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The Role of C-reactive Protein and Procalcitonin in Differentiating Complicated and Non-Complicated Acute Appendicitis in Babylon Province

A Thesis

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By

Raad Gazy Faisel Sheltag

B.Sc. in Chemistry (University of Babylon)

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Supervised by

Professor Dr.
Moaed E. AL-Gazally

Professor Dr.
Mohend A.N Alshalah

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيم

صَدَقَ اللهُ العَلِيُّ العَظيم
سورة يوسف: (الآية٧٦)

Dedication

To the awaited Imam, may God hasten his honorable reappearance

To my lovely father and mother

To all members of my family

To all friends and lovers

To every* knowledge *student

I dedicate this study

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Thanks and praise to Allah, and prayer and peace upon His messenger Mohammed, and his own pure relatives and best colleagues.

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Raad

Supervisor Certification

We certify that this thesis was prepared under our supervision at the College of Medicine, University of Babylon, as partial fulfillment of the requirement for the master degree of science (M.Sc.) in Clinical Biochemistry.

Prof. Dr.
Moaed E.AL-Gazally

Prof. Dr.
Mohend A.N Alshalah

In review of the available recommendation, I forward this thesis for debate by the examining committee.

Prof. Dr.
Abdulsamie H. Alta'ee
Head of Chemistry and Biochemistry Department

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية الطب

فرع الكيمياء والكيمياء الحياتية

دور بروتين سي التفاعلي والبروكالسيتونين للتفريق بين التهاب الزائدة الدودية البسيط والمعقد في محافظة بابل

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من قبل

رعد غازي فيصل شلتاغ

بكالوريوس علوم الكيمياء/2006-2007

بإشراف

الأستاذ الدكتور

مؤيد عمران الغزالي

الاستاذ الدكتور

مهند عباس الشلاه

Summary

Appendicitis is the inflammation of the vermiform appendix which is the most common cause of acute abdomen in both adults and children all over the world.

Appendicitis occurs most commonly between the ages of 10 and 20 years and it has a male-to-female ratio of 1.4:1. The lifetime risk is 8.6% for males and 6.7% for females in the United States , The global incidence of appendicitis is (100 in North America, 105 in Eastern Europe, and 150 in Western Europe) in 100.000 person per year . The incidence is 160/100.000 person per year for Turkey

The aim of this study is to determine the role of C-reactive protein and procalcitonin as a markers to support modified Alvarado Score in differentiating Complicated Acute Appendicitis from Non-Complicated Acute Appendicitis by estimating the sensitivity and specificity of each of them, as well as which is more powerful to add to the diagnostic protocol.

To achieve this aims, 100 patients suffer from acute appendicitis with ages range between (15-55) years are classified into two groups according to the severity , 60 patients with non-complicated acute appendicitis , 40 patients with complicated acute appendicitis. C-reactive protein, procalcitonin concentration were determined by enzyme linked Immunosorbent assay (ELISA) method, Also total serum bilirubin was estimated by spectrophotometric method .

The results of present study reveals a significant increase in C-reactive protein concentration ($p < 0.001$) in complicated acute appendicitis patients when compared to those of the non-complicated acute appendicitis patients ,the sensitivity and specificity of c-reactive protein was 75%,68.7% respectively .Also significant increase in procalcitonin concentration ($p < 0.001$) in complicated acute appendicitis patients when compared to those of the non-complicated acute appendicitis patients, the sensitivity and specificity of procalcitonin was 75%,77.8% respectively. There was non-significant increase in concentration of total serum bilirubin in complicated acute appendicitis patients more than normal range .

The results showed significant positive correlations between modified Alvarado score and C-reactive protein ($r = 0.216$, $p < 0.05$), significant positive correlation between modified Alvarado score and procalcitonin ($r = 0.265$, $p < 0.05$) and significant positive correlation between C-reactive protein and procalcitonin ($r=0.546$, $p<0.005$) ,

In conclusion, , C-reactive protein and Procalcitonin were more effective in patients with complicated acute appendicitis ,also positive correlations between C-reactive protein and Procalcitonin with Alvarado score strength the possibility of differentiating between complicated and non-complicated acute appendicitis and Procalcitonin was more powerful from C-reactive protein in supporting modified Alvarado score to differentiate complicated from non-complicated acute appendicitis according to their sensitivity and specificity .

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Abbreviations	Key
AA	Acute Appendicitis
AD	Alzheimer Disease
AS	Alvarado Score
AUC	Area Under the Curve
CAA	Complicated Acute Appendicitis
CT	Computed Tomography
CRP	C-Reactive Protein
CVDs	Cardiovascular Diseases
COVID19	Corona Virus Disease 2019
COPD	Chronic Obstructive Pulmonary Disease
EAES	European Association of Endoscopic Surgery
ESR	Erythrocyte Sedimentation Rate
GALT	Gut Associated Lymphoid Tissue
GUE	General Urine Examination
HRP	Horseradish Peroxidase
IgG	Immunoglobulin G
IL	Interleukin
IL-1β	Interleukin 1 Beta
LA	Laparoscopic Appendectomy
mCRP	Monomeric C-Reactive Protein

mRNA	Messenger Ribo Nucleic Acid
NCAA	Non-Complicated Acute Appendicitis
NLR	Neutrophil Lymphocyte Ratio
NOM	Non-Operative Management
OA	Open Appendectomy
OD	Optical Density
pCRP	pentameric C-Reactive Protein
PD	Parkinson Disease
PCT	Procalcitonin
ROC	Receiver operating characteristic
RIPASA	Raja Isteri Pengiran Anak Saleha Appendicitis
SAP	Serum Amyloid P
SD	Standard Deviation
T2DM	Type Tow Diabetic Mellitus
TNF	Tumor Necrosis Factor

Chapter One

Introduction and Literature Review

1.Introduction

Appendicitis is the inflammation of the vermiform appendix which is the most common cause of acute abdomen in both adults and children all over the world(1).

Appendicitis occurs most commonly between the ages of 10 and 20 years and it has a male-to-female ratio of 1.4:1. The lifetime risk is 8.6% for males and 6.7% for females in the United States , The global incidence of appendicitis is (100 in North America, 105 in Eastern Europe, and 150 in Western Europe) in 100.000 person per year . The incidence is 160/100.000 person per year for Turkey (2)**.**

Appendicitis is a frequent disease whose etiology cannot be explained by any single factor. Luminal obstruction is the trigger event that culminates in inflammation of the appendix , Faecoliths are found in one-third of specimens. In the other cases, obstruction is thought to be caused by hypertrophy of mural lymphoid follicles in response to diverse causes (3).

Appendicitis often is separated into non-complicated or complicated forms . Non-complicated is acute appendicitis without any signs of perforation, abscess, or phlegmon; complicated appendicitis is a result of appendiceal rupture with subsequent abscess or phlegmon formation. Non-complicated acute appendicitis is most common in adolescents and young adults and complicated appendicitis is most common in the very young and very old(4).

Although acute appendicitis affects males and females in a nearly equal distribution, complicated appendicitis may occur slightly more often in males. Other demographic differences between patients with non-complicated and complicated appendicitis include mean time with symptoms

(less than 24 hours for non-complicated appendicitis compared with 48 hours or more for complicated appendicitis) as well as the distance the patient may live from the hospital(5).

1.1. Appendix

The appendix (or vermiform appendix; also cecal [or caecal] appendix; vermix; or vermiform process) is a finger-like, blind-ended tube connected to the cecum, from which it develops in the embryo. The cecum is a pouch-like structure of the large intestine, located at the junction of the small and the large intestines. The term "vermiform" comes from Latin and means "worm-shaped". The appendix was once considered a vestigial organ, but this view has changed since the early 2000s (6). The fact that early anatomical investigations were mainly done on animal species that do not have an appendix accounts for the appendix's late discovery in the scientific world (7) .

1.1.1. Anatomy of Appendix

The vermiform appendix originates from posteromedial wall of caecum in the large intestine. It is a narrow blind end like tube extension from caecum in the right lower abdomen approximately about 2cm in length just below the ileocecal valve. It is supported by mesoappendix, which is a double layered fold of peritoneum suspending the organ from the terminal ileum, The appendix is most mobile structure of abdomen. An average length of appendix is 6-9 cm, longer in males than in females. Anatomical variations in position of vermiform appendix can be associated with acute appendicitis (8). As shown in Figure 1-1.

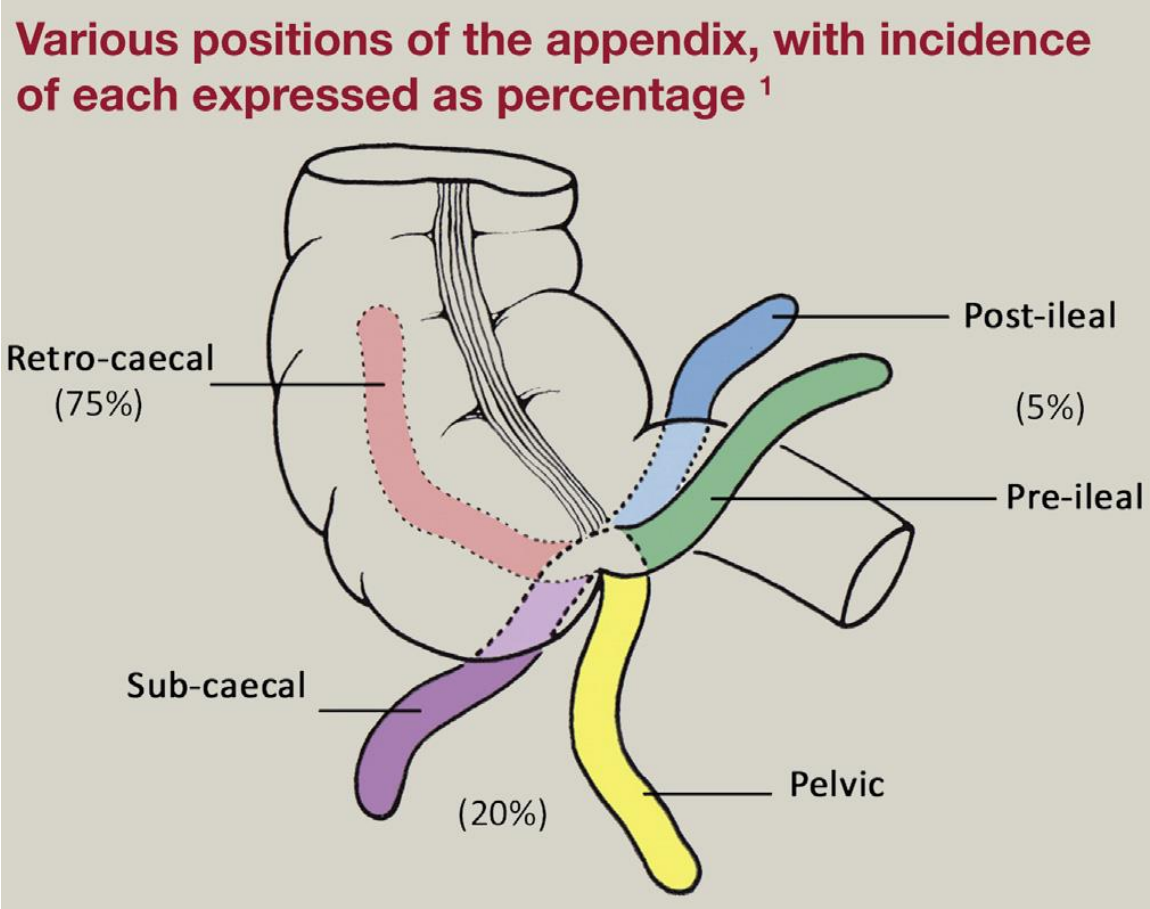

Figure 1- 1 different positions of vermiform (9).

According to anatomy textbooks, the appendix emerges at the cecum's posteromedial boundary, around 1.7 to 2.5 cm below the ileum's terminal section, Aside from this common root, there are various variations that Treves divides into four categories: Type 1 is a fetal appendix with a funnel-shaped origin; type 2 is an appendix that originates from the cecal fundus; form 3 is an appendix that develops dorsomedially out of the cecum (the most frequent type); type 4 is an appendix that originates alongside the ideal orifice (10) .

A vermiform appendix is not unique to the individual, it can be present in all hominoid apes, such as chimpanzees, gorillas, orangutans, and gibbons, as well as in variable degrees in several New World and Old World monkey species (11).

