Salmonella* infection and immune response. *Journal of Infectious Diseases, 225*(5), 822-830.

Luis, M., Dela, M., Pezzlo, M., Janet, T., Shigei, P., Peterson, & Ellena, M. (2004). *Color atlas of medical bacteriology*. Washington, D.C.: ASM Press.

M

MacFaddin, J. F. (2000). *Biochemical tests for identification of medical bacteria* (3rd ed.). Williams and Wilkins.

Majeed, A., & Al-Bakri, H. (1984). Acute appendicitis in Nineveh province. *Faculty of Medicine Baghdad, 26*(1), 71.

Mansueto, P., Iacono, G., Seidita, A., D'Alcamo, A., Sprini, D., & Carroccio, A. (2012). Review article: Intestinal lymphoid nodular hyperplasia in children and its relationship to food hypersensitivity. *Alimentary Pharmacology & Therapeutics, 35*(9), 1000–1009.

Manzoor, M; 2015. *Medical / Health Care / Pharmaceuticals.* [Online]Availableat:https://www.slideshare.net/drmohammadmanzoor/lacuteappendicits[Accessed 20 December 2015].

Marc, E., Ménager, C., Moulin, F., Stos, B., Chaleumeau, M., & Guérin, S. (2002). Procalcitonin and viral meningitis: Reduction of unnecessary antibiotics by measurement during an outbreak. *Archives of Pediatrics*, *9*, 358.

Marchand, F., Perretti, M., & McMahon, S. B. (2005). Role of the immune system in chronic pain. *Nature Reviews Neuroscience*, *6*(7), 521–532.

Marniok, B., Slusarczyk, K., Pastuszka, A., & Jarosz, R. (2004). Anatomical variations of the vermiform appendix. *Wiadomosci Lekarskie, 57*, 156-157.

Martin, B., Subramanian, T., Arul, S., Patel, M., & Jester, I. (2023). Using microbiology culture in pediatric appendicitis to risk stratify patients: A cohort study. *Surgical Infections*, *24*, 183–189. https://doi.org/10.1089/sur.2022.220

Mohammadi, S., Hedjazi, A., Sajjadian, M., Rahmani, M., Mohammadi, M., & Moghadam, M. D. (2017). Morphological variations of the vermiform appendix in Iranian cadavers: A study from developing countries. *Folia Morphologica*, *76*(4), 695-701.

Morfin-Otero, R., Noriega, E., & Dowzicky, M. (2015). Antimicrobial susceptibility trends among gram-positive and -negative clinical isolates collected between 2005 and 2012 in Mexico: Results from the Tigecycline Evaluation and Surveillance Trial. *Annals of Clinical Microbiology and Antimicrobials*, *14*, 53-60.

Mulholland, B. S., Forwood, M. R., & Morrison, N. A. (2019). Monocyte chemoattractant protein-1 (MCP-1/CCL2) drives activation of bone remodeling and skeletal metastasis. *Current Osteoporosis Reports*, *17*(6), 538-547. https://doi.org/10.1007/s11914-019-00545-7

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2016). Medical microbiology (8th ed.). Elsevier Health Sciences, Canada.

Myageri, A., Agnihotri, A. D., & Chauhan, L. D. (2019). Clinicopathologic study of appendix specimens: A two-year

retrospective study at a tertiary care center. *National Journal of Laboratory Medicine*, *8*(2).

N

Naher, H. S., & Ktab, F. K. (2013). Bacterial profile associated with appendicitis. *International Research Journal of Medical Sciences, 1*(2), 1-4.

Naqvi, S. A., Thompson, G. C., Joffe, A. R., Blackwood, J., Martin, D. A., Brindle, M., & Barkema, H. W. (2019). Cytokines and chemokines in pediatric appendicitis: A multiplex analysis of inflammatory protein mediators. *Mediators of Inflammation*, *2019*, 2359681.

Nia, A. A., & Zareifar, P. (2018). Mean platelet volume (MPV) in children with acute appendicitis. *8*(1), 3.

Nicola, Z., Virginia, M., Mauro, C., Amedeo, E., & Saverio, C. F. (2021). Quarantine and appendicitis: A macro-area experience. *Pediatric Gastroenterology, Hepatology and Nutrition*, *24*(1), 75–80.

