“EVALUATION OF HYPERBILIRUBINEMIA AS A NEW DIAGNOSTIC TOOL FOR ACUTE APPENDICITIS AND, ALSO AS A PREDICTOR OF APPENDICEAL PERFORATION”

REG.NO. BH0109001

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. S.
in
GENERAL SURGERY

DEPARTMENT OF SURGERY,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
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ENDORSEMENT BY THE HOD/PRINCIPAL/ HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"EVALUATION OF HYPERBILIRUBINEMIA AS A NEW DIAGNOSTIC TOOL FOR ACUTE APPENDICITIS AND, ALSO AS A PREDICTOR OF APPENDICEAL PERFORATION"** is a bonafide research work done by **CANDIDATE REG. NO. BH0109001**.

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LIST OF ABBREVIATIONS USED

ALP	-	Alkaline phosphatase
ALT	-	Alanine transaminase
AST	-	Aspartate transaminase
ATP	-	Adenosine triphosphate
Cm	-	Centimeters
CRP	-	C-reactive protein
CT	-	Computed tomography
dL	-	Deciliters
DLC	-	Differential leukocyte count
E. Coli	-	Escherichia coli
ELISA	-	Enzyme linked immunosorbent assay
g	-	Grams
HbsAg	-	Hepatitis B surface antigen
IL-6	-	Interleukin-6
LFT	-	Liver function tests
mg	-	Milligrams
mL	-	Milliliters
mm	-	Millimeters
n	-	Total number
NPV	-	Negative predictive value
OR	-	Odds ratio
PPV	-	Positive predictive value
SB	-	Serum bilirubin
SGOT	-	Serum glutamic oxaloacetic transaminase

SGPT	-	Serum glutamic pyruvic transaminase
SMV	-	Superior mesenteric vein
Sr.	-	Serum
TLC	-	Total leukocyte count
TNF	-	Tumor necrosis factor
TSB	-	Total serum bilirubin
USG	-	Ultrasonography
WBC	-	White blood cells

ABSTRACT

Background and Objectives

Acute appendicitis is the most common cause of an ‘acute abdomen’ in young adults. Diagnosis of Appendicitis still remains a dilemma in spite of the advances in various laboratory and radiological investigations. The present study was undertaken to assess relationship between hyperbilirubinemia and acute appendicitis and to evaluate its credibility as a diagnostic marker for acute appendicitis and also, to see whether elevated bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

Methodology

This one year cross sectional study was conducted in the Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period of January 2010 to December 2010. A total of 100 patients with clinical diagnosis of acute appendicitis or appendiceal perforation were studied. The serum bilirubin and liver function tests were carried out in all the patients.

Results

In this study, female (55%) outnumbered males (45%) and overall mean age was 23.6 ± 6.6 years. Of the 100 patients, 86% were diagnosed as acute appendicitis while 14% were diagnosed with appendiceal perforation. In patients with acute appendicitis, 80.23% had elevated bilirubin levels while 19.76% had normal bilirubin levels. Among patients with appendiceal perforation, 85.71% had elevated bilirubin levels while 14.28% had normal bilirubin levels. The

Sensitivity and Specificity of serum bilirubin as a marker in predicting acute appendicitis and appendiceal perforation was 80.23% and 14.28% respectively with positive predicative value and negative predicative value of 85.18% and 10.5% respectively (Odds ratio - 0.67).

Conclusion and interpretation

Serum bilirubin levels appears to be a promising new laboratory marker for diagnosing acute appendicitis and also promises to have a predictive potential for the diagnosis of appendiceal perforation. Clinical sings and symptoms of appendicitis with hyperbilirubinemia three times the normal range is suggestive of higher probability of appendiceal perforation.

Keywords

Acute Appendicitis; Appendiceal perforation; Hyperbilirubinemia; Serum Bilirubin;

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INTRODUCTION

Acute appendicitis is the most common cause of an 'acute abdomen' in young adults.[1] Appendicectomy is the most frequently performed urgent abdominal operation and is often the first major procedure performed by a surgeon in training.[1]

In the United States, 250,000 cases of appendicitis are reported annually, representing 1 million patient-days of admission. The incidence of appendicitis seems to have risen greatly in the first half of this century, particularly in Europe, America and Australasia, with up to 16% of the population undergoing appendicectomy. In the past 30 years, the incidence has fallen dramatically in these countries, such that the individual lifetime risk of appendicectomy is 8.6% and 6.7% among males and females respectively.[1]

Some familial predisposition exists.

The diagnosis of Appendicitis still remains a dilemma in spite of advances in the radiological and laboratory investigations. The clinical diagnosis of acute appendicitis is based primarily on symptoms and physical findings. However, this diagnosis is often difficult and up to 50% of the patients hospitalized for possible appendicitis do not actually have this disorder. Authors of large prospective studies reported a 22% to 30% removal rate of normal appendices at surgery.[2-5]

To supplement the clinical diagnosis and to reduce the frequency of unnecessary appendicectomy, the importance of laboratory investigations like White Blood Cell (WBC) counts and C-reactive protein (CRP) etc values has

been stressed.[6-8] The use of Ultrasonography (USG) as a diagnostic tool for appendicitis has been widely known and studied.[9-12] Various scores combining clinical features and laboratory investigations have also been developed and are good enough to reach the diagnosis. These are the Alvarado score[13] and the Modified Alvarado score.[14]

However up to date there is no confirmatory laboratory marker for the pre-operative diagnosis of acute appendicitis and appendiceal perforation.

Recently, elevation in serum bilirubin was reported, but the importance of the raised total has not been stressed in acute appendicitis and appendiceal perforation.[15] It is well established that when microbes invade the body, leukocytes defend it. This leads to increase in the leukocyte count. Bacterial invasion in the appendix leads to transmigration of bacteria and the release of TNF-alpha, IL6, and cytokines. These reach the liver via Superior mesenteric vein (SMV) and may produce inflammation, abscess or dysfunction of liver either directly or indirectly by altering the hepatic blood flow.[16-22]

In view of the above context, the present study was undertaken to assess relationship between hyperbilirubinemia and acute appendicitis and to evaluate its credibility as a diagnostic marker for acute appendicitis and also, to see whether elevated bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

OBJECTIVES

The objectives of the present study were;

1. To study the relationship between hyperbilirubinemia and acute appendicitis and to evaluate its credibility as a diagnostic marker for acute appendicitis.

2. To evaluate whether elevated bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

REVIEW OF LITERATURE

Historical Review

The vermiform appendix is considered by most to be a vestigial organ; its importance in surgery results only from its propensity for inflammation, which results in the clinical syndrome known as Acute appendicitis.[1]

That the appendix lay hidden in the right lower abdominal quadrant has been known for millennia, its function and role in disease however has remained obscure.

The appendix vermiformis as an anatomical structure was first described in 1521 by Jacopo Berengario da Carpi, (ca. 1470-1530) professor of anatomy at Bologna. In 1554 the French physician Jean Fernel (1497-1558) reported the first case of perforative appendicitis at autopsy.[23]

A classical post-mortem description is owed to Lorenz Heister (1683-1758), professor of medicine and also a practising surgeon at the universities of Altdorf-Nürnberg and Helmstedt in Germany (1712). Heister was the first to study the pathology of appendicitis (1711).[24]

The 19th century pathological concept is based on the notion ´perityphilitis´, that is inflammation of the cecum (typhlon, blind). The cecum rather than the appendix was considered as the site of the disease; this is easily explained by advanced stages of inflammation which were observed in autopsies.

The condition now called ‘appendicitis’ became a surgical problem once it was obvious that the starting point of the disease is the appendix vermiformis. The first to clearly recognize this was Harvard University’s pathologist Reginald Heber Fitz (1843-1913) who communicated his finding at the first meeting of the Association of American Physicians in 1886.

In his paper, Fitz pointed out that the frequent abscesses in the right iliac fossa were not due to typhilitis, perityphilitis or epityphlitis but to perforation of the vermiform appendix. Hence he gave the condition the name ‘appendicitis’ so as to avoid the possibility of misunderstanding and to localize the disease in its usual place of origin.[25]

Surgery for appendicitis

The first appendicectomy was performed at St. George’s Hospital, London, in 1736 by Claudius Amyand, a surgeon at St. George's Hospital in London and Sergeant Surgeon to Queen Ann, King George I, and King George II. The acutely inflamed appendix, perforated by a pin, and surrounding omentum was removed through a scrotal wound while dealing with a faecal fistula in a chronic scrotal hernia. The patient was 11-year-old boy and patient recovered.[26]

The first published account of appendicectomy for appendicitis was by Krönlein in 1886. However, the patient died two days postoperatively.

Fergus, in Canada, performed the first elective appendicectomy in 1883.[27]

Charles McBurney (1845-1913) was one of the surgeons pioneering the diagnostics and operative treatment of appendicitis. McBurney's classic report on early operative interference in cases of appendicitis was presented before the New York Surgical Society in 1889. In it he described the area of greatest abdominal pain in this disease process, now known as McBurney's point.

Five years later in 1894, he set forth in another paper the incision that he used in cases of appendicitis, now called McBurney's incision.[28]

However, McBurney later credited McArthur with first describing this incision.[28]

The US surgeon John Benjamin Murphy introduced and popularized early removal of the appendix in all cases of suspected appendicitis. In 1904 he described the triad of pain in abdomen, vomiting and fever, which remains a sound basis for diagnosis even today.[29]

Dawbarn suggested the use of a purse string suture, placed around the base of the appendix. In 1889, Senn first drew attention to the risks of ligature slipping off the appendix stump with subsequent peritoneal contamination.

On 13 September 1983 the gynaecologist Professor Kurt Semm performed the world's first laparoscopic appendicectomy at the University of Kiel in Germany.[30]

Laproscopic appendicectomy is now as widely used as Open appendicectomy and their comparison has been a matter of great debate.

Anatomy of Vermiform Appendix[31]

The vermiform appendix is a narrow, vermian (worm-shaped) tube which arises from the posteromedial caecal wall, 2 cm below the end of the ileum. It may occupy one of several positions.It may be retrocaecal, retrocolic (behind the caecum or lower ascending colon respectively), pelvic or descending (when it hangs dependently over the pelvic brim, in close relation to the right uterine tube and ovary in females). These are the commonest positions seen in clinical practice. Other positions are occasionally seen especially when there is a long appendix mesentery allowing greater mobility. These include subcaecal (below the caecum); preilial (anterior to the teminal ileum); postileal (behind the teminal ileum).

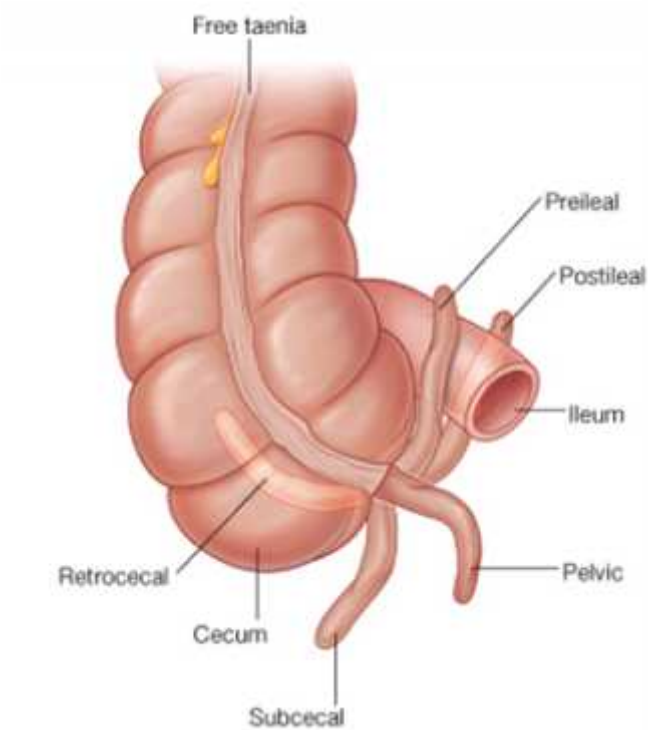


Figure 1. Various position of appendix

The three taeniae coli on the ascending colon and caecum converge on the base of the appendix, and merge into its longitudinal muscle. The anterior caecal taenia is usually distinct and can be traced to the appendix, which affords a guide to its location in clinical practice.

The appendix varies from 2 to 20 cm in length: it is often relatively longer in children and may atrophy and shorten after mid-adult life. It is connected by a short mesoappendix to lie in lower part of the ileal mesentery. This fold is usually triangular, extending almost to the appendiceal tip along the whole viscus.

The lumen of the appendix is small and opens into the caecum by an orifice lying below and slightly posterior to the ileocaecal opening. The orifice is sometimes guarded by a semilunar mucosal fold forming a valve. The lumen may be widely patent in early childhood and is often partially or wholly obliterated in the later decades of life. The appendix usually contains numerous patches of lymphoid tissue although these tend to decrease in size from early adulthood.