1.1.2.Function of Appendix

The function of the appendix has been described by authors in 1994 that unknown since it was first discovered in humans over 400 years ago and until the twenty-first century. On the other hand, a slew of biological evidence from histology and evolutionary investigations has clung to the notion that some yet to be discovered function must exist. Bollinger and colleagues have inferred that the cecal appendix is well equipped to aid the maintenance of biofilms containing mutualistic intestinal flora, which is an apparent purpose for which its tube-like form is seemingly well adapted (12). Function of vermiform appendix is immunological. Its function is similar to tonsil which provides protection to upper GI tract, The appendix acts as a guard for small intestine from different microbes which are present in large intestine (13).

Despite the fact that the appendix is an important part of the gut associated lymphoid tissue (GALT) system, lymphoid tissue first emerges in the appendix about 2 weeks after birth , Its endocrine cells produce amines and hormones to assist with various biological control mechanisms, whereas the lymphoid tissue is involved with maturation of B-lymphocytes and production of IgA antibodies (14). Every day, the appendix secretes 2–3 mL of mucus (15).

1.1.3.Appendicitis

Mc Burney of New York was the first to diagnose appendicitis early and to emphasize the significance of early surgical surgery in 1889. Early detection of appendicitis has been a joint goal of surgical publication since the time of Mc Burney and till now. The failure to make an early diagnosis is a major contributor to the high rate of morbidity and mortality (16).

1.1.3.1. Acute Appendicitis

Acute appendicitis (AA) is the most common cause of an acute abdomen needing surgery. However, the symptoms of AA overlap with those of a number of other illnesses, making identification difficult, especially early on (17) . For decades, appendicectomy surgery has been the typical classical urgent or emergent procedure of choice to avoid the developing inflammation that leads to perforation (18).

1.1.3.2.Chronic Appendicitis

When a patient is presented with chronic right iliac fossa pain, the chronic appendicitis is usually expected . An appendectomy is then recommended, as would be expected. (19).

The following criteria for chronic appendicitis have been proposed by some authors:Symptoms that last longer than two weeks, pathologic confirmation of chronic appendicle inflammation, and symptom relief after appendectomy. The symptoms of chronic appendicitis are similar to those of acute appendicitis, however they last longer (20).

1.1.4.Epidemiology of Appendicitis

A prevalence of appendicitis is 7–8% in worldwide , In United States, 250,000 cases of appendicitis are reported annually. Appendicitis is more common in older children and young adults, but it can theoretically show up at any age. A male to female ratio of 1.4:1 is common among young children (21).

In Asian and African countries, the incidence of acute appendicitis is probably lower because of dietary habits of the inhabitants of these geographic areas. Dietary fiber is thought to decrease the viscosity of faeces, decrease bowel transit time and discourage the formation of faecolith, which predispose individuals to obstructions of the appendiceal lumen. The incidence of appendicitis gradually rises from birth, peaks in the late 10 years and gradually declines in the geriatric years. It is most prevalent in the 10-19-year-old age group. In previous years, the number of cases in patients aged 30-69 has increased to 6.3% (22).

There is a difference in incidence between countries, with an increase in incidence being the most common. In newly industrialized countries, this has been observed. Appendicitis is thought to be caused by a lack of dietary fiber, This could explain why Western countries have a higher prevalence (23). However, appendicitis is becoming more common in newly industrialized countries at the turn of the century (24,25).

1.1.5. Etiology of Acute Appendicitis

The most common cause of Acute appendicitis is a faecolith (a hard lump of blocked feces) or lymphoid tissue obstructing the lumen, This causes appendix distension, bacterial overgrowth and infection, venous and lymphatic congestion, rupture, and infection transmission via the appendicular wall transmural (26).

Appendicitis can be caused by faecoliths, lymphoid hyperplasia, foreign bodies, cancer (most commonly carcinoid and adenocarcinoma), parasite and fungal infections, inflammatory bowel disease, and trauma The infection is by multi-organism, the most common bacteria cultured are Escherichia coli, Bacteroides, and Klebsiella. Fusobacterium growth appears to be linked to a very aggressive process and perforated appendicitis (27).

Perforation is infrequent in patients with simple appendicitis, even if treatment is delayed for 24 to 36 hours, implying that progression from simple to severe disease is varied (28).

1.1.6.Risk Factors for Acute Appendicitis

Appendicitis is caused by no direct factor according to Some previous studies, Despite the fact that certain factors appear to be linked to appendicitis or increase the risk of infection, they do not necessarily lead to infection (29). Anumber of studies have been conducted to identify factors that may help to prevent this disease or associated to cause it (30):

A-Diet

Dietary fiber has long been recognized as an important component of a well-balanced diet that is frequently disregarded. A sufficient amount of fiber in the diet not only helps to maintain proper gut function, but it also helps to avoid a variety of clinical conditions. Recent research has emphasized the importance of a high-fiber diet for an individual's general well-being and health (31).

Few researches on the function of dietary fiber in the prevention of acute appendicitis in adults and children have been conducted in Western populations. In 1998, Naeedar *et al*. discussed this issue and conducted a study that concluded that dietary fiber may not be the most important factor in acute appendicitis and that other luminal and/or morphological variables may be predisposing factors. Nelson *et al*. evaluated this phenomena in 1984 and found no statistically significant changes in average daily cereal intake between cases and controls, providing minimal support for reduced cereal fiber intake as a driver of appendicitis. Low water intake could also be a contributing factor. Infection and a hereditary predisposition to the disease may enhance susceptibility to the disease, according to some studies (32).

In 2016, Damanic *et al*. conducted a similar study on children and found that there is a significant link between a low-fiber diet and the incidence of appendicitis (p=0.0001), 14 (73.7 %) of the 19 patients on a low-fiber diet suffer acute appendicitis, while acute appendicitis affects just two people (12.5 %) on a high-fiber diet (33). All appendicitis patients had less portions of fruits and vegetables, whereas servings of fast/junk food

were substantially greater than those suggested by WHO in the Food Guide Pyramid (32).

B- Genetic Factors

Authors believe that appendicitis, like other inflammatory disorders, is caused by a combination of genetic and environmental factors (34–36) . Researchers report for the first time an association of appendicitis with polymorphism in five genes (IL-13, IL-17, CCL22, CTLA4, and CD44).They found a non-significant association with complicated appendicitis for SNP IL-6 rs1800975 which can give support to a previously reported association in a larger sample and confirmed previous reports of no association of SNP TLR4 rs4986790 with appendicitis(37) .

1.1.7.Complicated and Non-Complicated Acute Appendicitis

Simple or Non-complicated Acute Appendicitis(NCAA) is defined as a phlegmonous inflamed appendix without signs of necrosis or perforation, whereas complex or Complicated Acute Appendicitis (CAA) has focal or transmural necrosis, which eventually may lead to perforation. Differentiation between both entities is important, as non-complicated appendicitis may be treated conservatively with antibiotics without the need for surgery or may even resolve spontaneously without the need for antibiotic treatment (38) .

In contrast, patients with complicated appendicitis require emergency appendectomy with the exception of patients presenting with a per appendicular abscess .Multiple studies proposing that Non-complicated Acute Appendicitis and Complicated Acute Appendicitis are two distinct

entities with unique pathophysiology and that conservative treatment may be appropriate for the former (39,40). Several studies have shown that not all appendicitis leads to CAA, and that a significant percentage of individuals with appendicitis can be treated conservatively with antibiotics or monitoring (41).

Because epidemiological and immunological studies strongly support the view that histologically phlegmonous appendicitis (non-complicated acute appendicitis) and histologically gangrenous appendicitis possibly leading to perforation (complicated acute appendicitis), the distinction between different forms of AA through the use of abdominal ultrasound by radiologists will become more important in the near future (42–44).

Due to the high lifetime frequency of acute appendicitis, CAA (including abscess, harmfulness, and widespread peritonitis) continues to cause significant morbidity and mortality worldwide (45).

When perforation occurs, the morbidity and mortality rates linked with appendicitis increase, Tubal infertility can also be caused by appendiceal perforation, ruptured retrocecal appendicitis might manifest as a very severe variant of a common condition, such as a large retroperitoneal abscess or a right thigh abscess. As a result, it is self-evident that the surgeon's goal must be to avoid perforation (46).

A number of studies have attempted to assess CAA , its contributing elements at-risk patients and the influence on healthcare resource consumption. Diabetes mellitus, the duration of symptoms prior to surgery, extremes of age, various laboratory markers or other novel parameters, such as intra-abdominal pressure, types of medical insurance, imaging findings,

and the underlying pathology of inflamed appendix are some of the proposed associations with this condition (47). Perforation occurs at a rate ranging from 16% to 40%, with a larger frequency appearing in younger age groups (40–57%) and in people over the age of 50 (55–70%) (39).

1.1.8.Diagnosis of Acute Appendicitis

The majority of AA diagnoses are made on the basis of clinical characteristics, with radiography [ultrasonography (USG) and computed tomography (CT)] examinations preserved for selected individuals. Inability to diagnose AA at a beginning period can lead to complications such as perforation, which can cause considerable morbidity and even death (48).

The modified Alvarado score (AS) and the Raja Isteri Pengiran Anak Saleha appendicitis(RIPASA) score are two of the many screening and scoring methods available to help in the diagnosis of AA (49,50). However, scoring tools like these have been criticized for their lack of sensitivity and specificity, as well as their inability to predict the severity of AA , Many illnesses have symptoms that are similar to appendicitis such as Acute cystitis, acute pancreatitis, diverticulitis, ulcerative colitis, peritonitis, intestinal obstruction, trauma, hepatitis, dissecting aortic aneurysm, ovarian cyst and ectopic pregnancy are the most prevalent causes of non-specific abdominal pain (51).

The modified AS is a clinical performance indicator for appendicitis diagnosis, The score developed a 9-point clinical scoring system, in 1986 for the diagnosis of acute appendicitis based on symptoms, signs, and diagnostic tests in patients with suspected AA Table 1-1.

The Appendicitis Inflammatory Response score has largely replaced the modified AS as a clinical prediction too (52).

Table 1-1 : Modified Alvarado score criteria (50)

Modified Alvarado score	
Feature	**Score**
Migration of pain	1
Anorexia	1
Nausea	1
Tenderness in right lower quadrant	2
Rebound tenderness	1
Elevated temperature	1
Leukocytosis	2
Total	**9**

Many researchers are still working on developing a tool or marker that may predict the diagnosis of AA and differentiate between NCAA and CAA with high sensitivity and specificity. White blood cell (WBC) levels are usually elevated in appendicitis patients (53) .

Although elevated serum bilirubin has been reported to be a possible marker for appendix perforation, its sensitivity and specificity are insufficient (54–56) . For predicting perforation in AA , C-reactive protein (CRP) was found to be superior to bilirubin (57).

Some researchers have looked into the predictive significance of Neutrophil-to-lymphocyte ratio (NLR) in identifying AA , Neutrophil-to-lymphocyte ratio is a low-cost marker of subclinical inflammation that may be estimated quickly from differential WBC counts , NLR provides information on two different immunological and inflammatory pathways, making it a possible marker for predicting the severity of appendicitis. The lymphocyte count represents the regulatory route, whereas the neutrophil count indicates active and continuous inflammation (58).

1.1.9.Treatment of Appendicitis

Surgery has been the most generally acknowledged treatment since surgeons began performing appendectomies in the nineteenth century, with over 300,000 appendectomies performed annually in the United States (59) . When compared to open appendectomy (OA), current evidence demonstrates that laparoscopic appendectomy (LA) is the most successful surgical treatment, with a lower incidence of wound infection and post-intervention morbidity, a shorter hospital stay, and higher quality of life scores (60).

Despite all of the advancements in the diagnostic procedure, the critical decision of whether to operate or not remains difficult. Over the last 20 years, there has been renewed interest in the non-operative management of NCAA, most likely due to a more reliable analysis of postoperative

complications and costs of surgical interventions, which are mostly related to the increasing use of minimally invasive techniques (61).

Although conservative antibiotic management of other intra-abdominal infections (particularly uncomplicated acute sigmoid diverticulitis) has been well established, nonoperative management (NOM) of NCAA is still limited by conflicting results from recent studies with a high risk of bias (62).

However, the recently published Jerusalem Guidelines, as well as the European Association of Endoscopic Surgery (EAES) guidelines, state that there is insufficient evidence to advocate routine NOM, and hence appendectomy remains the treatment of choice in Europe and the United States (63).

1.2. C-Reactive Protein

C-reactive protein (CRP) is a homopentameric acute-phase inflammatory protein that was first discovered in 1930 by Tillet and Francis while studying the serum of patients with acute Pneumococcus infection. It was named after its reaction with the capsular (C)-polysaccharide of Pneumococcus (64).