Nielubowicz, G., & Mobley, H. (2010). Host–pathogen interactions in urinary tract infection. *Nature Reviews Urology*, *7*, 430–441. https://doi.org/10.1038/nrurol.2010.101

Nikaido, H. (1998). Multiple antibiotic resistance and efflux. *Current Opinion in Microbiology*, *1*, 516-523.

O

Okoro, I. (1998). The role of *Yersinia enterocolitica* in appendicitis in Zaria. *East African Medical Journal*, *65*(9), 625.

Oktay, M. M., Boğan, M., Çolak, S. T., Sabak, M., Gümüşboğa, H., & Eren, S. H. (2020). Evaluation of the diagnostic value of platelet indices in pediatric acute appendicitis. *Journal of International Medical Research*, *48*(9). https://doi.org/10.1177/0300060520946515

Orlova, E., Yeh, A., Shi, M., Firek, B., Ranganathan, S., Whitcomb, D. C., Finegold, D. N., Ferrell, R. E., Barmada, M. M., Marazita, M. L., Hinds, D. A., Shaffer, J. R., Morowitz, M. J., & 23andMe Research Team. (2019). Genetic association and differential expression of PITX2 with acute appendicitis. *Human Genetics*, *138*(1), 37–47.

Otto, M. (2004). Virulence factors of the coagulase-negative staphylococci. *Frontiers in Bioscience*, *9*, 841-863.

Ouyang, W., Rutz, S., Crellin, N. K., et al. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annual Review of Immunology*, *29*, 711-109. https://doi.org/10.1146/annurev-immunol-031210-101312

P

Paajanen, H., Mansikka, A., Laaaton, M., Ristamaki, R., Pulkki, K., & Kostiainen, S. (2002). Novel serum inflammatory markers in acute appendicitis. *Scandinavian Journal of Clinical and Laboratory Investigation*, *62*(8), 579-584.

Paczosa, M. K., & Mecsas, J. (2016). *Klebsiella pneumoniae*: Going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews*, *80*(3), 629-661. https://doi.org/10.1128/MMBR.00078-15

Pakbin, B., Brück, W. M., & Rossen, J. W. A. (2021). Virulence factors of enteric pathogenic *Escherichia coli*: A review. *International Journal of Molecular Sciences*, *22*(18), 9922. https://doi.org/10.3390/ijms22189922

Parija, S. C. (2012). *Textbook of Microbiology and Immunology* (2nd ed.). Elsevier, EIH Unit Ltd. Press.

Parkhomenko, I. U., Lozovskaia, L. S., & Giunko, V. A. (1991). The immunomorphological characteristic of the appendix in a viral-bacterial lesion in children with appendicitis. *Arkhiv Patologii*, *53*(5), 33.

Paterson-Brown, S. (2012). The Acute Abdomen and Intestinal Obstruction. In O. J. Garden, A. W. Bradbury, J. L. R. Forsythe, & R. W. Parks (Eds.), *Principles and practice of surgery* (6th ed.). Elsevier Churchill Livingstone, China.

Pathan, N. A., Shaikh, A. A., & Shaikh, M. A. (2018). Appendectomy. *The Professional Medical Journal, 25*(09), 1301–1305.

Prestwich, S. E., Cartwright, R., Lynch, J., Furstenau, T., School of Geographical Sciences and Urban Planning, School of Life Sciences, & Barrett, The Honors College. (2018). A review of the human vermiform appendix and its proposed function. In *Barrett, the Honors College Thesis/Creative Project Collection*.

Primasta, P., & Coldacre, M. J. (1994). Appendectomy for other *Salmonella typhimurium* appendicitis. *Journal of Pediatric Gastroenterology and Nutrition, 13*(1), 101.

R

Radenković, G., Petrović, V., Nedin-Ranković, G., Denčić, T., Živković, V., & Jocić, M. (2021). CD11C immunopositive cells in the human fetal vermiform appendix. *Vojnosanitetski pregled, 78*(8), 882-886.

Rafiei, M., Mohammad Gholi Mezerji, N., Shayan, Z., & Mosayebi, G. (2019). The accuracy of white blood cell count in the diagnosis of acute appendicitis using age-sex-adjusted receiver operating characteristic approach. *Medical Journal of the Islamic Republic of Iran, 33*, 139.