Vascular Supply - Appendiceal Artery

The main appendiceal artery, a branch from the lower division of the ileocolic artery, runs behind the terminal ileum and enters the mesoappendix a short distance from the appendiceal base. Here it gives off a recurrent branch, which anastomoses at the base of the appendix with a branch of the posterior caecal artery: the anastomosis is sometimes extensive. The main appendiceal artery approaches the tip of the organ, at first near to, and then in the edge of, the mesoappendix. The terminal part of the artery lies on the wall of the appendix and may be thrombosed in appendicitis, which results in distal gangrene or

necrosis. Accessory arteries are common, and many individuals possess two or more arteries of supply.

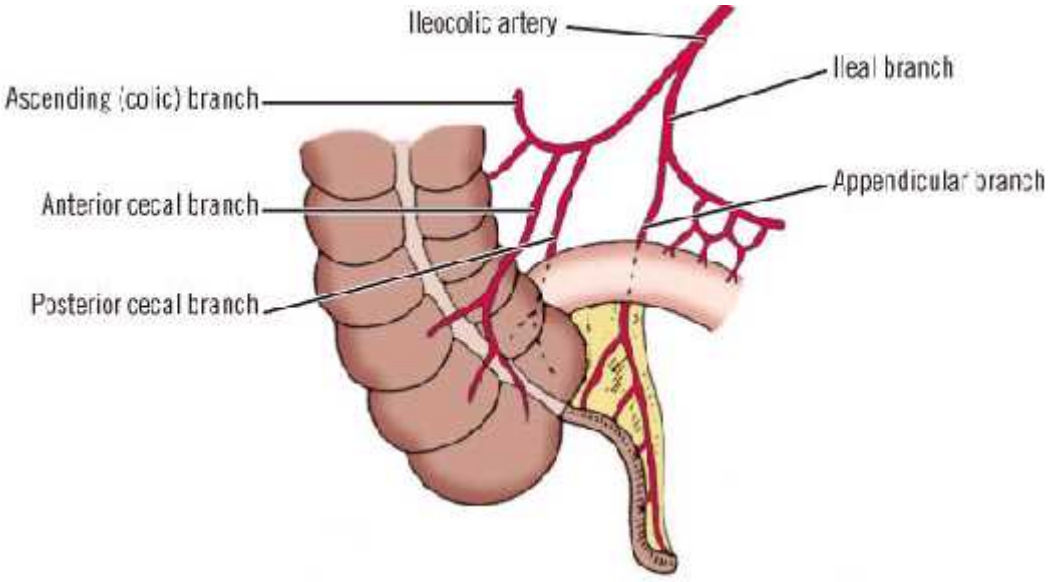


Figure 2. Blood supply of appendix

Appendiceal Veins

The appendix is drained via one or more appendiceal veins into the posterior caecal or ileocolic vein and thence into the superior mesenteric vein.

Lymphatic drainage

Lymphatic vessels in the appendix are numerous: there is abundant lymphoid tissue in its walls. From the body and apex of the appendix 8 to 15 vessels ascend in the mesoappendix, and are occasionally interrupted by one or more nodes. They unite to form three or four larger vessels which run into the lymphatic vessels draining the ascending colon, and end in the inferior and superior nodes of the ileocolic chain.

Innervation

The appendix and overlying visceral peritoneum are innervated by sympathetic and parasympathetic nerves from the superior mesenteric plexus. Visceral afferent fibres carrying sensation of distension and pressure mediate the symptoms of pain felt during the initial stages of appendiceal inflammation. In keeping with other structures derived from the midgut, these sensations are poorly localized initially, and referred to the central (periumbilical) region of the abdomen. It is not until parietal tissues adjacent to the appendix become involved in any inflammatory process that somatic nociceptors are stimulated, and there is an associated change in the nature and localization of päin.

Mesoappendix

The mesentery of the appendix is a triangular fold of peritoneum around the vermiform appendix. It is attached to the posterior surface of the lower end of the mesentery of the small intestine close to the ileocaecal junction. It usually reaches the tip of the appendix but some times fails to reach the distal third, in which case a vestigial low peritoneal ridge containing fat is present over the distal third. It encloses the blood vessels, nerves and lymph vessels of the vermiform appendix, and usually contains a lymph node.

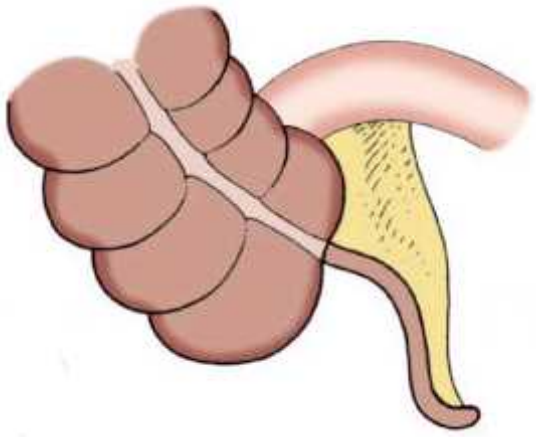


Figure 3. Mesoappendix

Caecal recesses

Several folds of peritoneum may exist around the caecum and form recesses. Paracaecal recesses are common sites for abscess formation following acute appendicitis.

Others include, Superior ileocaecal recess, Inferior ileocaecal recess and Retrocaecal recess

Microstructure of The Appendix

Mucosa

The mucosa is covered by a columnar epithelium , and M cells are present in the epithelium that overlies the mucosal lymphoid tissue. Glands (crypts) are fewer in number and thus less densely packed. They penetrate deep into the lymphoid tissue of the mucosal lamina propria

Sub-Mucosa

The submucosa typically contains many large lymphoid aggregates that extend from the mucosa and obscure the muscularis mucosae layer: consequently this becomes discontinuous. These aggregates also cause the mucosa to bulge into the lumen of the appendix, so that it narrows irregularly . They are absent at birth but accumulate over the first 10 years of life to become a prominent feature. The submucosal lymphoid tissue frequently exhibits germinal centres within its follicles, indicative of B-cell activation, as it is in secondary lymphoid tissue elsewhere . In adults, the normal layered structure of the appendix is lost and the lymphoid follicles atrophy and are replaced by collagenous tissue. In the elderly, the appendix may be filled with fibrous scar tissue.

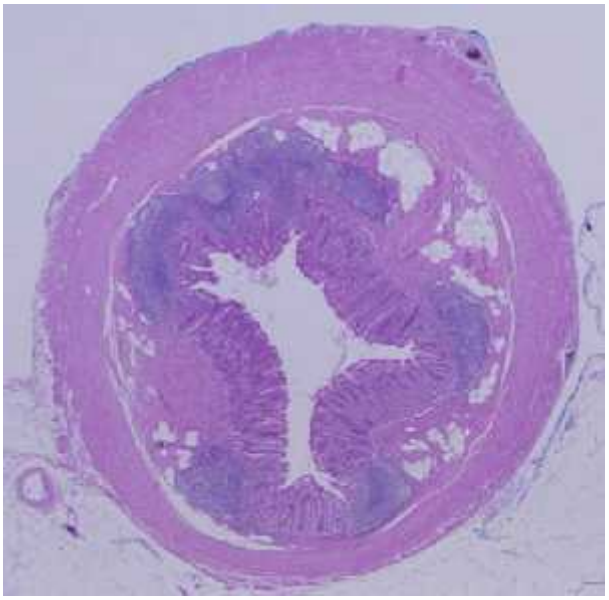


Figure.4. Normal histology of appendix

Muscularis Externa

The muscularis externa has outer longitudinal and inner circular layers of smooth muscle. The longitudinal fibres form a continuous layer but, with the exception of the uniform outer muscle layer of most of the appendix, macroscopically these are aggregated as longitudinal bands or taeniae coli. At the base of the appendix, the longitudinal muscle thickens to form rudimentary taeniae that are continuous with those of the caecum and colon. Between the taeniae coli the longitudinal layer is much thinner, less than half the circular layer in thickness.

Serosa

The serosa forms a complete covering, except along the mesenteric attachment. The longitudinal muscular fibres form a complete layer of uniform thickness, except over a few small areas where both muscular layers are deficient, leaving the serosa and submucosa in contact.

Embryology of The Appendix[32]

The appendix first becomes visible in the eighth week of embryologic development as a protuberance off the terminal portion of the caecum. During both antenatal and postnatal development, the growth rate of the caecum exceeds that of the appendix, so that the appendix is displaced medially toward the ileocecal valve. Congenital absence of the appendix is extremely rare.

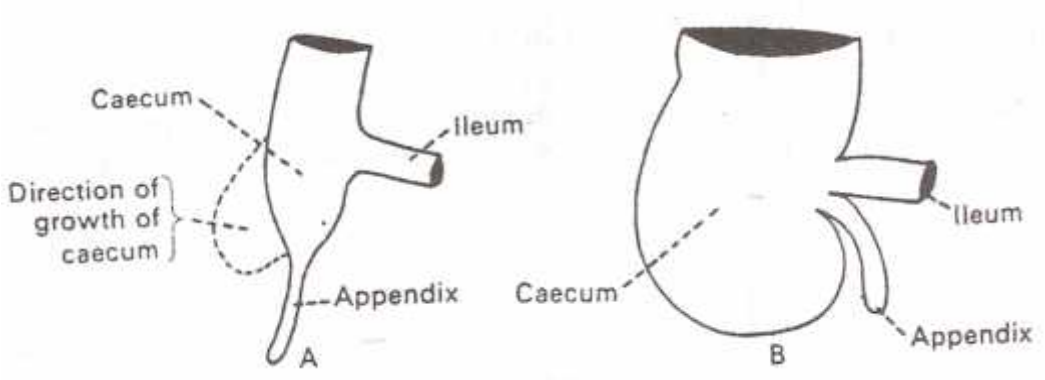


Figure 5. Development of the appendix

Pathology[33]

Morphology

Appendiceal inflammation is associated with obstruction in 50% to 80% of cases, usually in the form of a faecolith and, less commonly, a gallstone, tumor, or ball of worms (oxyuriasis vermicularis).

Continued secretion of mucinous fluid in the obstructed viscus presumably leads to a progressive increase in intraluminal pressure sufficient to cause eventual collapse of the draining veins. Ischemic injury then favors bacterial proliferation with additional inflammatory edema and exudation, further embarrassing the blood supply. Nevertheless, a significant minority of inflamed appendices have no demonstrable luminal obstruction, and the pathogenesis of the inflammation remains unknown.

At the earliest stages, only a scant neutrophilic exudate may be found throughout the mucosa, submucosa, and muscularis propria. Subserosal vessels are congested, and often there is a modest perivascular neutrophilic infiltrate. The

inflammatory reaction transforms the normal glistening serosa into a dull, granular, red membrane; this transformation signifies early acute appendicitis for the operating surgeon. At a later stage, a prominent neutrophilic exudate generates a fibrinopurulent reaction over the serosa.

As the inflammatory process worsens, there is abscess formation within the wall, along with ulcerations and foci of suppurative necrosis in the mucosa. This state constitutes acute suppurative appendicitis.

Further appendiceal compromise leads to large areas of hemorrhagic green ulceration of the mucosa and green-black gangrenous necrosis through the wall, extending to the serosa, creating acute gangrenous appendicitis, which is quickly followed by rupture and suppurative peritonitis.

The histologic criterion for the diagnosis of acute appendicitis is neutrophilic infiltration of the muscularis propria. Usually, neutrophils and ulcerations are also present within the mucosa. Since drainage of an exudate into the appendix from alimentary tract infection may also induce a mucosal neutrophilic infiltrate, evidence of muscular wall inflammation is requisite for the diagnosis.

Figure 6. Histology of inflamed appendicitis

Etio-pathogenesis[34]

Obstruction of the lumen is the dominant etiologic factor in acute appendicitis. Faecoliths are the most common cause of appendiceal obstruction. Less common causes are hypertrophy of lymphoid tissue, inspissated barium from previous x-ray studies, tumors, vegetable and fruit seeds, and intestinal parasites. The frequency of obstruction rises with the severity of the inflammatory process. Faecoliths are found in 40% of cases of simple acute appendicitis, in 65% of cases of gangrenous appendicitis without rupture, and in nearly 90% of cases of gangrenous appendicitis with rupture.