Unlike antibodies, CRP was detectable from the initiation of infection and decreased significantly as the disease resolved. These modifications happened prior to the appearance of the immunoglobulin (IgG) response. According to recent research, CRP is a component of the innate immune system, where it functions as a pattern recognition molecule to activate the adaptive immunological response. In humans, CRP is regarded as the

quintessential acute phase reactant. Acute phase reactants are proteins that the liver produces in reaction to a number of clinical situations such as infection, inflammation, and trauma. C-Reactive protein is used clinically to evaluate the inflammatory response in disorders such as rheumatoid arthritis and vasculitis . C-Reactive protein generation has also been utilized to detect the presence of infection and the efficacy of treatment. The proinflammatory cytokine interleukin 6 (1L- 6) is the primary inducer of its release by the liver. C-Reactive protein belongs to the pentraxin protein family (65).

1.2.1. Synthesis and Structure of C-Reactive protein

C-Reactive protein is a 206-amino acid member of the short pentraxin group, which includes serum amyloid P component (SAP) and has significant evolutionary conservation (66). Pentraxins have a unique structure that consists of five identical non glycosylated globular subunits, each of which is made up of two β-pleated sheets that are non-covalently linked and arranged in a symmetric cyclic pattern around a central pore, resulting in a pentameric, discoidal, and flattened configuration (67).

C-reactive protein is mostly formed in the liver (68), generally during the transcriptional phase of a proinflammatory cytokine response. Interleukin -6 appears to be a major regulator, increasing de novo CRP production via overexpression of C/EBPβ and C/EBPδ, two essential transcription factors in this process (69). Furthermore, IL-6 signaling may be augmented by IL-1β and Tumor Necrosis Factor (TNF-α), both of which enhance CRP transcription rate (70), as shown in Figure 1- 2 .

Serum CRP levels tend to increase markedly 6–8 hours after first stimulation, peaking at 24–48 hours . C-Reactive protein concentration in

the blood is predominantly determined by its rate of production (71). Although the liver is the primary site for CRP production and release, its mRNA has been found in a variety of extra hepatic sites, as shown in Figure (1- 3) , including adipose tissue, lungs, renal cortical tubule epithelial cells, lymphocytes, and atherosclerotic lesions, in both macrophages and smooth muscle cells (72).

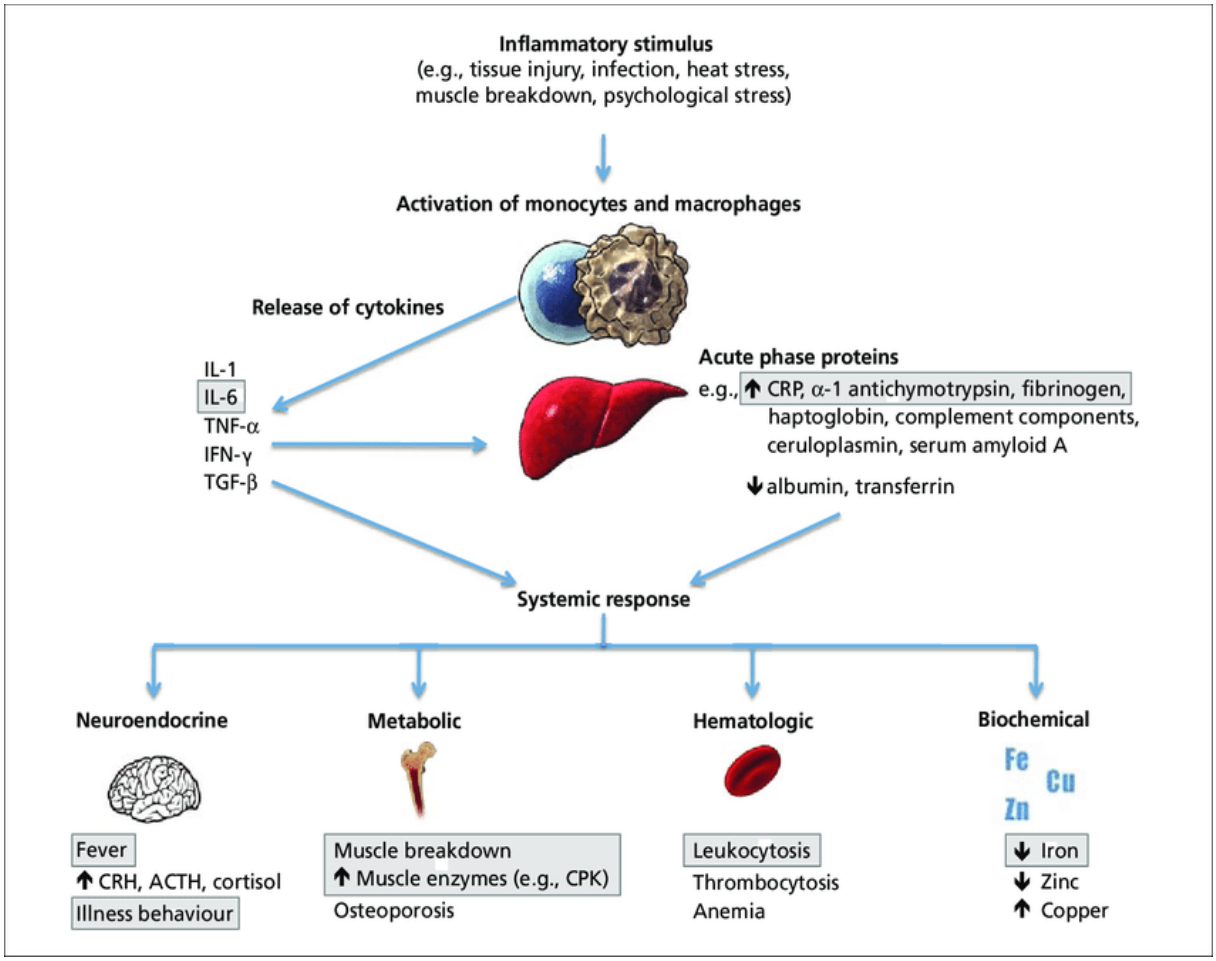

Figure 1- 2 :The acute phase reaction. An inflammatory stimulation activates monocytes and macrophages, causing them to produce cytokines. Cytokines increase the production of acute phase proteins in the liver. Cytokines, in conjunction with acute phase proteins, cause a systemic response that includes neuroendocrine, metabolic, hematologic, and biochemical alterations(73),[IL-1=interleukin-1,TNF-α=tumor necrosis alpha, IFN-ϒ=inter feron gamma,TGF-β=transforming growth factor beta, CRH=corticotropic hormone, ACTH=adrenocorticotropic hormone]

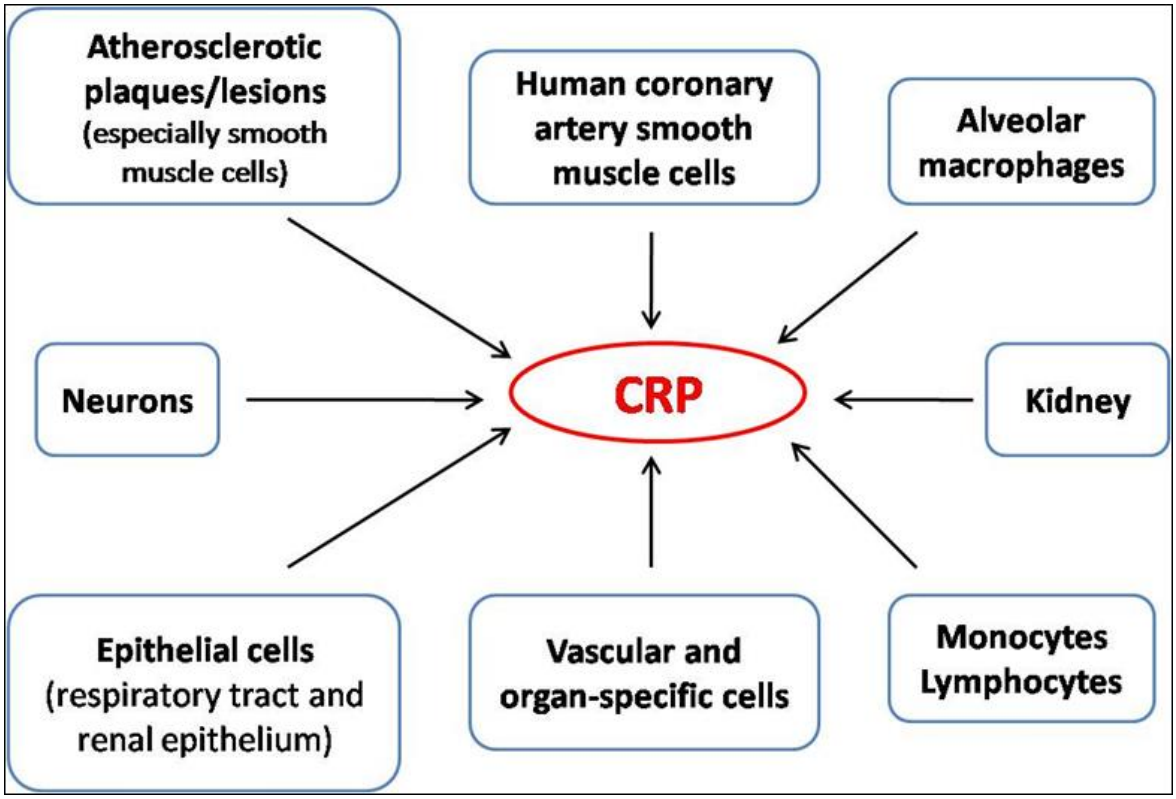

Figure 1-3: Extra hepatic sites of C-reactive protein (CRP) production (72).

1.2.2. Function of C-Reactive protein

C-reactive protein binds to phosphocholine, which is expressed on the surface of dead or dying cells as well as some bacteria, this activates the complement system, which promotes macrophage phagocytosis, which clears necrotic and apoptotic cells and bacteria (74).

On microorganisms, CRP binds to phosphocholine. It is hypothesized to help complement bind to foreign and destroyed cells and to improve phagocytosis by macrophages (opsonin-mediated phagocytosis) that express a CRP receptor. It functions as an early defensive system against pathogens in innate immunity (75).

1.2.3. Role of C-reactive protein in inflammation

C-reactive protein (CRP) is a pentraxin that comes in at least two different structural forms, such as native pentameric CRP (pCRP) and monomeric CRP (mCRP). According to studies, pCRP has both pro-inflammatory and anti-inflammatory effects depending on the setting . By contrast, mCRP exerts potent pro-inflammatory actions on endothelial cells, endothelial progenitor cells, leukocytes, and platelets and may amplify the inflammatory response (76).

In individuals with an acute inflammatory response, CRP is used as a serum biomarker (77). The increase in baseline CRP level has been reported to be effective in detecting chronic inflammation and tissue damage caused by excessive inflammation or failure of the inflammatory response . Higher CRP levels may indicate an acute infection or inflammation, hence they are frequently eliminated from chronic inflammation investigations. Cardiovascular diseases (CVDs) and complications leading to atherosclerosis may be caused by higher CRP concentrations over time rather than spikes in CRP (78).

CRP production is also linked to various chronic inflammatory disorders, such as hemorrhagic stroke, Alzheimer's disease (AD), and Parkinson's disease (PD) (79–81). Increased CRP levels have been linked to an increased risk of Type2 Diabetes Mellitus (T2DM) in cross-sectional and prospective studies (82) .

1.2.4. Role of C-reactive protein in acute appendicitis diagnosis

C-Reactive protein has been shown to be helpful in the diagnosis of appendicitis, however it lacks specificity and cannot be used to differentiate between infection locations. CRP values more than 1 mg/dL are typical in appendicitis patients, but very high levels of CRP in appendicitis patients signal gangrene, perforation, or suppurative disease progression, especially if the condition is accompanied by leukocytosis and neutrophilia. C-Reactive protein normalization, on the other hand, occurs 12 hours following the onset of symptoms. A normal CRP level has a negative predictive value of 97-100 percent for appendicitis in adults who have experienced symptoms for more than 24 hours, according to several prospective investigations (83).

1.3.Procalcitonin

Moya F *et al*., proposed the discovery of a calcitonin precursor in chicken in 1975. The hormone is produced when a big biosynthetic molecule divides intracellularly, and it was given the name Procalcitonin (PCT) (84). In 1981, a similar molecule was discovered in human thyroid medullary carcinoma tissue, leading to the identification of PCT's precise structure (85).

Procalcitonin levels in healthy individuals are generally lower than the detection threshold, and it has only been observed to elevate in patients with medullary thyroid cancer and small cell lung cancer. For the first time in 1993, elevated levels of PCT were discovered in patients with bacterial illnesses (86).