Rahman, M. A., & Karim, F. (2019). Histomorphological study of the wall thickness and luminal diameter of cadaveric vermiform appendix in Bangladeshi people. *American Journal of Medical Sciences and Medicine, 7*(3), 89-93.

Rahman, M. A., Azim, M. A., & Karim, F. (2019). Anatomical positions of vermiform appendix in Bangladeshi people: A postmortem study. *American Journal of Medical Sciences, 7*(3), 64-66.

Raoof Rahed, K., Abdulahmeed Midhat, T., & Falah Raheef, N. (2022). Use of procalcitonin and C-reactive protein as predictors and diagnostic tool of acute appendicitis. *Al-Kitab Journal for Pure Sciences, 3*(2), 163–170. https://doi.org/10.32441/kjps.03.02.p14

Rasquinha, M. T., Sur, M., Lasrado, N., & Reddy, J. (2021). IL-10 as a Th2 cytokine: Differences between mice and humans. *Journal of*

Immunology, 207(9), 2205-2215. https://doi.org/10.4049/jimmunol.2100565

Redmond, J. M., Smith, G. W., Wilasrusmee, C., & Kittur, D. S. (2002). A new perspective in appendicitis: Calculation of half-time (T(1/2)) for perforation. *American Surgeon, 68*(7), 593-597.

Riese, J., Niedobitek, G., Lisner, R., Jung, A., Hohenberger, W., & Haupt, W. (2004). Expression of interleukin-6 and monocyte chemoattractant protein-1 by peritoneal sub-mesothelial cells during abdominal operations. *Journal of Pathology, 202*(1), 34-40.

Riese, J., Schoolmann, S., Denzel, C., Herrmann, O., Hohenberger, W., & Haupt, W. (2002). Effect of abdominal infections on peritoneal and systemic production of interleukin 6 and monocyte chemoattractant protein-1. *Shock, 17*, 361–364.

Robbins, S. L., Cotran, R. S., & Kumar, V. E. (1996). *Pathologic Bases Of Disease* (4th ed.). Philadelphia: W.B. Saunders Company.

Roberts, M. C. (1996). Tetracycline resistance determinants: Mechanisms of action, regulation of expression, genetic mobility, and distribution. *FEMS Microbiology Reviews, 19*, 1-24.

Ryan, K. J., & Ray, C. G. (2014). Sherris. In *Medical Microbiology* (6th ed.). McGraw-Hill Education, USA.

S

Saliakellis, E., & Thapar, N. (2013). Paediatric GI emergencies. *Best Practice & Research Clinical Gastroenterology, 27*, 799–817.

Salih, M., Mehdi, A. J., & Abdullah, S. (2020). Human vermiform appendix of different age groups. *Egyptian Academic Journal of Biological Sciences, D. Histology & Histochemistry, 12*(1), 15-20.

Sarkar, A., Saha, A., Roy, S., Pathak, S., & Mandal, S. (2015). A glimpse towards the vestigiality and fate of human vermiform appendix: A histomorphometric study. *Journal of Clinical and Diagnostic Research, 9*(2), AC11.

Sarla, G. (2018). Acute appendicitis: Age, sex and seasonal variation. *Journal of Medical Science and Clinical Research*, *6*(6), 262–264.

Sastry, A. S., & Bhat, K. S. (2016). *Essentials of Medical Microbiology (1st* ed.). JAYPEE Brothers Medical Publisher (P) Ltd.

Shahmoradi, M. K., Zarei, F., Beiranvand, M., & Hosseinnia, Z. (2021). A retrospective descriptive study based on etiology of appendicitis among patients undergoing appendectomy. *International Journal of Surgery Open*, *31*, 100326.

Shaikh, M., & Gurukkal, C. M. K. (2018). Anatomical variations in the position of the vermiform appendix: An anatomical study of aborted fetuses. *International Journal of Scientific Study*, *6*(1), 147-150.

Shaker, Z. N., Mahdi, D. S., & Alsaimary, I. E. (2024). Demographical study of appendicitis patients in Basra/Iraq. *European Journal of Medical Sciences*, *6*(4), 1–5. https://doi.org/10.24018/ejmed.2024.6.4.2136

Sharma, J., Chaudhary, N., Bhargava, S., & Agrawal, I. (2017). Role of mast cells in appendicitis. *International Journal of Health Sciences Research*, *7*(5), 37–41.