Traditionally the belief has been that there is a predictable sequence of events leading to eventual appendiceal rupture. The proximal obstruction of the appendiceal lumen produces a closed-loop obstruction, and continuing normal secretion by the appendiceal mucosa rapidly produces distention. The luminal

capacity of the normal appendix is only 0.1 mL. Secretion of as little as 0.5 mL of fluid distal to an obstruction raises the intraluminal pressure to 60 cm H_2O. Distention of the appendix stimulates the nerve endings of visceral afferent stretch fibers, producing vague, dull, diffuse pain in the midabdomen or lower epigastrium. Peristalsis also is stimulated by the rather sudden distention, so that some cramping may be superimposed on the visceral pain early in the course of appendicitis. Distention increases from continued mucosal secretion and from rapid multiplication of the resident bacteria of the appendix. Distention of this magnitude usually causes reflex nausea and vomiting, and the diffuse visceral pain becomes more severe. As pressure in the organ increases, venous pressure is exceeded. Capillaries and venules are occluded, but arteriolar inflow continues, resulting in engorgement and vascular congestion. The inflammatory process soon involves the serosa of the appendix and in turn parietal peritoneum in the region, which produces the characteristic shift in pain to the right lower quadrant.

The mucosa of the GI tract, including the appendix, is susceptible to impairment of blood supply; thus its integrity is compromised early in the process, which allows bacterial invasion. As progressive distention encroaches on first the venous return and subsequently the arteriolar inflow, the area with the poorest blood supply suffers most: ellipsoidal infarcts develop in the antimesenteric border. As distention, bacterial invasion, compromise of vascular supply, and infarction progress, perforation occurs, usually through one of the infarcted areas on the antimesenteric border. Perforation generally occurs just beyond the point of obstruction rather than at the tip because of the effect of diameter on intraluminal tension.

This sequence is not inevitable, however, and some episodes of acute appendicitis apparently subside spontaneously. Many patients who are found at operation to have acute appendicitis give a history of previous similar, but less severe, attacks of right lower quadrant pain. Pathologic examination of the appendices removed from these patients often reveals thickening and scarring, suggesting old, healed acute inflammation.[35,36]

The strong association between delay in presentation and appendiceal perforation supported the proposition that appendiceal perforation is the advanced stage of acute appendicitis; however, recent epidemiologic studies have suggested that nonperforated and perforated appendicitis may, in fact, be different diseases.[37]

Bacteriology

The bacterial population of the normal appendix is similar to that of the normal colon. The appendiceal flora remains constant throughout life with the exception of *Porphyromonas gingivalis.* This bacterium is seen only in adults.[40] The bacteria cultured in cases of appendicitis are therefore similar to those seen in other colonic infections such as diverticulitis. The principal organisms seen in the normal appendix, in acute appendicitis, and in perforated appendicitis are *Escherichia coli* and *Bacteroides fragilis.*[38-41] However, a wide variety of both facultative and anaerobic bacteria and mycobacteria may be present (Table 1). Appendicitis is a polymicrobial infection, with some series reporting the culture of up to 14 different organisms in patients with perforation.[38]

Table 1. Common Organisms seen in Patients with Acute appendicitis

Aerobic and Facultative	**Anaerobic**
Gram-negative bacilli	**Gram-negative bacilli**
Escherichia coli	Other *Bacteroide*s species
Pseudomonas aeruginosa	*Bacteroides fragilis*
Klebsiella species	*Fusobacterium* species
Gram-positive cocci	**Gram-positive cocci**
Streptococcus anginosus	*Peptostreptococcus* species
Other *Streptococcu*s species	**Gram-positive bacilli**
Enterococcus species	*Clostridium* species

Epidemiology

Acute appendicitis is the most common general surgical emergency, and early surgical intervention improves outcomes. About 8% of people in Western countries have appendicitis at some time during their lifetime.[42]

Appendicitis is most frequently seen in patients in their second through fourth decades of life, with a mean age of 31.3 years and a median age of 22 years.[43,44]

In teenagers and young adults - there is a slight male preponderance of 3:2. While in adults, the incidence of appendicitis is approximately 1.4 times greater in men than in women.

The incidence of appendicitis gradually rises from birth, peaks in the late teen years, and gradually declines in the geriatric years. The mean age when appendicitis occurs in the pediatric population is 6 to 10 years. Lymphoid hyperplasia is observed more often among infants and adults and is responsible for the increased incidence of appendicitis in these age groups. Younger children have a higher rate of perforation, with reported rates of 50 to 85%. The median age at appendicectomy is 22 years. Although rare, neonatal and even prenatal appendicitis have been reported. Clinicians must maintain a high index of suspicion in all age groups.

The lifetime rate of appendicectomy is 12% for men and 25% for women, with approximately seven percent of all people undergoing appendicectomy for acute appendicitis during their lifetime. Over the 10 year period from 1987 to 1997, the overall appendicectomy rate decreased in parallel with a decrease in incidental appendicectomy.[43,44] However, the rate of appendicectomy for appendicitis has remained constant at 10 per 10,000 patients per year.[45] Despite the increased use of ultrasonography, computed tomography (CT), and laparoscopy, the rate of misdiagnosis of appendicitis has remained constant (15.3%), as has the rate of appendiceal rupture. The percentage of misdiagnosed cases of appendicitis is significantly higher among women than among men (22.2 vs. 9.3%). The negative appendicectomy rate for women of reproductive age is 23.2%, with the highest rates in women aged 40 to 49 years. The highest negative appendicectomy rate is reported for women >80 years of age.[44,45]

In the United States, 250,000 cases of appendicitis are reported annually, representing one million patient-days of admission. The incidence of acute

appendicitis has been declining steadily since the late 1940s, and the current annual incidence is 10 cases per 100,000 population. Appendicitis occurs in seven percent of the US population, with an incidence of 1.1 cases per 1000 people per year. Some familial predisposition exists.

In Asian and African countries, the incidence of acute appendicitis is probably lower because of the dietary habits of the inhabitants of these geographic areas. The incidence of appendicitis is lower in cultures with a higher intake of dietary fiber. Dietary fiber is thought to decrease the viscosity of feces, decrease bowel transit time, and discourage formation of faecoliths, which predispose individuals to obstructions of the appendiceal lumen.

In the last few years, a decrease in frequency of appendicitis has also been reported in Western countries, which may be related to changes in dietary fiber intake. In fact, the higher incidence of appendicitis is believed to be related to poor fiber intake in such countries.

Diagnosis

History[1]

The classical features of acute appendicitis begin with poorly localised colicky abdominal pain. This is due to mid-gut visceral discomfort in response to appendiceal inflammation and obstruction. The pain is frequently first noticed in the peri-umbilical region and is similar to, but less intense than, the colic of small bowel obstruction. Central abdominal pain is associated with anorexia, nausea and usually one or two episodes of vomiting that follow the onset of pain

(Murphy's triad). Anorexia is a useful and constant clinical feature, particularly in children. The patient often gives a history of similar discomfort that settled spontaneously. A family history is also useful as up to one-third of children with appendicitis have a first-degree relative with a similar history.

With progressive inflammation of the appendix, the parietal peritoneum in the right iliac fossa becomes irritated, producing more intense, constant and localised somatic pain that begins to predominate. Patients often report this as an abdominal pain that has shifted and changed in character. Typically, coughing or sudden movement exacerbates the right iliac fossa pain. The classic visceral–somatic sequence of pain is present in only about half of those patients subsequently proven to have acute appendicitis. Atypical presentations include pain that is predominantly somatic or visceral and poorly localised. Atypical pain is more common in the elderly, in whom localisation to the right iliac fossa is unusual. An inflamed appendix in the pelvis may never produce somatic pain involving the anterior abdominal wall, but may instead cause suprapubic discomfort and tenesmus. In this circumstance, tenderness may be elicited only on rectal examination and is the basis for the recommendation that a rectal examination should be performed on every patient who presents with acute lower abdominal pain. During the first six hours, there is rarely any alteration in temperature or pulse rate. After that time, slight pyrexia (37.2 to 37.7°C) with a corresponding increase in the pulse rate to 80 or 90 is usual. However, in 20% of patients, there is no pyrexia or tachycardia in the early stages. In children, a temperature greater than 38.5°C suggests other causes, for example mesenteric adenitis.

Because appendicitis is so common, a high index of suspicion for appendicitis is warranted in all patients with abdominal pain.[46]

Physical examination[1]

The diagnosis of appendicitis rests more on thorough clinical examination of the abdomen than on any aspect of the history or laboratory investigation. The cardinal features are those of an unwell patient with low-grade pyrexia, localised abdominal tenderness, muscle guarding and rebound tenderness. Inspection of the abdomen may show limitation of respiratory movement in the lower abdomen.

Gentle superficial palpation of the abdomen, beginning in the left iliac fossa moving anticlockwise to the right iliac fossa will detect muscle guarding over the point of maximum tenderness, classically McBurney's point. Asking the patient to cough or gentle percussion over the site of maximum tenderness will elicit rebound tenderness. Cutaneous hyperaesthesia may be demonstrable in the right iliac fossa, but is rarely of diagnostic value.

Multiple signs can be detected on physical examination to contribute to the diagnosis of appendicitis.

1. *The pointing sign:* The patient is then asked to point to where the pain began and where it moved.

2. *Rovsing's sign:* Pain in the right lower quadrant on palpation of the left lower quadrant, is further evidence of localized peritoneal inflammation in the right lower quadrant.

3. *Psoas sign:* Pain with flexion of the leg at the right hip, can be seen with a retrocecal appendix due to inflammation adjacent to the psoas muscle.

4. *The obturator sign:* Pain with rotating the flexed right thigh internally, indicates inflammation adjacent to the obturator muscle in the pelvis.

Investigations

The diagnosis of acute appendicitis is essentially clinical; however, a decision to operate based on clinical suspicion alone can lead to the removal of a normal appendix in 15 to 30% of cases. The premise that it is better to remove a normal appendix than to delay diagnosis does not stand up to close scrutiny, particularly in the elderly.[1]

A number of Laboratory and Imaging studies have been devised to assist diagnosis.

Laboratory Tests

Laboratory studies can be helpful in the diagnosis of appendicitis, but no single test is definitive.

White Blood Cell Count (WBC)

A White Blood Cell count (WBC) is perhaps the most useful laboratory test. The white blood cell count is elevated with more than 75% neutrophils in most patients. A completely normal leukocyte count and differential is found in about 10% of patients with acute appendicitis. A high white blood cell count

(>20,000/mL) suggests complicated appendicitis with either gangrene or perforation.[42]

The clinician must remember, however, that the WBC count can be normal in patients with acute appendicitis, particularly in early cases. Serial WBC measurements improve the diagnostic accuracy, with a rising value over time commonly seen in patients with appendicitis.[47]

C-reactive protein

C-reactive protein (CRP) is an acute-phase reactant synthesized by the liver in response to infection or inflammation and rapidly increases within the first 12 hours. CRP has been reported to be useful in the diagnosis of appendicitis; however, it lacks specificity and cannot be used to distinguish between sites of infection. CRP levels of greater than 1 mg/dl are commonly reported in patients with appendicitis, but very high levels of CRP in patients with appendicitis indicate gangrenous evolution of the disease, especially if it is associated with leukocytosis and neutrophilia.

However, CRP normalization is known to occur 12 hours after onset of symptoms. Several prospective studies have shown that in adults who have had symptoms for longer than 24 hours, a normal CRP level has a negative predictive value of 97-100% for appendicitis.[48-50]

Multiple studies have been done evaluating the sensitivity of CRP level alone for the diagnosis of appendicitis in patients selected to undergo

appendicectomy. Gurleyik et al noted a CRP sensitivity of 96.6% in 87 of 90 patients with histologically proven disease.[51]

Urinalysis

Urinalysis is performed to diagnose other potential causes for abdominal pain, specifically urinary tract infection and ureteral stone. Significant hematuria with colicky abdominal pain suggests ureterolithiasis, and testing directed at this diagnosis is indicated. A urinary tract infection, on the other hand, is not uncommon in patients with appendicitis. Its presence does not exclude the diagnosis of acute appendicitis, but it should be identified and treated. Although pyuria suggests urinary tract infection, it is not uncommon for the urinalysis in a patient with appendicitis to show a few white blood cells solely due to inflammation of the ureter by the adjacent appendix.

In certain patient populations, other laboratory tests are indicated. In women of childbearing age, the urine human chorionic gonadotropin should be checked to alert the clinician to the possibility of ectopic or concurrent pregnancy. Ectopic pregnancy is another cause of right lower quadrant pain that demands emergent diagnosis and treatment.

Imaging Studies

The potential imaging modalities for diagnosis of acute appendicitis include plain radiographs, ultrasound, and computed tomography.

Plain radiographs

Prior to the wide-spread use of modern imaging techniques, plain abdominal films were often obtained in patients with abdominal pain, and a right lower quadrant faecolith (or appendicolith) was considered pathognomonic for acute appendicitis.[46] A calcified appendicolith is visible on plain films in only 10% to 15% of patients with acute appendicitis.[42] Studies show that faecoliths are not pathognomonic for appendicitis, as some patients with abdominal pain and a faecolith have a normal appendix. In addition, faecoliths are not common enough in patients with appendicitis to be used as a reliable sign.