1.3.1. Synthesis and structure of procalcitonin

Procalcitonin is generated in the thyroid C-cells and cleaved from pre-procalcitonin by an endopeptidase in the endoplasmic reticulum in healthy people Figure 1- 4, N-terminal PCT, C-terminal katacalcin, and active calcitonin are formed once PCT is further broken down. The serum PCT level in healthy people is below detectable because all PCT generated in C-cells is broken down into the above-mentioned products and no PCT reaches the circulation. Furthermore, no plasma enzymes can break down PCT once it reaches the circulation, therefore it has a half-life of 25-30 hours and remains unaltered (87).

Procalcitonin levels are significantly elevated in the event of a microbial infection, as it is released by all parenchymal tissue under the influence of endotoxins and pro-inflammatory cytokines (88).

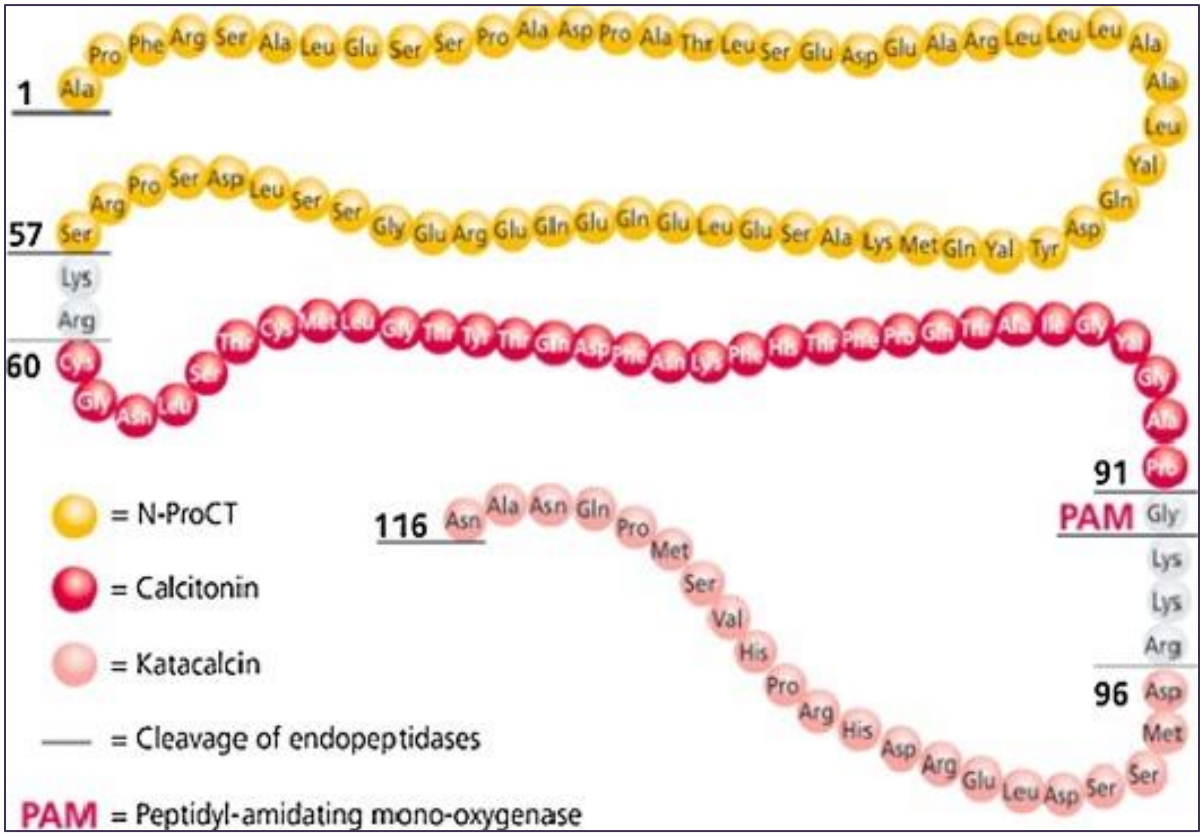

Figure 1- 4: Structure and production of procalcitonin : precursor of Calcitonin is procalcitonin (PCT). The calc-1 gene on chromosome 11 of the human genome is the site of development. The initial translation product after CT-DNA to mRNA is pre-procalcitonin, which then undergoes many modifications to become PCT (89) .

1.3.2. Role of procalcitonin in different situations

Procalcitonin has been found to be one of the most important biochemical indicators that correlates with the severity of the inflammatory host response to microbial infections in recent research . Procalcitonin concentration rises preferentially in cases of bacterial infections, but it remains normal in cases of viral infection . The level PCT of has been found to be related to the severity of inflammation . procalcitonin has a diagnostic value that far outweighs commonly used indicators like fever, leukocyte

count, and Erythrocyte Sedimentation Rate (ESR). It is a more accurate indicator than CRP , which increases in inflammatory situations (90).

1.3.2.1.Procalcitonin in sepsis

Procalcitonin is one of the most studied host-directed markers. Its synthesis mechanism can change depending on the severity of the inflammation. Because the protein is not released into the blood in the absence of systematic inflammation, serum PCT is undetectable in healthy people . However, when sepsis is caused by bacterial infections, PCT synthesis is stimulated in almost all tissues, making it detectable in the blood. Bacterial toxins such as endotoxin and cytokines [e.g., interleukin (IL)-1beta, interleukin-6, and tumor necrosis factor (TNF)-alpha] stimulate PCT production . PCT synthesis is not triggered in the majority of viral infections due to cytokines generated during viral infections that block TNF-alpha production . Furthermore, PCT has a broad biological range and a quick time to induction following administration (91).

1.3.2.2. Procalcitonin in Lower Respiratory Tract Infection

Procalcitonin guided antibiotic treatment choice techniques have gotten a lot of attention since the initial pilot research in 2004 . This strategy, for example, could be used to guide antibiotic treatment in patients with acute exacerbations of chronic obstructive pulmonary disease (COPD) , as well as treatment duration in patients with community-acquired pneumonia and severe infectious diseases in intensive care units (92).

1.3.2.3.Procalcitonin in appendicitis

Biomarkers such as (PCT) have recently been demonstrated to have potentially good diagnostic accuracy and reliability, suggesting that they could be more relevant indices in the diagnosis of appendicitis and in certain situations, predict the severity of the condition (93).

Procalcitonin is generally undetectable in healthy people (serum concentration less than (0.05 ng/ml). Procalcitonin is generated rapidly by most parenchymal tissues throughout the body when activated by endotoxin or inflammatory cytokines. Procalcitonin, unlike CRP, does not react to sterile inflammation or viral infection, This distinguishes it as an useful biomarker with a broad range of clinical applications, including AA (94).

Aims of study :

1. Determine the effective of C-reactive protein and Procalcitonin in complicated and non-complicated acute appendicitis.
2. Assessment the correlation of C-reactive protein and Procalcitonin with Alvarado score for supporting the differentiating complicated acute appendicitis from non-complicated acute.
3. Estimation of sensitivity and specificity of C-reactive protein and procalcitonin to determine which is more diagnostic to support the differentiation between complicated and non-complicated acute appendicitis.

Chapter Two

Material and methods

2.1. Materials

2.1.1. Study settings

This study was carried out from patients collected from Emergency Department in AL-Hilla general teaching hospital in Babylon province in Hilla city. The samples were collected from October 2021 till January 2022. Patients with Alvarado score 5 or more were enrolled in this study. All patients had their histories and physical examinations been done by specialist doctor on call, and investigations such as (WBC, GUE, and occasionally US) were performed. The practical side of the study was performed at the laboratory of biochemistry department in College of Medicine / University of Babylon.

2.1.2. Study design

This study was designed as a Cross sectional study.

2.1.3. Study population

This study includes 100 diagnosed acute appendicitis patients, the age was ranged between (15 - 55) years.

These patients were divided into two groups, the first group included 60 patients with NCAA (25 male and 35 female) and the second group included 40 patients with CAA (22 male and 18 female),as shown in Figure 2-1.

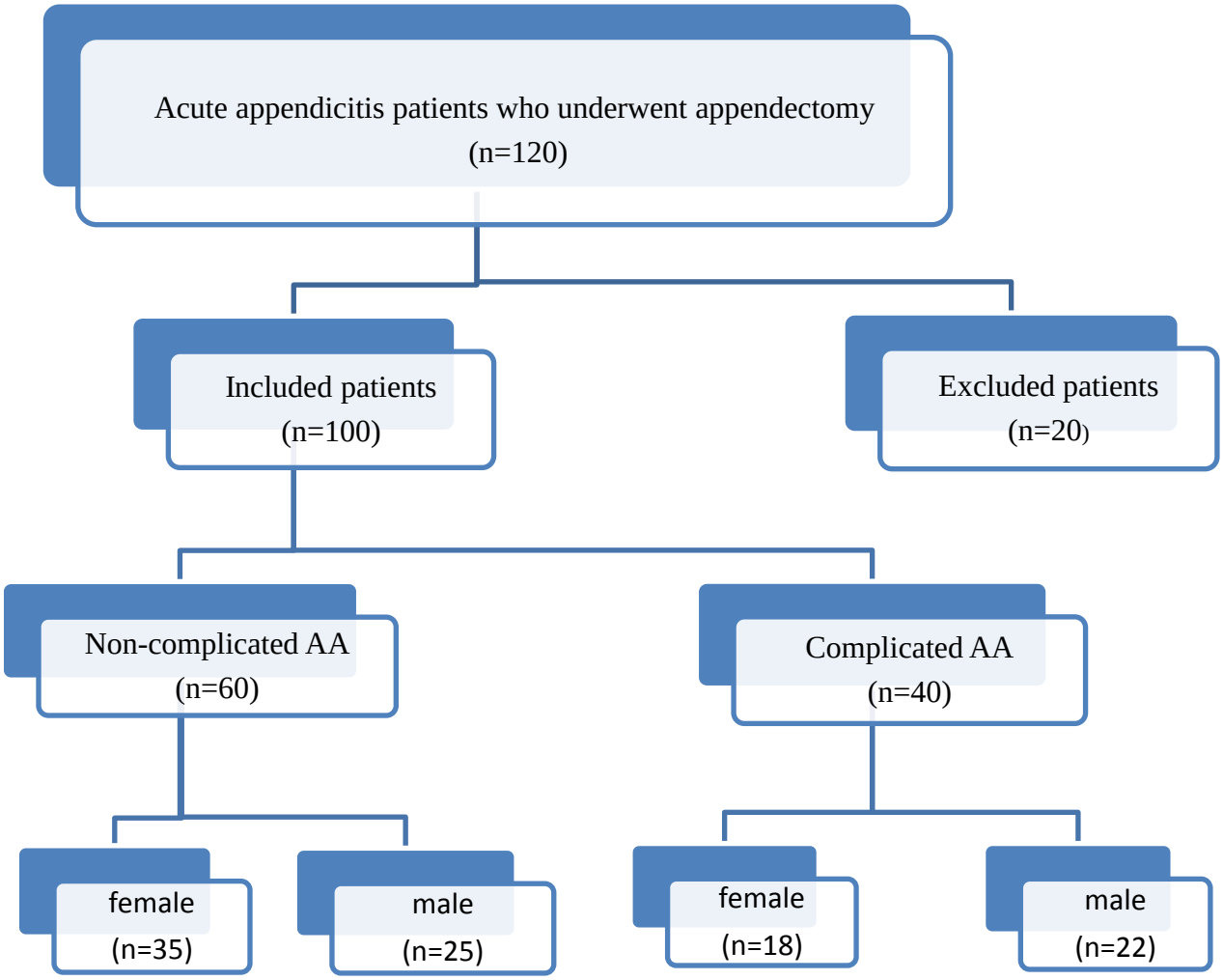

Figure 2-1 : patient distribution in this study.

2.1.4. Ethical issues

Depends on the following:

a. Approval by the Babylon Medical College (University of Babylon, Iraq) scientific committee and the Biochemistry Department of the same college.

b. The medical committee of AL-Hilla General Teaching Hospital in Babylon province, Hilla city, has given their approval.

c. All patients in the present study were verbally accepted participation once the purposes and methodology of the investigation were explained to them.

2.1.5. Data collection

The inclusion and exclusion criteria for this study are as follows:

a- **Inclusion criteria:** The following were the inclusion criteria:

(I) Patients who agreed to participate .

(II) Patients with histologically confirmed acute appendicitis.

b- **Exclusion criteria:** The following were the exclusion criteria:

(I) Patients treated with anti-Inflammatory drugs .

(II) Patients with a known chronic inflammatory disease.

(III) Patients with a known acute inflammatory disease.

(IV) Patients with normal appendix.

(V) Pregnant women .

(VI) Patients with liver disease.