Sharma, S. B., & Gupta, V. (2004). Acute appendicitis presenting as acute hemi-scrotum in a boy. *Indian Journal of Gastroenterology*, *23*, 150-151.

Shelton, T., Lefering, R., & Schwartz, R. W. (2003). Acute appendicitis: Current diagnosis and treatment. *60*, 502-505.

Shen, G., Li, S., Shao, Z., Liu, L., Liu, Q., Yu, H., & Mei, Z. (2021). Platelet indices in patients with acute appendicitis: A systematic review with meta-analysis. *Updates in Surgery*, *73*(4), 1327-1341.

Sherry, B., Tekamp-Olson, P., Gallegos, C., Bauer, D., Davatelis, G., Wolpe, S. D., Masiarz, F., Coit, D., & Cerami, A. (1988). Resolution of the two components of macrophage inflammatory protein-1, and cloning and characterization of one of those components, macrophage inflammatory protein-1 beta. *Journal of*

Experimental Medicine, *168*(6), 2251–2259. https://doi.org/10.1084/jem.168.6.2251

Short, A. R. (1920). The causation of appendicitis. *British Journal of Surgery, 8*, 171.

Simó Alari, F., Gutiérrez, I., & Gimenéz Pérez, J. (2017). Familial history aggregation on acute appendicitis. *BMJ Case Reports, 2017*.

Singhal, V., & Jadhav, V. (2007). Acute appendicitis: Are we over diagnosing it? *The Annals of The Royal College of Surgeons of England*, *89*(8), 766-769.

Siraj, F., Qureshi, M. A., Ahmad, M., Siddiqi, O., & Hassan, B. (2020). Relationship between fiber in diet and acute appendicitis. *Medical Forum Monthly, 31*(9), 181–184.

Soehnlein, O., Lindbom, L., & Weber, C. (2009). Mechanisms underlying neutrophil-mediated monocyte recruitment. *Blood, 114*(21), 4613–4623.

Soliman, G. A. (2019). Dietary fiber, atherosclerosis, and cardiovascular disease. *Nutrients*, *11*(5), 1155.

Soybel, D. I. (2008). Surgery basic science and clinical evidence. In Norton, H. A. (Ed.), *Appendix* (2nd ed., pp. 991-1010). New York: Springer Science and Business Media, LLC.

Standring, S. (2016). *Gray's Anatomy: The Anatomical Basis of Clinical practice* (41st ed.). Elsevier Limited.

Sucu, A., Tolunay, O., Banlı Cesur, İ., Özçelik, Z., Çelik, T., Reşitoğlu, S., & Çelik, Ü. (2018). Relationship between acute appendicitis and platelet indices in childhood. *Turkish Journal of Pediatric Emergency and Intensive Care Medicine, 64–68*.

Suvara, K., Layton, C., & B, J. D. (2019). Bancroft's theory and practice of histological techniques (8th ed.). Churchill Livingstone Elsevier.

Suzuki, Y., Yamashita, R., Shirota, M., *et al*. (2004). Sequence comparison of human and mouse genes reveals a homologous

block structure in the promoter regions. *Genome Research, 14*(9), 1711-1718. https://doi.org/10.1101/gr.2435604.

T

Takahashi, Y., Obata, S., Matsuura, T., Kawano, Y., Yanagi, Y., Yoshimaru, K., Izaki, T., & Taguchi, T. (2021). The experiences of interval appendectomy for inflammatory appendiceal mass. *Pediatrics International*, *63*, 88–93.

Tamura, R., Nakamura, K., Hirotani, T., Yasui, Y., & Okajima, H. (2022). Differences in isolated bacteria between perforated and non-perforated appendicitis: An analysis of 680 consecutive appendectomies in a single institution. *Pediatric Surgery International*, *38*(12), 1887–1893. https://doi.org/10.1007/s00383-022-05236-6

Teng, T. Z. J., Thong, X. R., Lau, K. Y., Balasubramaniam, S., & Shelat, V. G. (2021). Acute appendicitis: Advances and controversies. *World Journal of Gastrointestinal Surgery, 13*, 1293.