As a result, plain abdominal radiographs are neither helpful nor cost effective and are not recommended for the diagnosis of acute appendicitis. Plain abdominal films may be useful for the detection of ureteral calculi, small bowel obstruction, or perforated ulcer, but such conditions are rarely confused with appendicitis.[42]

Ultrasonography (USG)

Among patients with abdominal pain, *Abdominal ultrasonography* has a sensitivity of about 85% and a specificity of more than 90% for the diagnosis of acute appendicitis.[42]

Sonographic findings consistent with acute appendicitis include:

1. Appendix of seven mm or more in antero-posterior diameter,
2. A thick-walled, noncompressible luminal structure seen in cross section referred to as a *target lesion,*

3. Increased echogenicity of the surrounding fat signifying inflammation, or
4. Presence of an appendicolith
5. In more advanced cases, peri-appendiceal fluid or a mass may be found.

Ultrasonography has the advantages of being a noninvasive modality requiring no patient preparation that also avoids exposure to ionizing radiation. For these reasons, it is commonly used in children and in pregnant patients with equivocal clinical findings suggestive of acute appendicitis. Disadvantage of ultrasonography is that it is highly operator-dependent, and it is frequently unable to visualize the normal appendix.[52]

Pelvic ultrasound can be especially useful in excluding pelvic pathology, such as tubo-ovarian abscess or ovarian torsion, that may mimic acute appendicitis.[42]

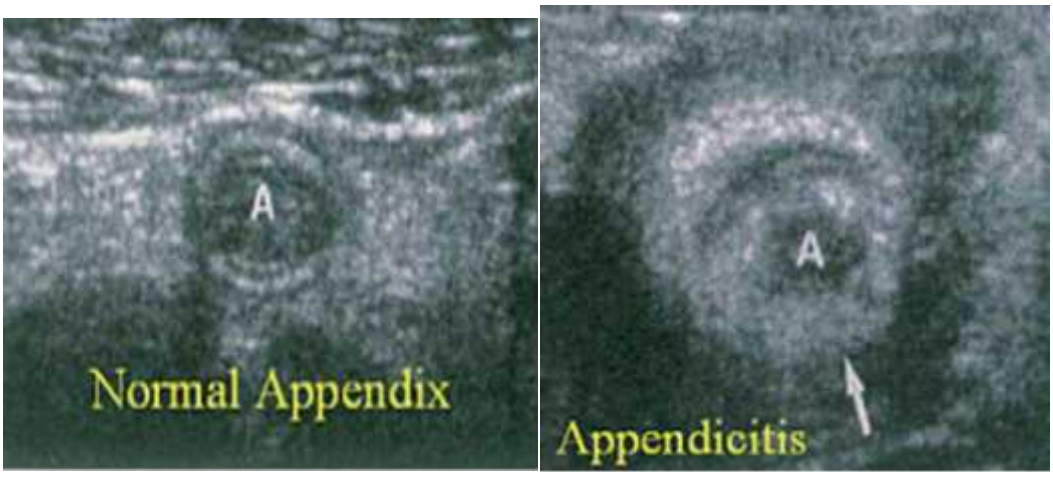


Figure 7. USG finding of a normal appendix and inflammed appendix (Appendicitis)

Computed tomography

Computed tomography (CT) is commonly used in the evaluation of adult patients with suspected acute appendicitis, especially so in the elderly.[42] CT benefits has a high diagnostic accuracy for appendicitis,[53] and visualization and diagnosis of many of the other causes of abdominal pain that can be confused with appendicitis.

Improved imaging techniques, including the use of 5-mm sections, have resulted in increased accuracy of CT scanning,[54] which has a sensitivity of about 90% and a specificity of 80% to 90% for the diagnosis of acute appendicitis among patients with abdominal pain.

Controversy remains as to the importance of intravenous, oral gastrointestinal, and rectal contrast in improving diagnostic accuracy.

In general, CT findings of appendicitis increase with the severity of the disease. Classic findings include a distended appendix greater than seven mm in diameter and circumferential wall thickening, which may give the appearance of a halo or target. As inflammation progresses, one may see periappendiceal fat stranding, edema, peritoneal fluid, phlegmon, or a periappendiceal abscess. CT detects appendicoliths in about 50% of patients with appendicitis and also in a small percentage of people without appendicitis. Among patients with abdominal pain, the positive predictive value of the finding of an appendicolith on CT remains high at about 75%.

In prospective studies, CT demonstrated a sensitivity of 0.94 and a specificity of 0.95.[53] CT thus has a high negative predictive value, making it particularly useful in excluding appendicitis in patients for whom the diagnosis is in doubt. Appendicitis is highly unlikely if enteric contrast fills the lumen of the appendix and no surrounding inflammation is present. The clinician must remember, however, that a CT performed early in the course of appendicitis might not show the typical radiographic findings.[1]

The rational approach is – the selective use of CT scanning.

Laproscopy

Although most patients with appendicitis will be accurately diagnosed based on history, physical exam, laboratory studies, and if necessary, imaging techniques, there are a small number in whom the diagnosis remains elusive. For these patients, diagnostic laparoscopy can provide both a direct examination of the appendix and a survey of the abdominal cavity for other possible causes of pain.

Laparoscopy can serve as both a diagnostic and therapeutic maneuver for patients with acute abdominal pain and suspected acute appendicitis.

Laparoscopy is probably most useful in the evaluation of females with lower abdominal complaints, because appendicectomy is performed on a normal appendix in as many as 30 to 40% of these patients. Differentiating acute gynecologic pathology from acute appendicitis can be effectively accomplished using the laparoscope.[34]

Barium enema studies

In the past, barium enema examination was used to diagnose appendicitis. However in the era of ultrasonography and CT scanning, barium enema study has absolutely no role in the diagnosis of acute appendicitis.

Scoring Systems

A number of clinical and laboratory-based scoring systems have been devised to assist diagnosis. The most widely used is the Alvarado score. A score of seven or more is strongly predictive of acute appendicitis.[1]

Liver Function Tests

Importance of hyperbilirubinemia or elevated Serum Bilirubin (serum bilirubin) and its association in acute appendicitis has being postulated recently. It is hypothesized that an association exists between hyperbilirubinemia and acute appenditics and its complications such as appendiceal perforation.[55]

Bilirubin

Bilirubin (a tetrapyrrole, formerly referred to as hematoidin) is the end product of the metabolic degradation of haem, prosthetic group of haemoglobin, myoglobin, the cytochrome P450s and various other haemo-proteins.[56] The serum level of bilirubin represents the balance between production and excretion (destruction) of this breakdown product. Laboratory evaluation of serum bilirubin allows detection in two forms

1. Indirect or Unconjugated bilirubin (i.e. before hepatic metabolism)

2. Direct or Conjugated (i.e. after hepatic metabolism)[57]

Since bilirubin is potentially toxic waste product, hepatic handling is designed to eliminate it from the body via biliary tract. There are various steps involved in this process namely; hepatocellular uptake, intracellular binding, conjugation and excretion.[56] Modern analytical methods document that normal plasma contains virtually no bilirubin conjugate. The 10 to 20% of the bilirubin in normal plasma that gives rise prompt (Diazo) reaction is an artifact of kinetic of the Van Den Berg reaction which with along various modifications is the method most commonly used to quantitate bilirubin in clinical laboratories. Indeed, when direct reacting fraction is less than 15% of total bilirubin at virtually any total bilirubin concentration, the bilirubin in the sample can be considered as essentially all unconjugated.[56]

Conjugated bilirubin (mono- and di-glucronide) is excreted across canalicular plasma membrane into the canaliculus by an ATP dependant transport process mediated by a canalicular membrane protein called multi-drug resistant-associated-protein-2. The canalicular transport mechanism of excretion of bilirubin conjugate is very sensitive to injury. Accordingly, in hepatocellular disease, as well as with either cholestasis or mechanical obstruction to the bile duct, bilirubin conjugates within the hepatocytes, prevented from taking their normal pathway into the canaliculi and down the bile duct, may reflux into blood stream, resulting in mixed or less often a truly conjugated hyperbilirubinemia.[56]

Hyperbilirubinemia occurs either due to cholestatic, hepatocellular or haemolytic diseases. Cholestatic and hepatocellular hyperbilirubinemia are

associated with a rise in liver enzymes. In these cases the bilirubin is predominantly conjugated in type (mixed type). An isolated rise in serum bilirubin (without enzyme elevation) may be familial or due to hemolysis.[58] Cholestasis is the failure of normal bile to reach duodenum. This may be due to pathology any where between the hepatocyte and ampulla of Vater. Intrahepatic cholestasis includes those conditions where there is no demonstrable obstruction to major bile duct. The causes are drugs, hormones, primary billiary cirrhosis and sepsis.[58] Sepsis reaches to the liver by various routes but one of the commonest routes is through portal vein from the gastro-intestinal tract. Any inflammatory condition may cause transmigration/translocation of bacteria; its toxin or cytokines may cause suppression of hepatocellular function and reduced excretion of bile from billiary canaliculi.[59]

Hyperbilirubinemia and appendicitis

Hyperbilirubinemia, defined as an excessive amount of bilirubin in the blood, either because of increased bilirubin production or alteration of bilirubin clearance, has not been well recognized as a potential laboratory marker for aiding preoperative diagnosis of acute appendicitis and appendiceal perforation. Both mechanisms, increased production and alteration of bilirubin clearance, lead to an accumulation of bilirubin and might play a role in the observed hyperbilirubinemia of patients with appendiceal perforation.

First a variety of bacterial infections have been shown frequently to accompany hepatic dysfunction to the point of abnormalities in bile acid formation and bile flow. This ultimately can result in hyperbilirubinemia, which

is a well-known side effect in the setting of bacterial infection and especially in septic patients.[60] Septic patients those with extrahepatic bacterial infection, such as in perforated appendicitis, show a proinflammatory cytokine and nitric oxide – triggered cholestasis by impairing hepatocellular and bile duct formation.[61] Further, the most common bacterial species cultured from appendiceal wall of patients with acute appendicitis has been *Escherichia coli* and *Bacteroides fragile,* both of which have been shown to interfere with hepatocyte microcirculation, including sinusoidal damage to as shown in a rat liver model.[62,63] *E Coli* associated lipopolysaccharides have been shown to have an effect on hepatocyte uptake and excretion of bile acids.[64] *E. Coli* endotoxin leads to a dose-dependent impairment of choleresis, which has been shown in a rat model.[65] In addition, *E Coli* infection has been shown to induce hemolysis of regular erythrocytes.[66] This leads to an increased bilirubin load in infected individuals, which likely promotes hyperbilirubinemia.

<u>In summary, the patho-physiology behind the elevation of Sr. Bilirubin in Acute Appendicitis/Appendiceal perforation:</u>[15,55]

Acute Appendicitis/Appendiceal perforation (Inflammatory response causes appendix to become more oedematous and ischmeic)

↓

Causes transmigration/translocation of bacteria/toxins/cytokines

↓

Leading to endotoxemia / bacteremia

↓

Invasion of Bacteria into the hepatic parenchyma interferes with the physiology of excretion of bile

↓

Hyperbilirubinemia

The mechanism of hepatic injury in sepsis is not completely understood. This could be because of bacteria, its toxin or cytokines. In early sepsis with hyperdynamic circulation bacteria, its toxin or cytokines are involved where as in late sepsis; ischemia due to decreased hepatic blood flow to the liver is the mechanism of hepatic injury. In both above situations the hepatic injury leads to dysfunction of hepatocyte and tubule leading to mixed type of hyperbilirubinemia (hepatocellular and intra hepatic cholestasis).[22]

Cholestasis in severe bacterial infection, particularly in childhood or post operatively, is presumably hepatocellular in nature. It can also be related to cholestatic effect of endotoxin on sodium-potassium-ATPase.[58]

All the constituents of bile show an increased level in serum. Conjugation of billiary substance is intact but excretion is defective. Serum alkaline phosphatase is raised. The rise is due to increase synthesis or release of enzymes from liver or biliary plasma membrane. The minimal hepatocellular damage may be suspected by noting minimal elevated transaminase value and some times serum bilirubin.

A few case reports describe hyperbilirubinemia and jaundice as a clinical observation in patients with appendicitis.[67,68] However there is a lack of studies with a larger group of patients.