(VII) Patients with thyroid disease.

(VIII) Children .

2.1.6 Questionnaire

A questionnaire sheet is attached in appendix at the end of the thesis

2.1.7 Blood collection

Venous blood samples were obtained from all subjects included in present study with a disposable sterile syringe (five milliliters). Each patient had five milliliters of blood been drawn from their veins and slowly put into two parts, first part 3 ml put in gel tubes and allowed to clot for 10-15 minutes at 37°C before being centrifuged at 3000 x g for 10-15 minutes.

And the residual 2ml of hole blood put in (EDTA) tubes. The serum was then split into two parts and stored at -20°C until analysis [CRP , PCT and total bilirubin].

2.2. Chemicals: The chemicals used in this research are shown in the following Table 2-1

Table 2- 1: kits used for this study

No.	Chemical compound	Origin
1.	Human C-Reactive protein ELISA Kit	Bioassay (China)
2.	Human Procalcitonin ELISA Kit	Bioassay (China)
3.	Total serum bilirubin spectrophotometric kit	China

2.3. Instruments

The instruments and tools used in this study are shown in Table 2-2.

Table 2-2: Tools and Instruments Used in this Study

No.	Tools and Instruments	Company/ Origin
1.	Autoclave	Germany
2.	Centrifuge	Hitachi / Germany
3.	Deep Freeze	GFL / Germany
4.	Disposable syringes	Medical jet (Syria)
5.	Eppendorf tube	China
6.	EDTA tube	AFCOVAC (Jordan)
7.	Micropipettes	Slamed (Germany)
8.	Pipette tips	China
9.	ELISA reader and washer	Biotech/ USA
10.	Gel tube (5ml)	AFCOVAC (Jordan)
11.	UV transiluminator	E-Graph / Japan
12.	Spectrophotometer	Japan

2.4. Methods

2.4.1. Determination of Human C-Reactive Protein concentration

The Enzyme linked immuno sorbent assay (ELISA) technology was used to assess C-Reactive protein in the kit produced by Bioassay-china.

Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human CRP antibody. CRP present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human CRP Antibody is added and binds to CRP in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated CRP antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human CRP. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm (95).

Reagent Preparation

1. All reagents was brought at room temperature before use.

2. Standard Reconstitute the 120µl of the standard (96ng/ml) with 120µl of standard diluent was generate a 48ng/ml standard stock solution. the standard was sit for 15 min with gentle agitation prior to making dilutions. therefore, must Prepare duplicate standard points by serially diluting the standard stock solution (48ng/ml) 1:2 with standard diluent to produce 24ng/ml, 12ng/ml, 6ng/ml and 3ng/ml solutions. Standard diluent serves as

the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions using as described in Table 2-3.

Table 2-3:Standard preparation steps

Standard Concentration	**Standard No.5**	**Standard No.4**	**Standard No.3**	**Standard No.2**	**Standard No.1**
96ng/ml	**48ng/ml**	**24ng/ml**	**12ng/ml**	**6ng/ml**	**3ng/ml**
	120µl Original Standard + 120µl Standard Diluent	120µl Standard No.5 + 120µl Standard Diluent	120µl Standard No.4 + 120µl Standard Diluent	120µl Standard No.3 + 120µl Standard Diluent	120µl Standard No.2 + 120µl Standard Diluent

3. Wash Buffer : 20ml of Wash Buffer Concentration 25x was dilute in to deionized or distilled water to yield 500 ml of 1x Wash Buffer. and mixed gently until the crystals have completely dissolved.

Reagent

Table 2-4: Reagent provided in kit.

Components	Quantity
Standard Solution (96ng/ml)	**0.5ml x1**
Pre-coated ELISA Plate	**12 * 8 well strips x1**
Standard Diluent	**3ml x1**
Streptavidin-HRP	**6ml x1**
Stop Solution	**6ml x1**
Substrate Solution A	**6ml x1**
Substrate Solution B	**6ml x1**
Wash Buffer Concentrate (25x)	**20ml x1**
Biotinylated Human CRP Antibody	**1ml x1**
User Instruction	**1**
Plate Sealer	**2 pic**
Zipper bag	**1 pic**

Procedure

1. All reagents, standard solutions and samples were prepared according to the instructions . All reagents was brought at room temperature before use. all assay was done at room temperature.

2. The number of strips required for the assay was determined. the strips was inserted in the frames for use. The unused strips were stored at 2-8°C.

3. Standard adding : A volume of 50µl standard was added to standard wells. Antibody was not added to standard wells because the standard solution contains biotinylated antibody.

4. Sample: A volume of 40µl sample was added to the sample wells and, then added 10µl anti-CRP antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well)and mixed well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. The sealer was removed and washed the plate 5 times with washing buffer. Soak wells with at least 350 µl washing buffer for 30 seconds for each wash. Blot the plate on to paper towels or other absorbent material.

6. Substrate: A volume of 50µl substrate solution A was added to each well and then 50µl substrate solution B were added to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. A volume of 50µl Stop Solution was added to each well, the blue color would immediately change into yellow.

8. The optical density (OD value) of each well was determined instantly using a micro plate reader set to 450 nm within 10 minutes after the stop solution was added.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and was draw a best fit curve through the points on the graph.

This standard curve is depicted in Figure 2-2.

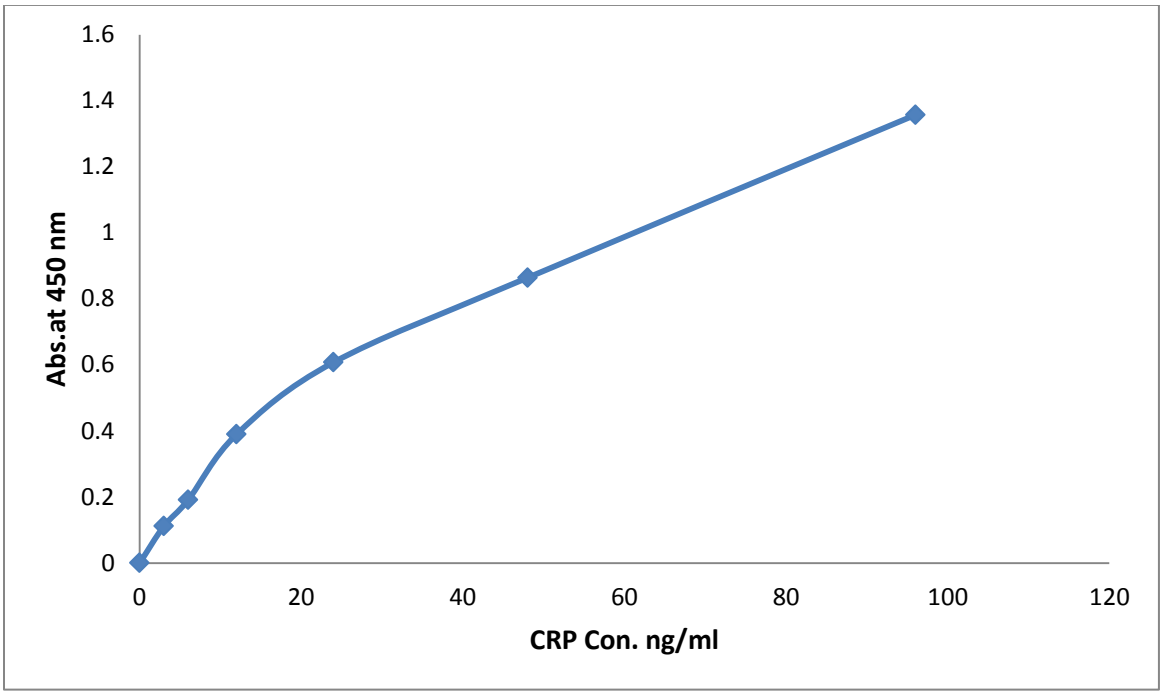

Figure 2-2:standard curve for C-reactive protein**.**

2.4.2. Determination of Human procalcitonin concentration

The Enzyme linked immune sorbent assay (ELISA) technology was used to assess procalcitonin in the kit produced by Bioassay-china.

Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human PCT antibody. PCT present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human PCT Antibody is added and binds to PCT in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated PCT antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of Human PCT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm. (96).

Reagent Preparation

1. All reagents was brought at room temperature before use.

2. Standard Reconstitute the 120µl of the standard (2400pg/ml) with 120µl of standard diluent was generate a 1200pg/ml standard stock solution. the standard was sit for 15 min with gentle agitation prior to making dilutions. therefore, duplicate standard points was prepared by serially dilution the standard stock solution (1200pg/ml) 1:2 with standard diluent to produce 600pg/ml, 300pg/ml, 150pg/ml and 75pg/ml solutions. Standard diluent serves as the zero standard(0 pg/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions using as described in Table 2-5.

Table 2-5:standard preparation steps

Standard Concentration	**Standard No.5**	**Standard No.4**	**Standard No.3**	**Standard No.2**	**Standard No.1**
2400g/ml	**1200g/ml**	**600g/ml**	**300pg/ml**	**150pg/ml**	**75pg/ml**
	120µl Original Standard + 120µl Standard Diluent	120µl Standard No.5 + 120µl Standard Diluent	120µl Standard No.4 + 120µl Standard Diluent	120µl Standard No.3 + 120µl Standard Diluent	120µl Standard No.2 + 120µl Standard Diluent

3. Wash Buffer : A volume of 20ml of Wash Buffer Concentration 25x was dilute in to deionized or distilled water to yield 500 ml of 1x Wash Buffer. and mixed gently until the crystals have completely dissolved.

Reagent

Table 2-6: Reagent provided in kit.

Components	Quantity
Standard Solution (2400g/ml)	**0.5ml x1**
Pre-coated ELISA Plate	**12 * 8 well strips x1**
Standard Diluent	**3ml x1**
Streptavidin-HRP	**6ml x1**
Stop Solution	**6ml x1**
Substrate Solution A	**6ml x1**
Substrate Solution B	**6ml x1**
Wash Buffer Concentrate (25x)	**20ml x1**
Biotinylated Human PCT Antibody	**1ml x1**
User Instruction	**1**
Plate Sealer	**2 pic**
Zipper bag	**1 pic**

Procedure

1. All reagents, standard solutions and samples were prepared according to the instructions . All reagents was brought at room temperature before use. all assay is done at room temperature.

2. The number of strips required for the assay were determined. the strips were inserted in the frames for use. The unused strips was stored at 2-8°C.

3. Standard adding : A volume of 50µl standard was added to standard well, antibody was not added to standard well because the standard solution contains biotinylated antibody.

4. Sample: A volume of 40µl sample was added to the sample well and then we added 10µl anti-PCT antibody to sample well, then we added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well) and mixed well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. The sealer was removed and washed the plate 5 times with washing buffer. Soak wells with at least 350 µl washing buffer for 30 seconds to for each wash. Blot the plate on to paper towels or other absorbent material.

6. Substrate: A volume of 50µl substrate solution A was added to each well and then 50µl substrate solution B were added to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7. A volume of 50µl Stop Solution was added to each well, the blue color would immediately change into yellow.

8. The optical density (OD value) of each well was determined instantly using a micro plate reader set to 450 nm within 10 minutes after the stop solution was added.

Calculation of Result

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and was draw a best fit curve through the points on the graph. This standard curve is depicted in figure (2-3) .