Téoule, P., de Laffolie, J., Rolle, U., & Reissfelder, C. (2020). Acute appendicitis in childhood and adulthood. *Deutsches Ärzteblatt International*, *117*(45), 764–774.

Theodorou, C. M., Stokes, S. C., Hegazi, M. S., Brown, E. G., & Saadai, P. (2021). Is *Pseudomonas* infection associated with worse outcomes in pediatric perforated appendicitis? *Journal of Pediatric Surgery*, *56*, 1826–1830. https://doi.org/10.1016/j.jpedsurg.2020.10.031

Tille, P. M. (2014). *Bailey and Scott's Diagnostic Microbiology* (13th ed.). St. Louis: Mosby Elsevier.

Truty, M. J., Stulak, J. M., Utter, P. A., et al. (2008). Appendicitis after appendectomy. *Archives of Surgery*, *143*(4), 413-415.

Tzortzopoulou, A. K., Giamarelou, P., Tsolia, M., Spyridis, N., Vakaki, M., Passalides, A., & Zavras, N. (2019). The Jumping Up (J-Up) Test: Making the diagnosis of acute appendicitis easier in children. *Global Pediatric Health*, *6*, 2333794X19884824.

U

Ulukent, S. C., Sarici, I. S., & Ulutas, K. T. (2016). All CBC parameters in diagnosis of acute appendicitis. *International Journal of Clinical and Experimental Medicine, 9*(6), 11871-11876.

V

Vuong, C., & Otto, M. (2002). *Staphylococcus epidermidis* infections. *Microbes and Infection, 4*(4), 481-489.

W

Watson Ng, W., Hampartzoumian, T., Lloyd, A., & Grimm, M. (2007). A murine model of appendicitis and the impact of inflammation on appendiceal lymphocyte constituents. *Clinical & Experimental Immunology, 150*(1), 169-178.

Whittle, C., Pérez, L., Cortes, M., Switt, M., Aguirre, J., & Castro, A. (2020). Appendicular lymphoid hyperplasia in the differential diagnosis of acute appendicitis: Sonographic findings. *Journal of Diagnostic Medical Sonography, 36*(1), 19–23.

Widdowson, C. A., & Klugman, K. P. (1998). Emergence of the M phenotype of erythromycin-resistant pneumococci in South Africa. *Emerging Infectious Diseases, 4*(2), 277-281.

Wu, H. P., Chang, C. F., & Lin, C. Y. (2003). Predictive inflammatory parameters in the diagnosis of acute appendicitis in children. *Acta Paediatrica Taiwanica, 44*, 227–731.

X

Xu, Y., Jeffrey, R. B., DiMaio, M. A., & Olcott, E. W. (2016). Lymphoid hyperplasia of the appendix: A potential pitfall in the sonographic diagnosis of appendicitis. *American Journal of Roentgenology, 206*(1), 189-194.

Y

Yamashita, H., Yuasa, N., Takeuchi, E., Goto, Y., Miyake, H., Miyata, K., Kato, H., & Ito, M. (2016). Diagnostic value of procalcitonin

for acute complicated appendicitis. *Nagoya Journal of Medical Science*, *78*(1), 79-88.

Yang, J., Liu, C., He, Y., & Cai, Z. (2019). Laboratory markers in the prediction of acute perforated appendicitis in children. *Emergency Medicine International*, *2019*, e4608053.

Yang, X., Zeng, J., Yu, X., Wang, Z., Wang, D., Zhou, Q., Bai, T., & Xu, Y. (2023). PCT, IL-6, and IL-10 facilitate early diagnosis and pathogen classifications in bloodstream infection. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), 103.

Yilmaz, M., Akbulut, S., Kutluturk, K., Sahin, N., Arabaci, E., Ara, C., & Yilmaz, S. (2013). Unusual histopathological findings in appendectomy specimens from patients with suspected acute appendicitis. *World Journal of Gastroenterology: WJG*, *19*(25), 4015.

Yılmaz, Y., Kara, F., Gümüşdere, M., Arslan, H., & Üstebay, S. (2015). The platelet indices in pediatric patients with acute appendicitis. *International Journal of Research in Medical Sciences*, *3*(6), 1388–1391.