Literature review

It is hypothesized that an association exists between hyperbilirubinemia and acute appenditics and its complications.[55] There are only a few case reports in

the available literature that describe the finding of hyperbilirubinemia in patients of acute appendicitis.[55]

A retrospective analysis by Sand M et al, done at The Department of General and Visceral Surgery, Augusta Krankenanstalt, Academic Teaching Hospital of the Ruhr University, Bochum, Germany involving 538 patients (306 females: 232 males, mean age, 35.6 years) with histologically confirmed acute appendicitis who underwent conventional or laproscopic appendicectomy between January 2004 to December 2007 found the mean bilirubin level of all patients was 0.9mg/dl (±0.6 SD mg/dl; range 0.1 to 4.3mg/dl; median 0.7mg/dl). Patients with Appendiceal perforation, however had a mean bilirubin level of 1.5mg/dl (±0.9 SD mg/dl; range 0.4 to 4.3 mg/dl; median 1.4mg/dl), which was significantly higher than those with a non perforated appendicitis *($p<0.05$)*. The Specificity of hyperbilirubinemia for appendiceal perforation was 0.86 compared wwith 0.55 for white blood count and 0.96 for C-reactive protein.[69] The study concluded that the Patients with hyperbilirubinemia and clinical symptoms of appendicitis should be identified as having probability of appendiceal perforation than those with normal bilirubin levels.[69]

A retrospective analysis done at Department of Surgery, St. Luke's Hospital, Kilkenny, Ireland by Emmanuel A et al, whereby retrospective analysis of appendicectomies performed in two hospitals (n=472) was done. Data collected included laboratory and histological results. Patients were grouped according to histology findings and comparisons were made between the groups.[70] They found that the mean bilirubin levels were higher for patients with simple appendicitis compared to those with a non-inflamed appendix ($p<0.001$).

More patients with simple appendicitis had hyperbilirubinaemia on admission (30% vs 12%) and the odds of these patients having appendicitis were over three times higher (odds ratio: 3.25, $p<0.001$). Hyperbilirubinaemia had a specificity of 88% and a positive predictive value of 91% for acute appendicitis. Patients with appendicitis who had a perforated or gangrenous appendix had higher mean bilirubin levels ($p=0.01$) and were more likely to have hyperbilirubinemia ($p<0.001$). The specificity of hyperbilirubinaemia for perforation or gangrene was 70%. The specificities of white cell count and C-reactive protein were less than hyperbilirubinaemia for simple appendicitis (60% and 72%) and perforated or gangrenous appendicitis (19% and 36%). The authors concluded that hyperbilirubinaemia is a valuable marker for acute appendicitis. Patients with hyperbilirubinaemia are also more likely to have appendiceal perforation or gangrene. Bilirubin should be included in the assessment of patients with suspected appendicitis.[70]

A prospective study conducted at Department of Surgery, Nepalgunj Medical College, Teaching Hospital, Nepalgunj, Nepal by Khan S during Oct.2004-Oct.2005 in which 45 Consecutive cases of acute appendicitis admitted in surgical unit III were recruited for the study. Clinically suspected cases were subjected to investigations to confirm the diagnosis. These cases were also subjected to routine liver function tests. Subsequently these cases were operated and clinical diagnosis was confirmed per-operatively and post operatively by histopathological examination of the specimen. Of the total number of 45 cases25 were males and 20 were females. Their age ranged from 11years to 60 years. The average was 27.2 years. Duration of symptoms ranged from five hours to

maximum nine days. Among 45 cases diagnosed as acute appendicitis clinically (preoperatively), per operatively, 36 cases had inflamed appendix, three cases had gangrene, five cases had perforation with peritonitis (four localized and one generalized peritonitis) and only a single case was noted to be of normal appendix. Liver function tests (LFT) analysis revealed following results, Among 45 cases, serum bilirubin was raised in 39 cases where as six cases had normal serum bilirubin level. The raised serum bilirubin ranged from 1.2 mg/dL to 8.4 mg/dL. The average level of serum bilirubin was 2.38 mg/dL. All the cases had indirect fraction of serum bilirubin above 15%. The rise in serum bilirubin was without concomitant much rise in liver enzymes.[15] The author drew the following conclusions from its study. Firstly, There was Hyperbilirubinemia in 86.6% of the patients of acute inflammation of appendix (that is, acute appendicitis and its complications). Secondly, Raised serum bilirubin ranged from 1.2 mg/dL - 8.4 mg/dL. Thirdly, the rise in serum bilirubin was mixed in type (both indirect and direct). Finally, the hyperbiliubinemia was intra hepatic cholestatic in type due either to abnormality in permeability of hepatocyte or ductular membrane enzyme inhibition as the liver enzymes were not much elevated.[15]

A retrospective review done at the Department of Surgery, Keck School of Medicine of the University of Southern California and LosAngeles County, USC Medical Center, Los Angeles, CA, USA by Estrada J et al studied the relationship between hyperbilirubinemia and appendicitis. Patients with liver function tests on admission and pathologically confirmed appendicitis were included in the study. Age, duration of symptoms, temperature, white blood cell counts, systemic inflammatory response score, and bilirubin levels were

independent variables in a logistic regression analysis assessing factors predicting the presence or absence of appendiceal gangrene/perforation.[57] Elevated total bilirubin levels (>1mg/dl) were found in 59(38%) of 157 patients. Patients with gangrene/perforation were significantly *(p=0.004)* more likely to have hyperbilirubinemia than those with acute suppurative appendicitis. No statistical differences were observed for any of the other variables. On logistic regression the only significant relationship between the presence or absence of appendiceal gangrene and perforation was the presence of hyperbilirubinemia (p=0.031, 95% confidence interval 1.11–7.6). The odds of appendiceal perforation are three times higher (odds ratio 2.96) for patients with hyperbilirubinemia compared to those with normal bilirubin levels. Hyperbilirubinemia is frequently associated with appendicitis. Elevated bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.[55]

Another study conducted at Department of surgery, Nepalgunj Medical College, Nepalgunj, Nepal by Khan S to determine the role and predictive value of elevated total serum bilirubin (total serum bilirubin) in the diagnosis of acute appendicitis. In this study all patients admitted with clinical diagnosis of acute appendicitis were tested by laboratory investigations and ultrasonography of the abdomen. Preoperatively patient's blood was also collected for serum bilirubin and other liver enzymes estimation. Cases that underwent emergency appendicectomy from January 2004-May 2007 were included in present study.[75] It found that all the patients presented within five hours to seven days of onset of pain. Out of 110 patients studied, 71 (64.54%) were males and 39(35.45%) were females. Age distribution was between six years to 73 years with a mean of 29.5

years. Out off 110 cases, 106 cases had acute appendicitis (positive cases). Among 106 positive cases, total serum bilirubin was elevated in 87 (82.07%) cases. The mean of elevated total serum bilirubin was 2.26 mg/dL, ranged 1.2 to 11.5 mg/dL. An interesting finding was observed that patient's in whom the appendix was gangrenous or perforated; elevation of total serum bilirubin was found to be higher as compared to simple suppurative acute appendicitis. The specificity, sensitivity was 100%, 82.07%, respectively with predictive value of positive test 100%and predictive value of negative test 17.3%. The liver enzymes were either normal or marginally elevated (<1 time) in most of the cases. The Study concluded that elevated total serum bilirubin (without severe abnormalities in the value of liver enzymes) is good indicator of acute appendicitis. The specificity and sensitivity of elevated total serum bilirubin was 100% and 82.07% respectively with a predictive value for positive test 100%. If total serum bilirubin is added to already existing laboratory tests, then the diagnosis of acute appendicitis in clinically suspected cases can be made with fair degree of accuracy and unnecessary or delay in appendicectomy can be avoided.[71]

METHODOLOGY

The present study was conducted in the Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period of January 2010 to December 2010.

Study design

A one year cross sectional study

Place

The present study was conducted in the Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum attached to Jawaharlal Nehru Medical College, Belgaum.

Study period

One year from January 2010 to December 2010.

Source of data

Patients admitted with clinical diagnosis of acute appendicitis or appendiceal perforation under the Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period.

Sample size

A total of 100 patients with clinical diagnosis of acute appendicitis or appendiceal perforation were studied.

Sampling method

The sample size was calculated based on the following formula.

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where,

n = Sample size

Z = 1.96 ≈ 2 (considering confidence as 95%)

p = prevalence (Considered as 50% as exact prevalence is not known)

q = 100 – p that is, 50%

d = Absolute error which was 10%

Selection criteria

Inclusion

- All patients diagnosed as acute appendicitis clinically on admission.
- All patients diagnosed as appendiceal perforation clinically on admission.
- For both these groups, only patients with histopathological report suggestive of acute appendicitis or appendiceal perforation were included.

Exclusion

- All patients documented to have a past history of;
    - Jaundice or Liver disease.
    - Chronic alcoholism (that is intake of alcohol of > 40 g/day for Men and > 20 g/day in Women for 10 years).[72]

  - Hemolytic disease.
  - Acquired or congenital biliary disease.
- All patients with positive HBsAg.
- All patients with cholelithiasis.
- All patients with cancer of hepato-biliary system.

Procedure

Ethical clearance for the study was obtained from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum. Based on the selection criteria patients admitted with clinical diagnosis of acute appendicitis or appendiceal perforation under Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the study period were screened for eligibility. The eligible patients were briefed about the nature of the study and a written informed consent (Annexure I) was obtained from the consented patients. Thorough history was taken and clinical examination was done for all patients and findings were recorded on predesigned and pretested proforma (Annexure II).

The following tests were carried out on admission.

- Routine blood investigations (Complete blood count, platelet count, reticulocyte count, mini real profile).
- Peripheral smear to rule out hemolytic anemia.
- Serum haptoglobulin if peripheral smear and blood tests indicate features of hemolytic anemia.
- Serum Bilirubin (Total and Direct bilirubin).

- Liver Function Tests (LFTs) which include;
    - SGPT (Alanine transaminase).
    - SGOT (Aspartate transaminase).
    - ALP (Alkaline phosphatase).
- Seropositivity for HbsAg
- Urine analysis (routine and microscopy).

The serum bilirubin and LFTs were carried out using the Auto Analyser machine available in the hospital and HbsAg was tested by ELISA / Spot technique using HEPALISA© or HEPACARD© kit.

Reference Range of Serum Bilirubin and Liver Enzymes[34]

Test		Normal Range
Serum Bilirubin	Total	0.3 - 1.0 mg/dL
	Direct	0.1 – 0.3 mg/dL
Liver Enzymes	SGPT	0 – 35 U/L
	SGOT	0 – 35 U/L
	ALP	30 – 120 U/L

The results were grouped as ‘Normal’ or ‘Raised’ (hyperbilirubinemia) as per the above reference values.

Statistical analysis

The data obtained was tabulated on Microsoft excel spreadsheet and analysed as below.

- Patients with clinical diagnosis of acute appendicitis having hyperbilirubinemia were expressed in percentage as

$$= \frac{\text{Patients with clinical diagnosis of acute appendicitis with elevated Sr. bilirubin level}}{\text{All patients with clinical diagnosis of acute appendicitis}}$$

- Mean of the level of elevation of Sr. bilirubin was calculated for patients with clinical diagnosis of acute appendicitis.

- Patients with clinical diagnosis of appendiceal perforation having hyperbilirubinemia were expressed in percentage as;

$$= \frac{\text{Patients with clinical diagnosis of appendiceal perforation with elevated Sr. bilirubin}}{\text{All patients with clinical diagnosis of appendiceal perforation}}$$

- Mean of the level of elevation of serum bilirubin were calculated for patients with clinical diagnosis of appendiceal perforation.

- A hypothesis was made based on the observation of the level of the two means.

- Also, sensitivity, specificity, positive predictive value, negative predictive value and Odds ratio was determined by 2 x 2 table as below.