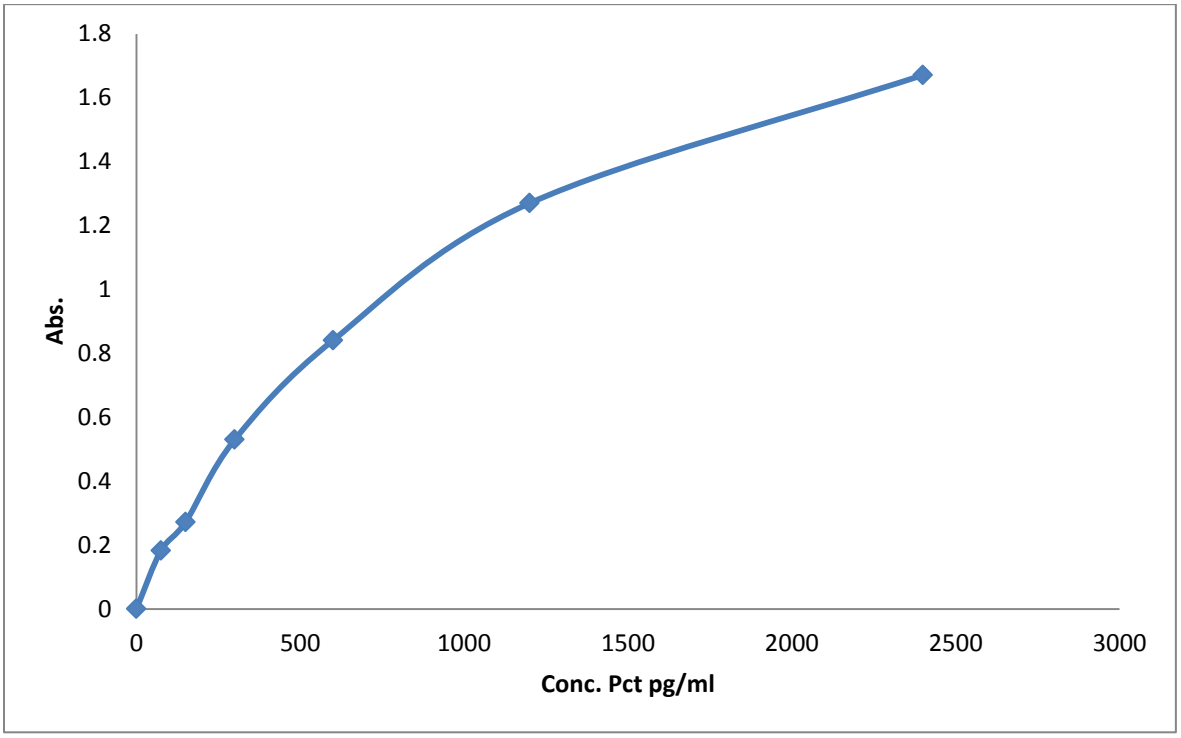

Figure 2-3 Standard curve for Procalcitonin

2.5. Data Analysis

Statistical analysis was carried out using SPSS software. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). Student t-test was used to compare means between two groups. Pearson chi-square and fisher-exact test were used to find the association between categorical variables. Pearson correlation coefficient was used to assess the relationship between two continuous variables. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of CRP and PCT. The area under the curve(AUC) provides a useful tool to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and predictive marker. A p-value of ≤ 0.05 was considered as significant.

Chapter Three

Results and Discussion

3. Results and Discussion

This study included 100 patients divided to two groups, 60patients with NCAA and 40 patients with CAA in different areas of Babylon Province in Iraq.

3.1.Demographic features of the Study groups

The demographic of both CAA and NCAA patients groups are shown in Table 3-1. The results showed no significant differences ($p > 0.05$) in age, gender and residence in CAA patients group when compared to those of the NCAA patients group.

Table 3-1: Demographic characteristics of the study groups

Characteristic	Sub-group	Non-complicated acute appendicitis group	Complicated acute appendicitis group	Total Patients	P–value
Age (years)	**15-35** **36-55**	**53 (88.3 %)** **7 (11.7 %)**	**31 (77.5 %)** **9 (22.5 %)**	**84 (84%)** **16 (16%)**	**0.215**
Gender	**Male** **Female**	**25 (41.7%)** **35 (58.3%)**	**22 (55%)** **18 (45%)**	**47 (47%)** **53 (53%)**	**0.173**
Residence	**Urban** **Rural**	**24 (40%)** **36 (60%)**	**14(35%)** **26 (65%)**	**38 (38%)** **62 (62%)**	**0.306**

3.1.1. Age

The mean age of CAA patients group was 24.09 ± 8.401 years and that of NCAA patients group was 26.64 ± 10.094 years. In this study, most of the cases (CAA and NCAA) fall within the age group of 15–35 years .

That's due to The appendix tissue has characteristics of a lymphoid organ, and there is more lymphoid tissue in young people. Any obstruction in the appendix lumen can produce lymphoid hyperplasia, which can progress to appendicitis if the condition is not treated. As a result, young people are more likely to develop appendicitis.

These results are supported by research from around the world were found in Lahore, Pakistan, where 66% of the population was between the ages of 15 and 30 years old (97).

Also, this study agrees with another conducted in Al-Aziziyah Hospital in Wasit Governorate, which states that The age group of 10 to 19 years had the highest incidence of appendicitis, while the age group of 50 and up had the lowest (98).

According to another study, only 6% of the instances were documented in the first decade of life, whereas 65.9% occurred between the ages of 11 _ 30 years. Males and females experienced the highest rates in the second and third decades, respectively (99).Another researches show that In individuals with AA, the incidence of appendiceal perforation is estimated to be 20-30%, increasing to 32-72 % in those over 60 years old(100).

3.1.2. Gender

Male patients group comprised of 47% from the total patients and female patients group comprised 53% ,as show in Table 3-1 . These patient distributed into CAA patients (22 male patients and 18 female patients) and NCAA patients (25 male patients and 35 female patients) .

The overall number of male patients in the present study was 47 (47%), and the total number of female patients was 53 (53%), which agrees with Thabit *et al*.,2012 findings of a high incidence in females approximately 53.3 % (101). Another finding agrees with present study, Seerwan *et.al.,* shown that female 51.4% and male 48.6% (102).

The present study disagree with study in Al-Aziziyah Hospital in Wasit Governorate, which states that male to female ratio was 1.27:1 (56% males and 44% females) (98) , also disagree with the findings in other studies (103,104).

3.1.3. Residency

The comparison of the percentage distribution of CAA patients and NCAA patients according to residency is shown in Table 3-1. Complicated Acute Appendicitis patients group included 14 (35 %) patients from urban areas and 26 (65 %) patients from rural areas; whereas, NCAA patients‘ group included 24 (40 %) patients from urban areas and 36 (60%) patients from rural areas. There was no significant difference in the frequency distribution of NCAA patients and CAA patients according to residency ($P > 0.05$).

Through the research, it was found that most patients with CAA are from rural areas, and these results are identical to the research that concluded "In comparison to urban individuals with appendicitis, rural patients are more prone to have their appendix perforated (105).

3.2. Modified Alvarado score

Modified Alvarado scored (AS) appendicitis patients from their clinical symptoms used laboratory results to divide them into three groups: patients with 7-9 points, who were highly suspected of having acute appendicitis and required immediate surgery, patients with 0-4 points, who were discharged without further evaluation due to the low chances of having acute appendicitis, and patients with 5-6 points, who were difficult to diagnose based on the scores alone (50). In present study, 15 patients (15%) had an Modified AS (5-6) points, and 85 patients (85%) had an Modified AS (7-9) points ,Thus, it was concluded that for patients with lower points(less than 6), their symptoms are nonspecific, resulting in delayed diagnosis and increased possibility of developing CAA

According to Alvarado scoring system, patients number distributed as in the Figure 3-1.

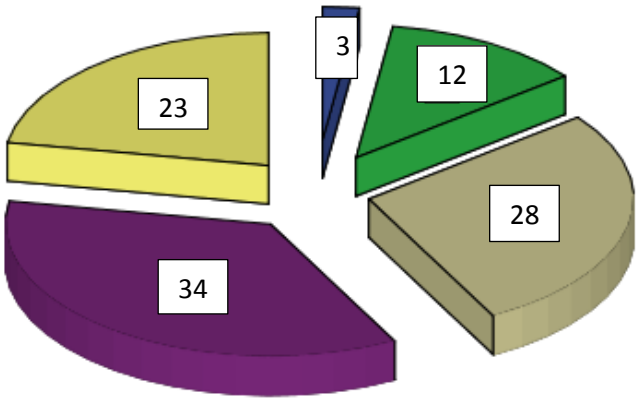

Figure 3-1: Explained the patients number distributed according the Modified Alvarado score system ,(5, 6, 7, 8 , 9)

3.2.1.Modified Alvarado score in complicated and non-complicated acute appendicitis

According to the Modified Alvarado scoring system, The distribution of patients with CAA and NCAA have shown in Figure 3-3 .

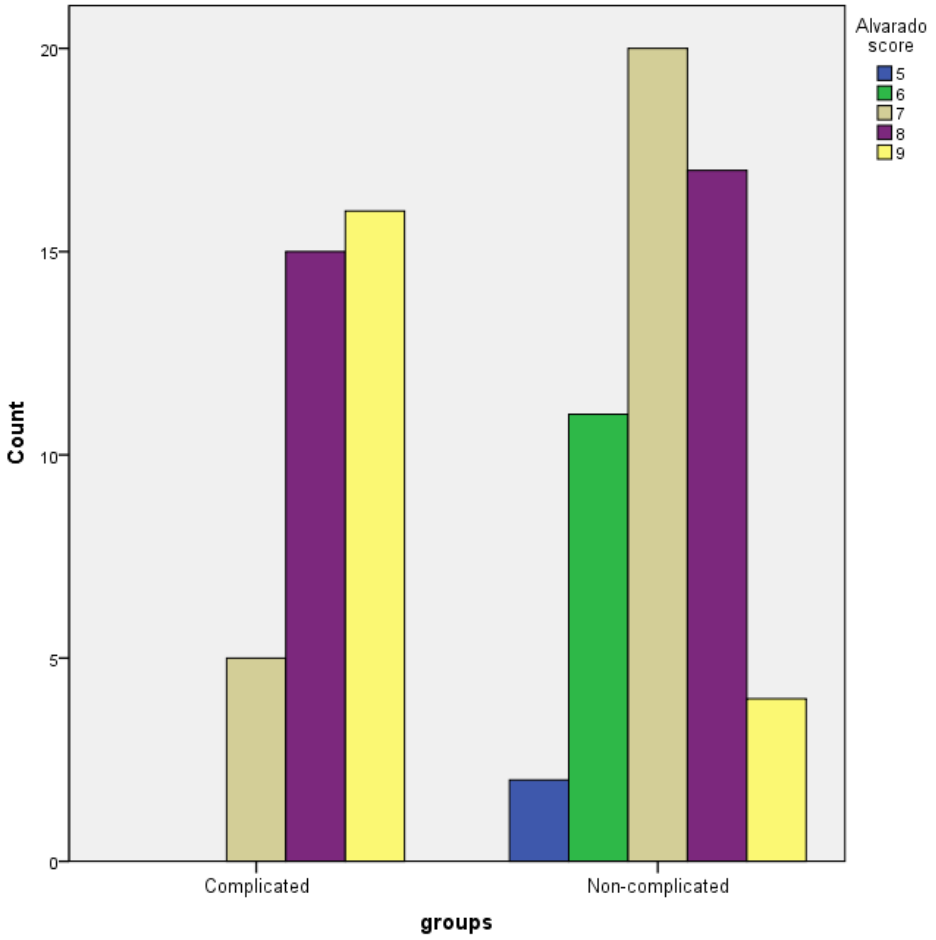

Figure 3-2: Distribution of patient groups according to their Modified Alvarado score degree.

Decision making in cases of acute appendicitis may be a problematic experience in developing countries where the facilities for investigations lack, especially in rural and semi-rural areas, which is why Modified (AS) may be used as a guide in diagnosis and treatment of patients with acute appendicitis (106).

3.2.2. Comparison of modified Alvarado score between complicated and non-complicated acute appendicitis.

The results show significant differences ($p < 0.05$) in CAA patients group when compared to those of the NCAA patients group.

The mean modified AS degree of CAA and NCAA patients group was is shown in Table 3-2.

Table 3-2: Descriptive statistic of modified Alvarado score between complicated and non-complicated acute appendicitis

Patient groups	Patient no.	Mean ± S.D	P value
Complicated acute appendicitis	40	8.31 ± 0.710	**<0.001**
Non-Complicated acute appendicitis	60	7.19 ± 0.973	

With a p value of <0.001, an independent t-test revealed a larger significant difference in modified Alvarado score degree in patients with CAA compared to NCAA, indicating a meaningful link between increased modified AS degree and CAA.

3.2.3. Receiver operating characteristic for modified Alvarado score(AS)

Receiver operating characteristic (ROC) analysis was used to identify the cut-off values for the AS as shown Figure 3-3 .