Yu, C., Juan, L., Wu, M., Shen, C., & Wu, H. (2003). Systematic review and meta-analysis of the diagnostic accuracy of procalcitonin, C-reactive protein and white blood cell count for suspected acute appendicitis. *British Journal of Surgery*, *100*(3), 322.

Z

Zachos, K., Kolonitsiou, F., Panagidis, A., Gkentzi, D., Fouzas, S., Alexopoulos, V., Kostopoulou, E., Roupakias, S., Vervenioti, A., Dassios, T., Georgiou, G., & Sinopidis, X. (2023). Association of the bacteria of the vermiform appendix and the peritoneal cavity with complicated acute appendicitis in children. *Diagnostics*, *13*(11), 1839.

Zeillemaker, A. M., Hoynck van Papendrecht, A. A., Hart, M. H., Roos, D., Verbrugh, H. A., & Leguit, P. (1996). Peritoneal interleukin-8 in acute appendicitis. *Journal of Surgical Research*, *62*(2), 273-277. https://doi.org/10.1006/jsre.0207

Zhang, Y., Lyu, F.-X., Kang, Q., Dong, Z.-X., Zhao, T.-X., Xie, S.-N., & Luo, Q. (2018). Association of meteorological factors with pediatric acute appendicitis in China: A 7-year retrospective analysis. *Medicine, 97*(42).

Zlotnik, A., & Yoshie, O. (2000). Chemokines: A new classification system and their role in immunity. *Immunity, 12*(2), 121–127.

الخلاصة

شملت الدراسة الحالية (135) عينة ، حيث تم استبعاد (35) مريضاً ممن يعانون من آلام في البطن في أماكن غير محددة دون اشتباه خاص بالتهاب الزائدة الدودية. كما استُبعد المرضى الذين لديهم التهاب الغشاء البريتوني الناتج عن انفجار الزائدة، وكذلك أولئك الذين أُزيلت لديهم الزائدة الدودية عرضيًا أثناء إجراء فتح البطن لأسباب لا تتعلق بالتهاب الزائدة الحاد.

اجريت الدراسة الحالية على (100) عينة قسمت إلى مجموعتين : المجموعة الاولى شملت (50) مريضا عانوا من اعراض التهاب الزائدة الدودية الحاد كالحمى والإسهال والغثيان والقيء وآلام البطن التي تمتد الى الجهة اليمنى اسفل البطن ، وتم جمع عينات من المرضى الذين كانوا يترددون على مدينة الامام الحسين الطبية في محافظة كربلاء في الفترة من كانون الثاني 2024 الى حزيران 2024. شملت عينات المرضى الزائدة الدودية والدم بالمقارنة مع (50) عينة دم تم جمعها من مجموعة السيطرة (المجموعة الثانية) والتي كانت متطابقة مع مجموعة المرضى ولكن لم يكن لديهم أي أعراض لالتهاب الزائدة الدودية الحاد.

كما تضمنت الدراسة الاسس الاحصائية التي تشمل :- العمر والجنس والاعراض السريرية الاخرى ، أظهرت نتائجنا أن التهاب الزائدة الدودية الحاد يؤثر على جميع الفئات العمرية . تم جمع العينات من المرضى الذين تتراوح أعمارهم من (8 الى 60) سنة. وكانت نسبة الإصابة بالتهاب الزائدة الدودية في الغالب بين (16 - 30) سنة بنسبة 44% من إجمالي الحالات ، ثم (1 - 15) سنة بنسبة 40% ، (31 – 45) سنة بنسبة 14% ونسبة الأقلية 2% والتي تنتمي إلى الفئة العمرية (46 - 60) ، كما أظهرت هذه الدراسة أن التهاب الزائدة الدودية الحاد يؤثر على كلا الجنسين مع عدم وجود فروق ذات دلالة إحصائية بين المجموعتين ولكنه يؤثر على الذكور (54%) أكثر من الإناث (46%) . سجلت الدراسة الحالية الأعراض السريرية الشائعة المصاحبة لالتهاب الزائدة الدودية الحاد مثل: آلام البطن والقيء والغثيان والحمى والإسهال 50 (100%) ، 38 (76%) ، 37 (74%) ، 13 (26%) ، 5 (10%) على التوالي وتظهر فروقا إحصائية بين المرضى الذين يعانون من التهاب الزائدة الدودية الحاد والأعراض المرتبطة به.