	Acute appendicitis	Appendiceal perforation
Raised Sr. Bilirubin	a	b
Normal Sr. Bilirubin	c	d
	a + c	b + d

Sensitivity: $\frac{a}{a + c}$ x 100

Specificity: $\frac{d}{b + d}$ x 100

Positive predictive value : $\frac{a}{a + b}$ x 100

Negative predictive value : $\frac{d}{c + d}$ x 100

Odds ratio: $\frac{ad}{bc}$

RESULTS

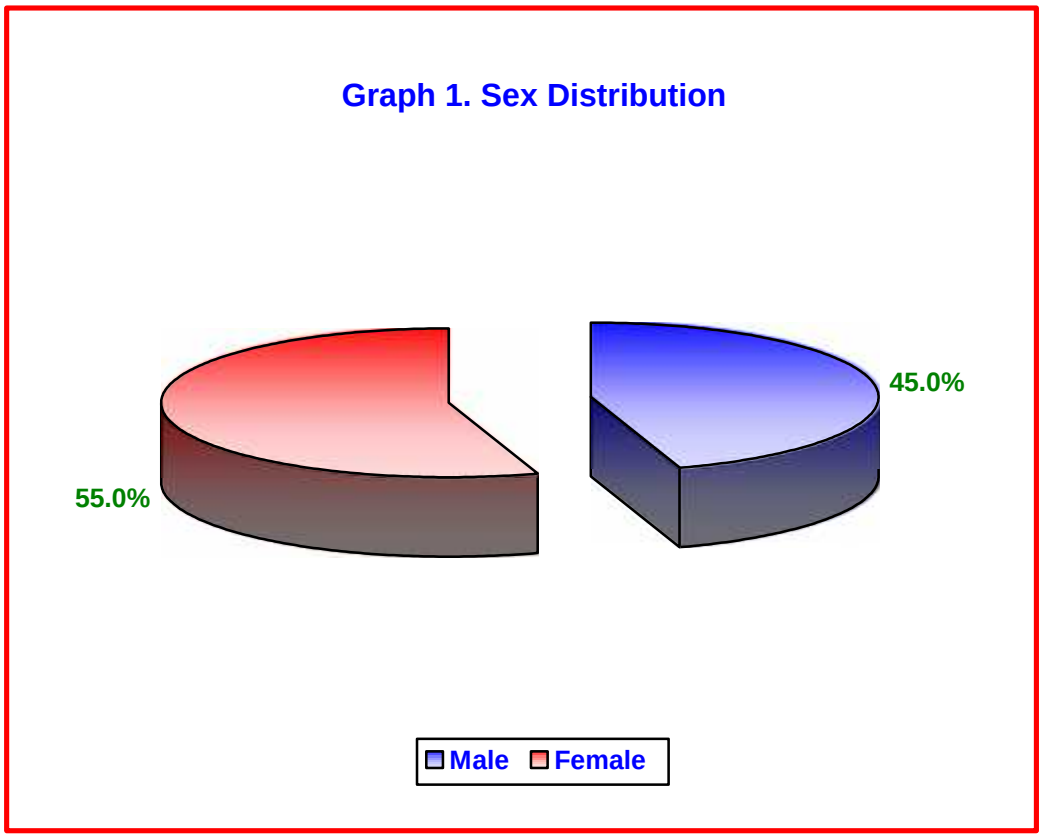
The present one year cross sectional study was conducted in the Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period of January 2010 to December 2010.

A total of 100 patients with clinical diagnosis of acute appendicitis or appendiceal perforation were enrolled in the study and studied.

The data obtained was tabulated on Microsoft excel spreadsheet and analyzed as below.

Table 2. Sex distribution

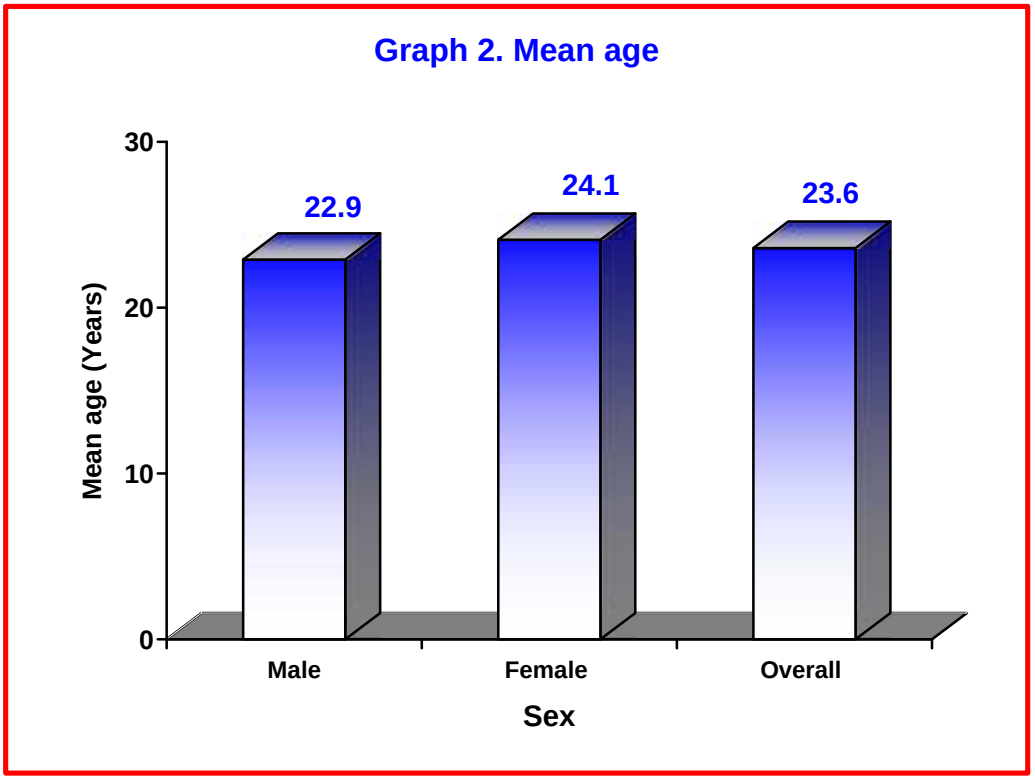
Sex	Distribution (n=100)	
	Number	**Percentage**
Male	45	45.00
Female	55	55.00
Total	**100**	**100.00**



Of the 100 patients enrolled for the study, 45 patients (45%) were males while the remaining 55 patients (55%) were females.

Table 3. Age distribution

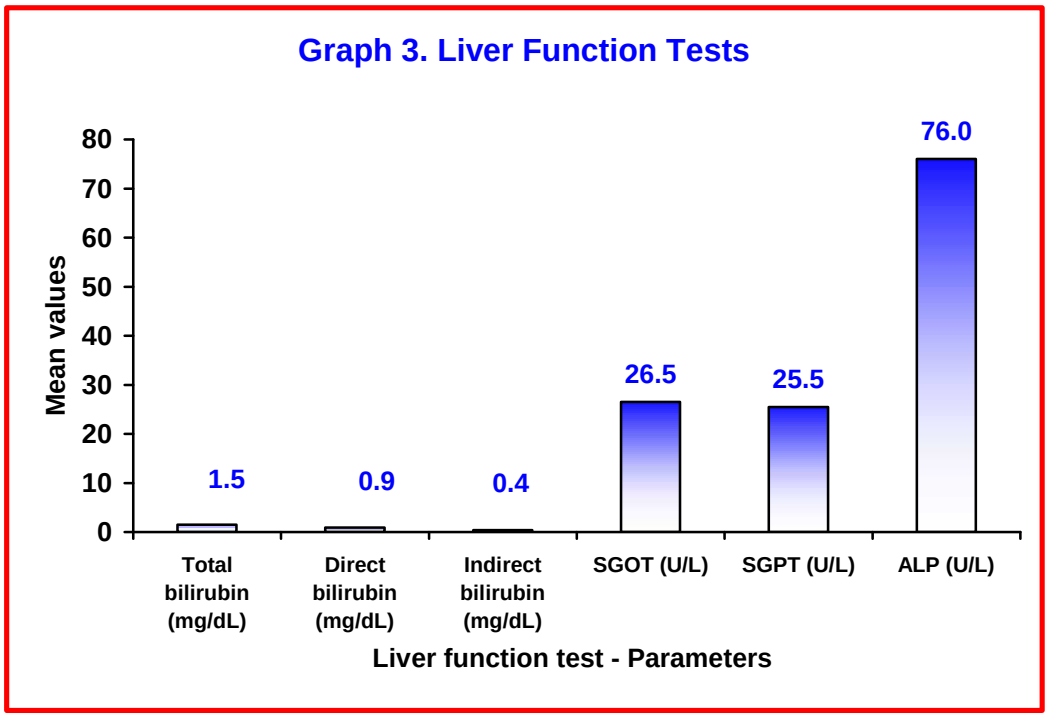
Sex	Mean age (Years)	
	Mean	**SD**
Male	22.9	6.27
Female	24.1	6.97
Overall	**23.6**	**6.66**



The overall mean age of all 100 patients was 23.6 ± 6.6 years (range, 17.0 – 30.2 years). The average age in males and females was 22.9 ± 6.27 years (range, 16.63 – 29.17 years) and 24.1 ± 6.97 years (range, 17.13 – 31.07 years) respectively.

Table 4. Liver Function Tests

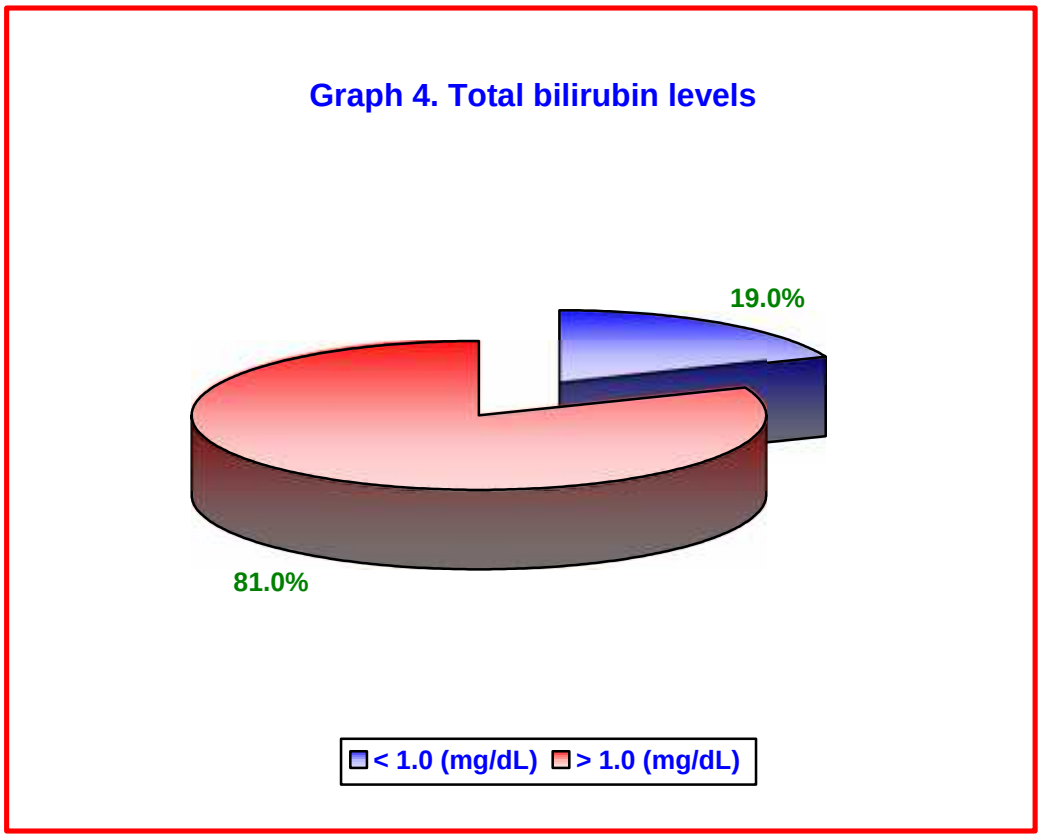
Parameters	Mean values	
	Mean	**SD**
Total bilirubin (mg/dL)	1.5	0.8
Direct bilirubin (mg/dL)	0.9	0.8
Indirect bilirubin (mg/dL)	0.4	0.2
SGOT (U/L)	26.5	11.3
SGPT (U/L)	25.5	10.0
ALP (U/L)	76.0	24.2



The mean Total bilirubin of all 100 patients was 1.5 ± 0.8 mg/dL (range, 0.7 – 2.3 mg/dL) while the Direct component (Direct bilirubin) was 0.9 ± 0.8 mg/dL (range, 0.1-1.7 mg/dL). The mean SGOT and SGPT were 26.5 ± 11.3 U/L (range, 15.2-37.6 U/L) and 25.5 ± 10.0 U/L (range, 15.5 – 35.5 U/L). The mean ALP values were 76.0 ± 24.2 U/L (range, 51.8 -100.2 U/L).

Table 5. Total bilirubin levels in All patients (n=100)

Total bilirubin (mg/dL)	Distribution (n=100)	
	Number	Percentage
< 1.0	19	19.0
> 1.0	81	81.0
Total	**100**	**100.00**

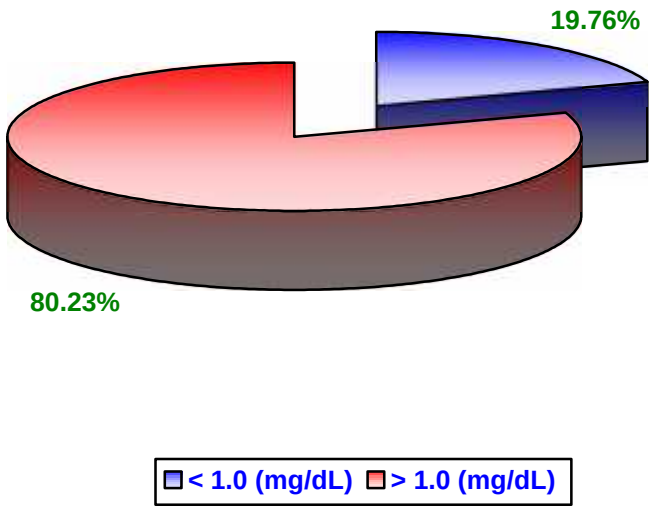


19 patients (19%) of all 100 patients were found to have normal bilirubin levels (≤ 1.0 mg/dL), while 81 patients (81%) had raised bilirubin levels (> 1.0 mg/dL).