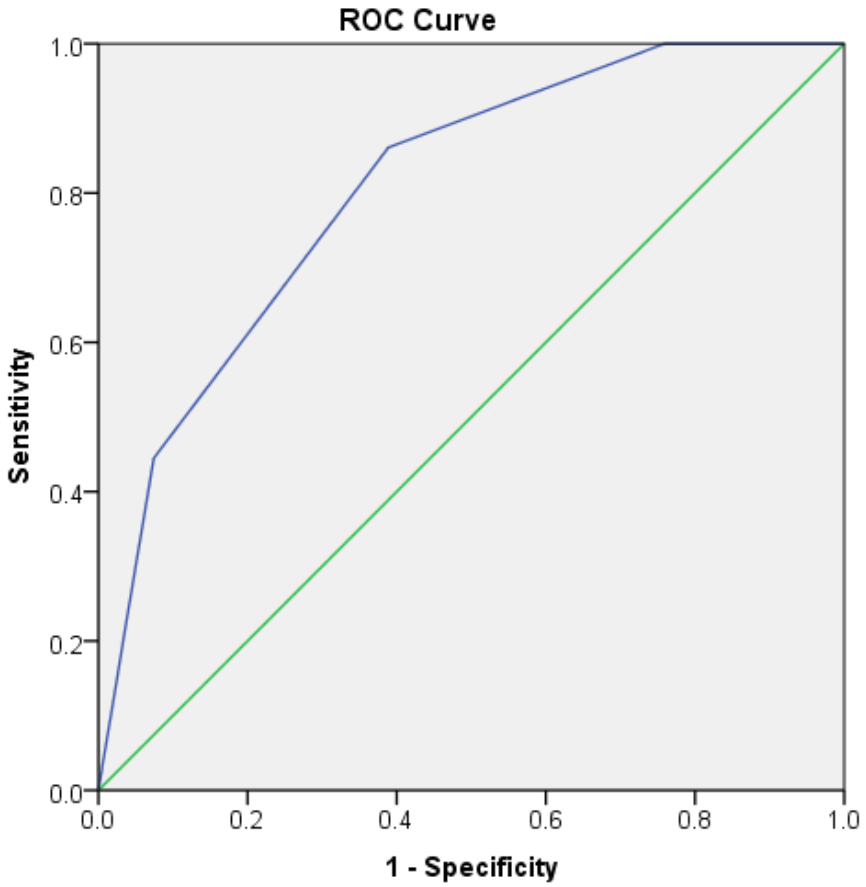

Figure 3-3: ROC-curves presenting the comparison modified Alvarado score between complicated and non-complicated acute appendicitis

Table 3- 3 : The result of ROC curve for modified Alvarado Score

AUC	Sensitivity	Specificity	CI 95%
0.807	86.1%	78.8%	0.719_0.896

The sensitivity of modified AS was 86.1 % in the present study, while The sensitivity of modified AS was 83.3 % in Khanafer *et al* study's (107) .

The sensitivity of modified AS was 76.3% in a research by Macklin et al (108) . Another study in a patients from India found that AS was sensitive but had a low specificity (109). The present study's modified AS specificity was 40.1%, which is slightly higher than Khanafer et al study's (107). In Macklin et al's study, modified AS had a specificity of 78.8%. AS≥7 specificity was calculated by Macklin *et al* (108).

3.3.Comparison of C-reactive protein value between complicated and Non-complicated acute appendicitis

The results show significant differences ($p < 0.05$) in CAA patients group when compared to those of the NCAA patients group.

The mean±SD for CRP value was of CAA and NCAA patients group respectively are shown in Table 3-4.

Table 3-4: Descriptive statistic for C-reactive protein(ng/ml) between complicated and non-complicated acute appendicitis

Parameter	Patient groups	Patient no.	Mean ± S.D	P value
CRP (ng/ml)	Complicated Acute appendicitis	40	8.50± 2.64	**<0.01**
	Non-complicated Acute appendicitis	60	6.51± 1.45	

A significant differences ($p < 0.05$) in CAA patients group when compared to those of the NCAA patients group this is due to that CRP is a biomarker that rises in the acute phase of many disorders as inflammation progresses. In the present study think that CRP levels under 7.05 ng/ml can

help prove a clinical diagnosis in patients without raising the probability of perforation or gangrene, on the other hand CRP levels more than 7.05 ng/ml help prove a clinical diagnosis in patients raising the probability of perforation or gangrene.

As a result, elevated CRP levels may help in predicting the detection of CAA and assist better surgical management (110).

3.3.1 Receiver operating characteristic for C-reactive protein (CRP)

Receiver operating characteristic (ROC) analysis was used to identify the cut-off values for the CRP in Figure 3-4 .

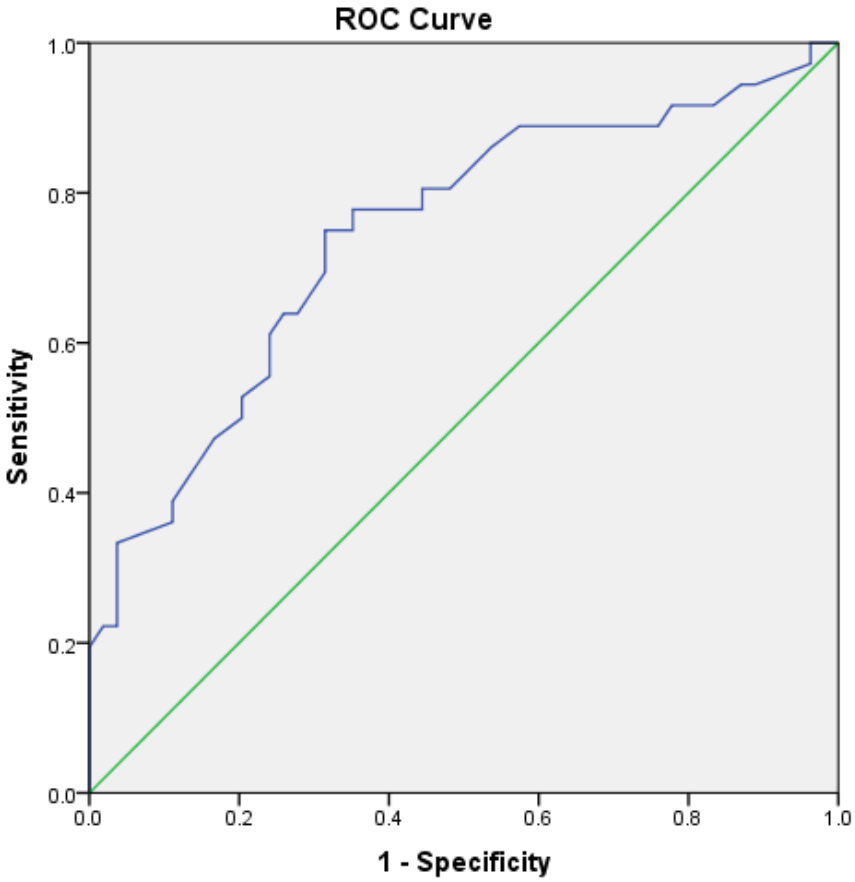

Figure 3-4 ROC-curves for C-reactive protein .

AUC	Sensitivity	Specificity	CI 95%
0.744	75%	68.7%	0.638_0.851

Table 3- 5 :The result of ROC curve for C_Reactive protein

The specificity and sensitivity of CRP's diagnostic accuracy in acute appendicitis were assigned to a wide range of values (94) . CRP's sensitivity and specificity range from 43.2% to 89.29% and 40% to 81.9%, respectively(111). In the present study, the CRP showed an identifying sensitivity of 75 % , a specificity of 68.7%, AUC=0.744 which was consistent with the literature, Moon and his friends demonstrated that a high CRP level was linked to CAA (112) .

3.4. Comparison of Procalcitonin value between complicated and Non-complicated acute appendicitis

The results show significant differences ($p < 0.05$) in CAA patients group when compared to those of the NCAA patients group. The mean±SD for PCT value of CAA and NCAA patients group are shown in Table 3-6.

Table 3-6 :Descriptive statistic for Procalcitonin(pg/ml) between complicated and non-complicated acute appendicitis

Parameter	Groups	Patient no.	Mean±SD	P value
PCT pg/ml	Complicated acute appendicitis	40	176.438±24.312	**<0.001**
	Non-Complicated acute appendicitis	60	147.759±27.766	

significant differences ($p < 0.05$) in CAA patients group when compared to those of the NCAA patients group these is due to Procalcitonin levels rise in response to a pro-inflammatory stimulus, particularly one of bacterial origin. As a result, it's frequently classified as an acute phase reactant (113) . Procalcitonin is generated rapidly by most parenchymal tissues throughout the body when activated by endotoxin or inflammatory cytokines , Blood levels of procalcitonin can rise by multiple orders of magnitude as a result of the inflammatory cascade and systemic response that a severe infection causes, with higher values indicating more severe disease (114).

3.4.1 Receiver operating characteristic for Procalcitonin (PCT)

Receiver operating characteristic (ROC) analysis was used to identify the cut-off values for the PCT in Figure 3-5 .

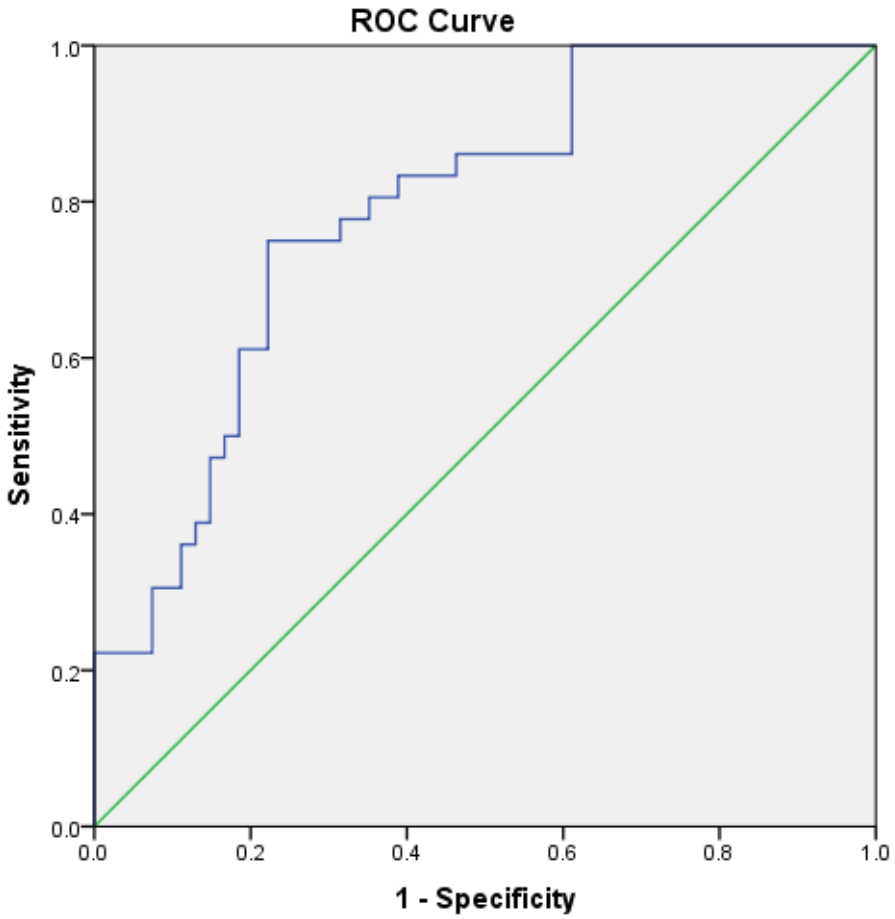

Figure 3-5 :ROC curve for procalcitonin

Table 3- 7 :The result of ROC curve for procalcitonin

AUC	Sensitivity	Specificity	CI 95%
0.789	75%	77.8%	0.696_0.881

Statistical analysis of present data shows a ROC curve analysis for cut-off value of PCT to be 164.606 pg/ml.

3.5.Estimation of Total Bilirubin

A previous studies was determine bilirubin as a marker for differentiation complicated acute appendicitis from non-complicated acute appendicitis , they found , The rise in serum bilirubin level in subjects with acute appendicitis should be considered as having higher probability of complication (gangrene or perforation). Together with clinical findings and other routine laboratory tests, presence of serum hyperbilirubinemia may help in managing subjects with complicated acute appendicitis earlier(115).

Another study conclude that there was no statistically significant difference in mean total serum bilirubin level between patients of complicated appendicitis and uncomplicated appendicitis (116).

In the present study There was no statistically significant difference in the mean total serum bilirubin level between patients with complicated and non-complicated appendicitis

3.6. Correlation between Alvarado score and biochemical parameters (CRP and PCT)

Table 3-8 :Correlation between Alvarado score and biochemical parameters

		AS	PCT(pg/ml)	CRP (ng/ml)
AS	Pearson correlation (r)	…….	0.265	0.216
	P. value	…….	0.012	0.040
	N	…….	100	100
PCT pg/ml	Pearson correlation (r)	…….	…….	0.546
	P. value	…….	…….	<0.001
	N	…….	…….	100

In this study, Although it is weak, there is a statistically significant positive correlation between the AS degree and the concentration of CRP (p-value= 0.040, r=0.216), as shown in Figure 3-6.