تضمنت الدراسة الحالية عزل أنواع من الاجناس البكتيرية من الزوائد الدودية وأظهرت جميع العينات نموا إيجابيا. تم عزل البكتيريا الموجبة لصبغة غرام بمعدل أقل (6%) من البكتيريا السالبة لصبغة غرام (94%) . *Escherichia coli* التي تمثل 29 (58%) عزلة

، تليها عزلات *Klebsiella pneumoniae* 8 (16%) ، 5 (10 %) عزلة من *Psedomonas aeruginosa* ، 2 (4%) عزلة *Enterococcus faecalis*، 1 (2 %) عزلة من *Enterobacter aerogenes*، 1 (2%) *Salmonella* 1 (2%) من *Proteus mirabilis* . كانت *Staphylococcus aureus* أكثر الكائنات الحية الدقيقة شيوعا بين البكتيريا إيجابية الجرام والتي تمثل 2 (4%). في حين كانت *Staphylococcus epidermidis* 1(2%).

تم إجراء اختبار حساسية المضادات الحياتية على جميع العزلات ، كان كل من Imipenem و Amikacin مضادات حياتية فعالة ضد البكتيريا سالبة لصبغة غرام . وفي الوقت نفسه كانت Ticarcillin ,Vancomycin , Penicillin , Rifampicin فعالة ضد البكتيريا الموجبة لصبغة غرام ، وتم عزل جميع أنواع البكتيريا إيجابية الجرام وكانت مقاومة Benzyl penicillin و Oxacillin ، وكانت معظم العزلات السالبة لصبغة غرام مقاومة Ticarcillinو Piperacillin 92.8٪ . أظهرت العزلات السالبة لصبغة غرام أنها أكثر مقاومة من العزلات الموجبة لصبغة غرام .

أظهرت نتائج فحص الدم وجود ارتفاع معنوي في عدد خلايا الدم البيضاء الكلية (WBC) والعدلات في مجموعة مرضى AA مقارنة بالمجموعة الضابطة ، وكان هناك انخفاضا لم يكن معنويا في عدد الصفائح الدموية في مجموعة مرضى AA مقارنة بمجموعة السيطرة. في حين أظهرت النتيجة انخفاضا كبيرا في عدد الخلايا الليمفاوية ، كريات الدم الحمراء وعدد HCT بين مجموعة المرضى مقارنة مع مجموعة السيطرة.

اظهرت نتائج الفحص المناعي وجود فروق ذات دلالة إحصائية كبيرة للبروكالسيتونين ، (IL-8) ، (IL-10) IL-1Ra ، (MCP-1) ، (MIP) -1α حيث ان (*p value* =0.0001 **) بين مستويات مصل الدم لمجموعة التهاب الزائدة الدودية ومجموعة السيطرة الصحية..

كما تضمنت الدراسة التغيرات النسيجية المرضية والتي شملت الفحص المظهري والفحص المجهري لجميع الزوائد الدودية حيث لوحظت بعض هذه الزوائد كانت متضخمة والأنسجة محاطة بحويصلات ، وبعضها له جدران ليفية ومتقرحة بألوان مختلفة. أظهرت تغيرات الأنسجة تغيرات في التركيب النسيجي للزائدة الدودية ، متمثلة في توسع تجويفها واحتقان الأوعية الدموية الوريدية في الاغشية المخاطية ، وزيادة كمية الأنسجة اللمفاوية المنتشرة عبر

طبقات الزائدة الدودية ، تضخم الاوعية اللمفاوية ، تسلل الخلايا الالتهابية التي تنتشر عبر طبقات الزائدة الدودية.

جـــامعة كربلاء
كلية التربية للعلوم الصرفة
قسم علوم الحياة

عزل وتوصيف البكتيريا المرتبطة بالتهاب الزائدة الدودية الحاد وبعض التغيرات النسجية المرضية

اطروحة مقدمة

الى مجلس كلية التربية للعلوم الصرفة - جامعة كربلاء

وهي جزء من متطلبات نيل شهادة الدكتوراه في علوم الحياة علم الحيوان

كتبت بواسطة

ندى جاسم محمد

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بإشراف

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1446هـ **2025 م**