Table 6. Bilirubin levels in patients with acute appendicitis diagnosis

Total bilirubin (mg/dL)	Distribution in Patients with Acute Appendicitis (n=86)	
	Number	**Percentage**
> 1.0	69	80.23
< 1.0	17	19.76
Total	**86**	**100.00**

Graph 5. Bilirubin levels in patients with acute appendicitis diagnosis

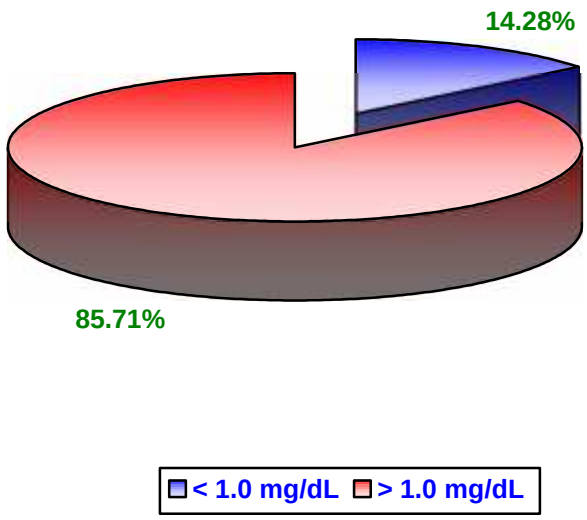


Of 86 patients diagnosed as acute appendicitis, 69 patients (80.23%) had raised bilirubin levels (> 1.0 mg/dL), while the remaining 17 patients (19.76%) had normal levels (≤ 1.0 mg/dL).

Table 7. Bilirubin levels in patients with appendiceal perforation diagnosis

Total bilirubin (mg/dL)	Distribution in Patients with Appendiceal perforation (n=14)	
	Number	**Percentage**
> 1.0	12	85.71
< 1.0	02	14.28
Total	**14**	**100.00**

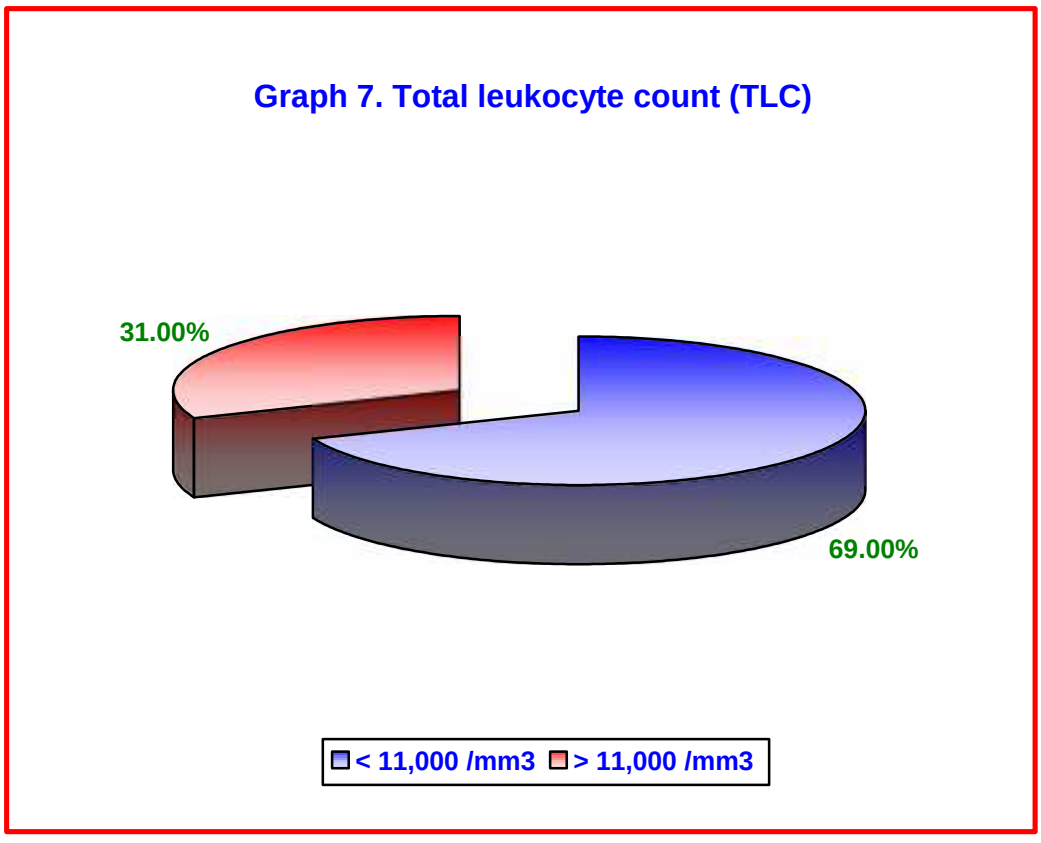
Graph 6. Bilirubin levels in patients with appendiceal perforation diagnosis



14 patients diagnosed as Appendiceal perforation, 12 patients (85.71%) had raised bilirubin levels (> 1.0 mg/dL), while the remaining 02 patients (14.28%) had normal levels (≤ 1.0 mg/dL).

Table 8. Total leukocyte count (TLC)

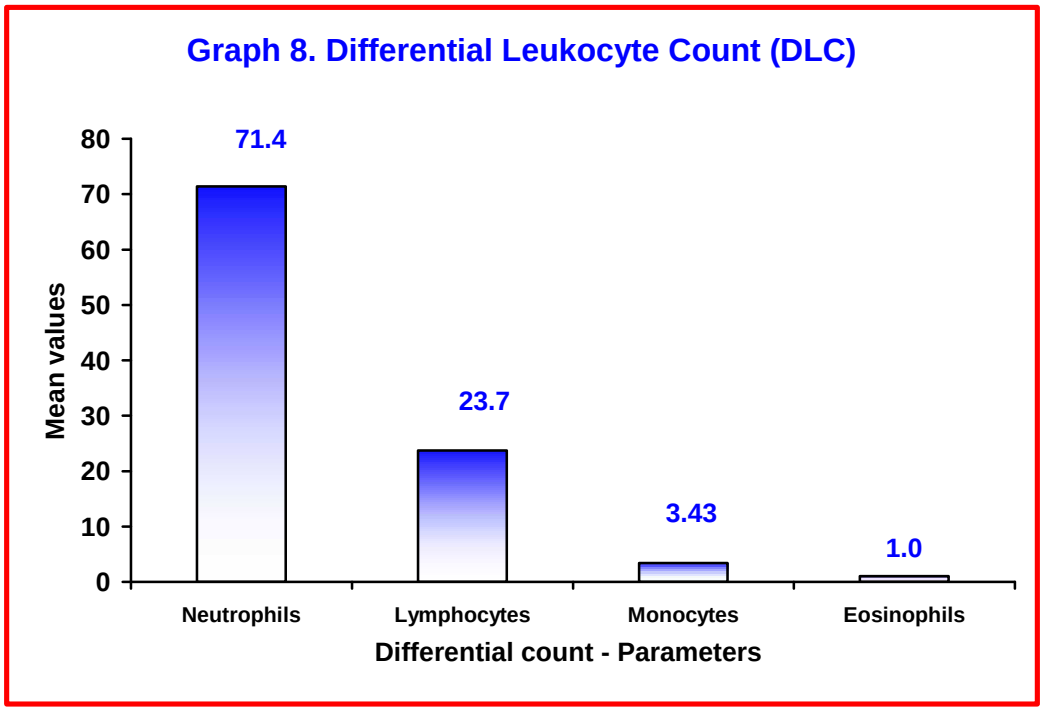
TLC count (/mm^3)	Distribution (n=100)	
	Number	**Percentage**
< 11,000	69	69.0
> 11,000	31	31.0
Total	**100**	**100.00**



69 patients (69%) had Total Leukocyte count less than 11,000/mm^3 while 31 patients (31%) counts above 11,000/mm^3

Table 9. Differential Leukocyte Count (DLC)

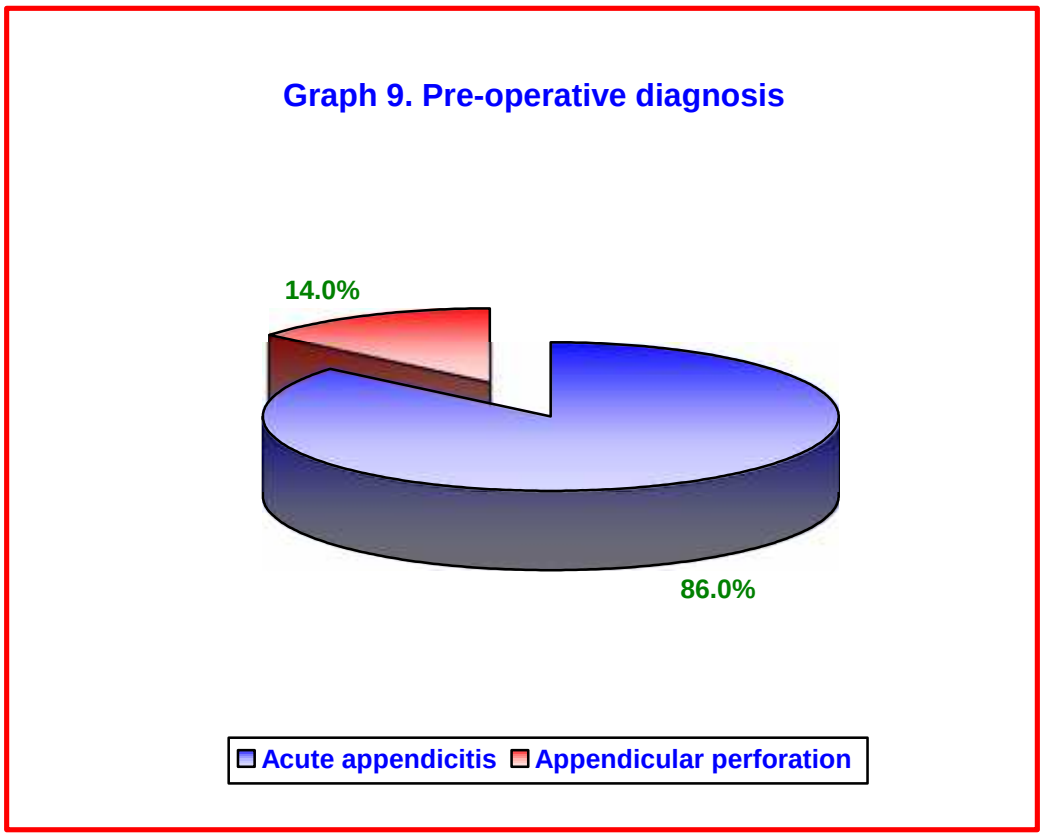
Differential Leukocyte Count (DLC)		Mean value	
		Mean	**SD**
Total count (/mm^3)		9800.5	3675.9
Differential count	Neutrophils	71.4	11.5
	Lymphocytes	23.7	10.7
	Monocytes	3.43	2.6
	Eosinophils	1.02	1.4



The mean of TLC count in all patients was 9800.5±3675.9 /mm^3 (range, 6124.6 - 13476.4 /mm^3), in which the highest percentage constituted neutrophils with 71.4% followed by 23.7% by Lymphocytes.