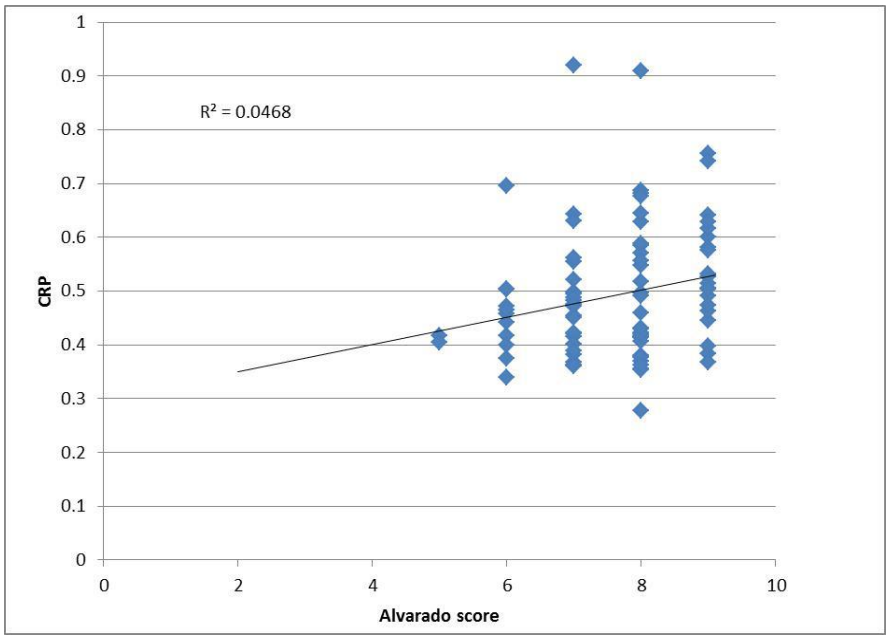

Figure 3-6:correlation between AS and CRP

While the correlation between PCT and AS was relatively more positive than that between CRP and AS , also this correlation was statistically significant (P.value=0.012, r=0.0265) as shown in Figure 3-7.

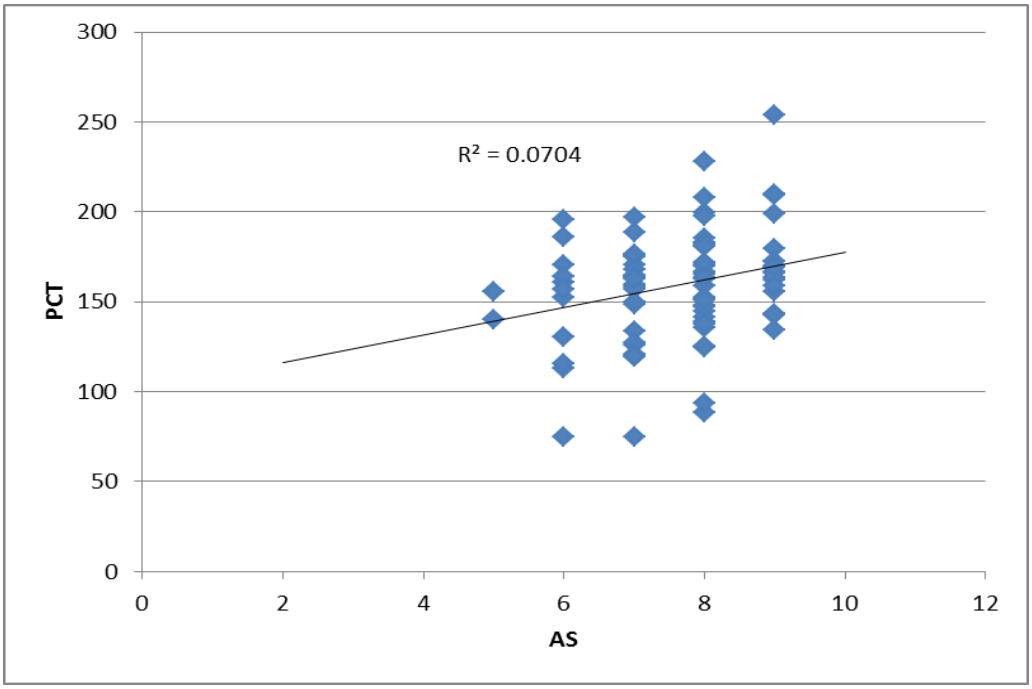

Figure 3-7:correlation between AS and PCT

These correlations between AS and (CRP , PCT) may be useful to support AS to differentiate between non-complicated and complicated AA

On the other hand, The correlation between PCT and CRP was relatively strong positive with (P.value<0.001, r=00.546) as shown in Figure 3-8.

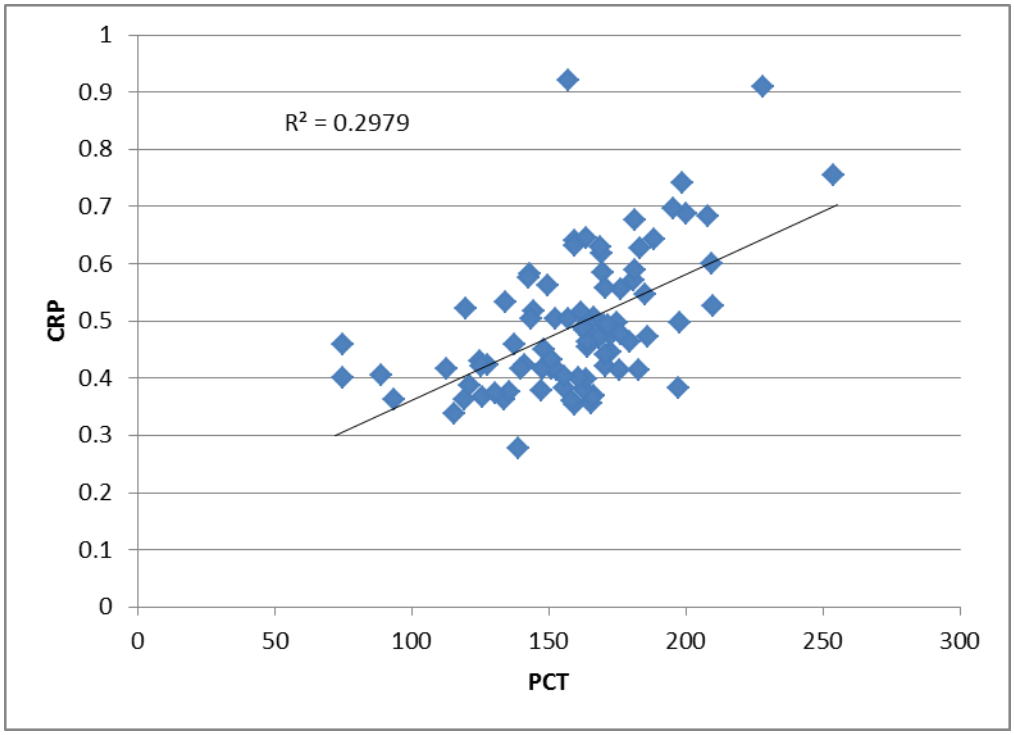

Figure 3-8: Correlation between CRP and PCT.

The correlation between CRP and PCT is significantly positive and the reason may be that the stimulation of the synthesis of the two parameters is affected by the same stimuli. Nevertheless, since the AUC, sensitivity and specificity of PCT are higher than that of CRP in the present study, it is considered PCT has more diagnostic accuracy to support AS in differentiation between complicated and non-complicated acute appendicitis.

Conclusions and Recommendations

Conclusions

1. According to the results , C-reactive protein and Procalcitonin were more effective in patients with complicated acute appendicitis.
2. Positive correlations between C-reactive protein and Procalcitonin with Alvarado score strength the possibility of differentiating between complicated and non-complicated acute appendicitis.
3. Procalcitonin was more powerful from C-reactive protein in supporting Alvarado score to differentiate complicated from non-complicated acute appendicitis according to their sensitivity and specificity .

Recommendations

1. A future study is to be done on large sample size to give results that are more accurate .
2. Collecting samples from more than one center.
3. Study of other biochemical parameters in addition to C-Reactive protein and Procalcitonin [e.g., interleukin (IL)-1beta, interleukin-6, and tumor necrosis factor (TNF)-alpha].

Limitation of study

There were certain limitations to our research. The following were the limitations:

1. It was based on data from a single center, If individuals were later identified with appendicitis at a different hospital, records from only one hospital system may have understated the missed appendicitis rate.
2. The number of patients was limited.
3. Some patients hide their infection with some other diseases, blaming them as a stigma.

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Appendix

Questionnaires

Name ☐ **ID** ☐

Age ☐ years Gender:- male ☐ female ☐

Residence:- Urban ☐ Rural ☐

Phone ☐

You have chronic disease:- yes ☐ NO ☐

Others inflammatory disease:- yes ☐ NO ☐

Symptoms:-

Pregnancy:- yes ☐ NO ☐

The duration of symptoms

Location of initial pain

Nausea

Vomiting

Genito urinary symptoms

Menstrual history

Vaginal discharge

Fever

Mc Burney's

Rebound tenderness

Pulse rate

WBC count

Operative finding

The pathological criteria

الخلاصة

يعتبر التهاب الزائدة الدودية الحاد السبب الاكثر شيوعا لألم البطن الحاد الذي يحتاج الى تداخل جراحي . يحدث التهاب الزائدة الدودية بشكل أكثر شيوعًا بين الأشخاص الذين تتراوح أعمارهم بين 10 و 20 عامًا وتبلغ نسبة الذكور إلى الإناث 1.4: 1 . نسبة انتشار مرض التهاب الزائدة هو 8.6٪ للذكور و 6.7٪ للإناث في الولايات المتحدة ، ويبلغ معدل الإصابة بالتهاب الزائدة الدودية عالميًا (100 اصابة في أمريكا الشمالية و 105اصابة في أوروبا الشرقية و 150 اصابة في أوروبا الغربية) لكل 100.000 شخص سنويًا. كذلك تبلغ الإصابة 160 / 100.000 شخص في السنة في تركيا.

الهدف من هذه الدراسة هو معرفة دور بروتين سي التفاعلي والبروكالسيتونين لدعم درجة الفارادو للتمييز بين التهاب الزائدة الدودية الحاد المعقد والتهاب الزائدة الدودية الحاد غير المعقد من خلال تقدير حساسية وخصوصية كل منهما ، وكذلك معرفة من هو أكثر قوة لإضافته إلى بروتوكول التشخيص.

ولتحقيق هذا الهدف تم تصنيف تسعين مريضا يعانون من التهاب الزائدة الدودية تتراوح أعمارهم بين (15-55) سنة إلى مجموعتين حسب الشدة ، أربعة وخمسون مريضا يعانون من التهاب الزائدة الدودية الحاد غير المعقد مع ستة وثلاثون مريضا كانوا يعانون من التهاب الزائدة الدودية الحاد المعقد . تم قياس تركيز البروتين التفاعلي C وتركيز البروكالسيتونين بواسطة طريقة ELISA،كذلك تم تقدير البيليروبين الكلي في الدم بطريقة القياس الطيفي.

كشفت نتائج الدراسة الحالية عن زيادة معنوية في تركيز CRP (P <0.00) ،و كانت حساسية وخصوصية بروتين سي التفاعلي 75٪ ، 68.7٪ على التوالي. تركيز PCT (P <0.001) في مرضى التهاب الزائدة الدودية الحاد المعقد بالمقارنة مع مرضى التهاب الزائدة الدودية الحاد غير المعقد، كانت حساسية وخصوصية البروكالسيتونين 75٪ ، 77.8٪ على التوالي.

كذلك لم تكن هناك زيادة في تركيز البيليروبين الكلي في الدم في مرضى التهاب الزائدة الدودية الحاد المعقد أكثر من المعدل الطبيعي.

أظهرت النتائج ارتباطًا إيجابيًا معنويًا بين درجة ألفارادو وبروتين C التفاعلي (r = 0.216، p <0.05) ، وارتباط إيجابي معنوي بين درجة ألفارادو والبروكالسيتونين (r = 0.265، p <0.05) وارتباط إيجابي معنوي بين بروتين C التفاعلي والبروكالسيتونين (r= 0.546، p<0.005).

في الختام كان البروتين C التفاعلي والبروكالسيتونين أكثر فعالية في المرضى الذين يعانون من التهاب الزائدة الدودية الحاد المعقد ، وكذلك الارتباطات الإيجابية بين البروتين C التفاعلي والبروكالسيتونين مع درجة ألفارادو تقوي إمكانية التمييز بين التهاب الزائدة الدودية الحاد المعقد وغير المعقد ، و ايضا كان البروكالسيتونين أقوى من C- البروتين التفاعلي في دعم درجة ألفارادو للتمييز بين التهاب الزائدة الدودية الحاد المعقد وغير المعقد وهذا واضح من خلال حساسيتهما وخصوصيتهما .