Table 10. Pre-Operative Diagnosis

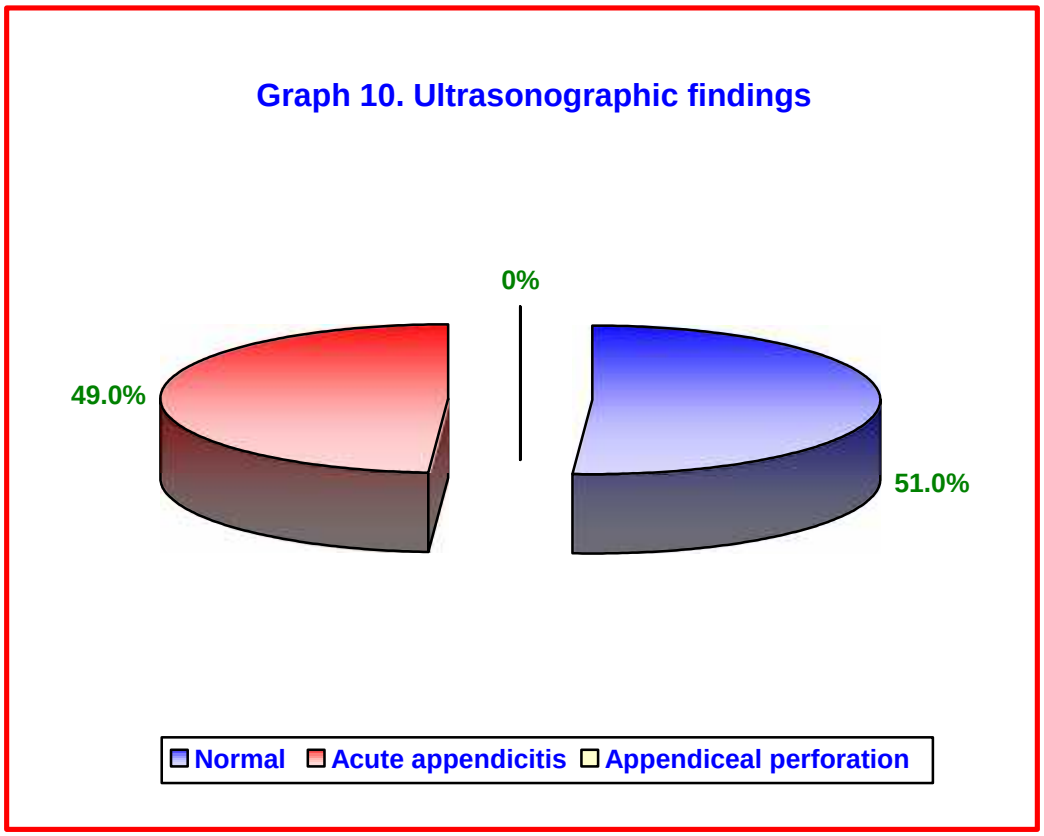
Pre – Operative Diagnosis	Distribution (n=100)	
	Number	**Percentage**
Acute appendicitis	86	86.0
Appendiceal perforation	14	14.0
Total	**100**	**100.00**



In the study population of 100 patients, 86 patients (86%) were diagnosed as acute appendicitis while 14 patients (14%) were diagnosed with appendiceal perforation.

Table 11. Ultrasonographic findings

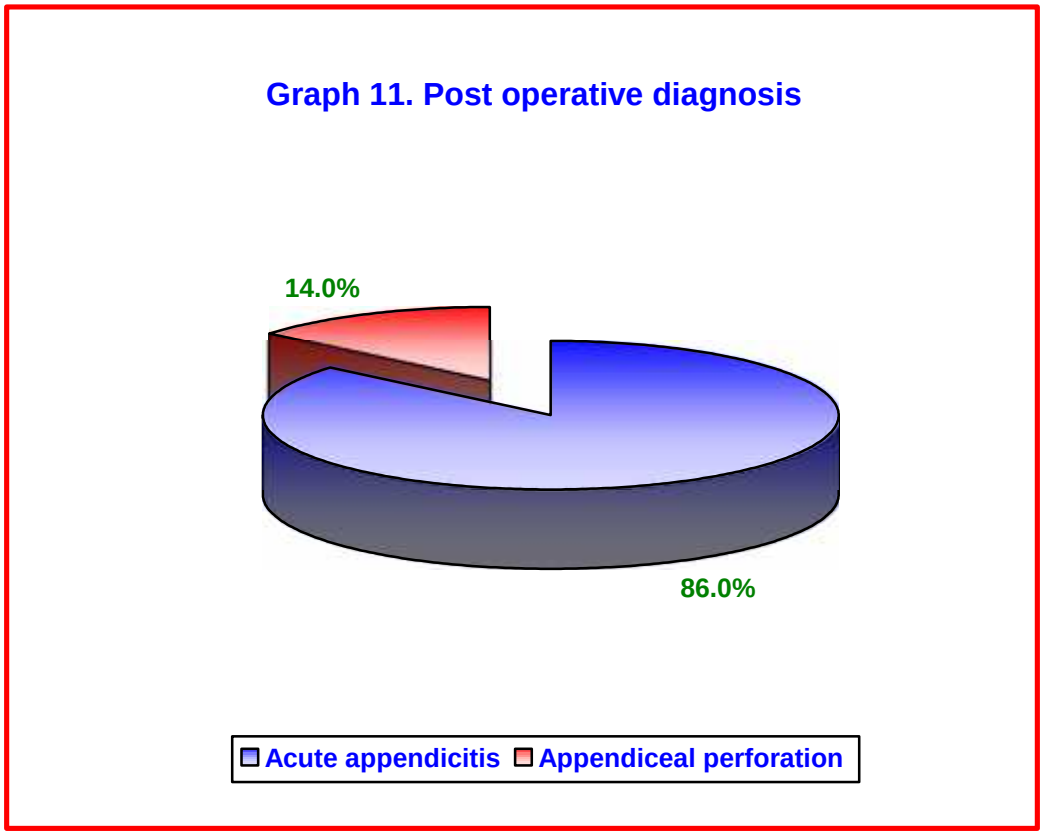
Findings	Distribution (n=100)	
	Number	**Percentage**
Normal	51	51.0
Acute Appendicitis	49	49.0
Appendiceal perforation	00	0.0
Total	**100**	**100.00**



On Ultrasonography, 49 patients (49%) were diagnosed as Acute appendicits while 51 patients (51%) were reported as normal ultrasonographic findings. None however were diagnosed as Appendiceal perforation on ultrasonography.

Table 12. Post-operative diagnosis

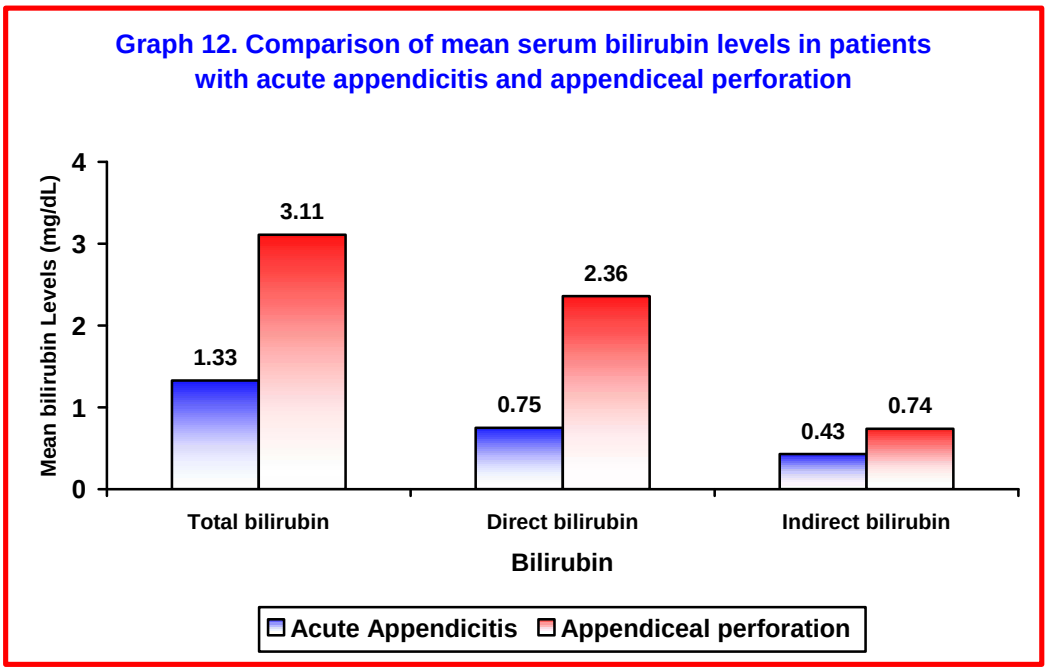
Diagnosis	Distribution (n=100)	
	Number	**Percentage**
Acute appendicitis	86	86.0
Appendiceal perforation	14	14.0
Total	**100**	**100.00**



Post-operatively 86 patients (86%) were confirmed as Acute appendicitis while 14 patients (14%) were diagnosed with Appendiceal perforation

Table 13. Comparison of mean serum bilirubin levels in patients with acute appendicitis and appendiceal perforation

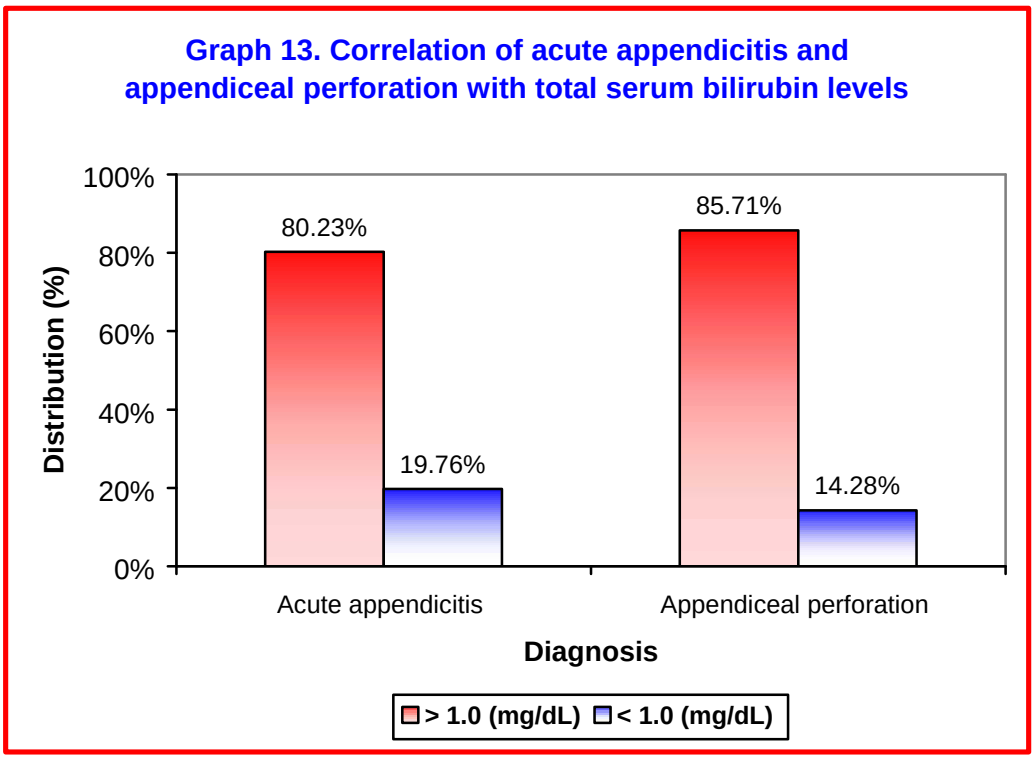
Bilirubin levels (mg/dL)	Diagnosis			
	Acute appendicitis		Appendiceal perforation	
	Mean	SD	Mean	SD
Total bilirubin	1.33	0.40	3.11	1.10
Direct bilirubin	0.75	0.40	2.36	1.00
Indirect bilirubin	0.43	0.20	0.74	0.30



The mean bilirubin levels in patients diagnosed with Acute appendicitis was 1.33 ±0.40 mg/dL (range, 1.29 – 1.73 mg/dL) while in patients diagnosed with Appendiceal perforation was 3.11±1.10 mg/dL (range, 2.01 – 4.21 mg/dL). The Direct bilirubin and Indirect bilirubin in patients diagnosed with Acute appendicitis was 0.75±0.40 mg/dL and 2.36±1.00 mg/dL respectively. The Direct bilirubin and Indirect bilirubin in patients diagnosed with Appendiceal perforation was 0.43±0.20 mg/dL and 0.74±0.30 mg/dL respectively.

Table 14. Correlation of acute appendicitis and appendiceal perforation with total serum bilirubin levels

Serum bilirubin (mg/dL)	Final diagnosis (n=100)			
	Acute appendicitis (n=86)		Appendiceal perforation (n=14)	
	No	%	No	%
> 1.0	69	80.23	12	85.71
< 1.0	17	19.76	2	14.28
Total	**86**	**100.00**	**14**	**100.00**



69 patients (80.23%) of the total patients diagnosed with Acute appendicitis (n=86) were found to have elevated bilirubin levels (> 1.0 mg/dL) while 17 patients (19.76%) had normal bilirubin levels (≤ 1.0 mg/dL). Similarly, 12 patients (85.71%) of the total patients diagnosed with Appendiceal perforation

(n=14) were found to have elevated bilirubin levels (> 1.0 mg/dL) while 02 patients (14.28%) had normal bilirubin levels (≤ 1.0 mg/dL).

From this table, following values were calculated as -

Sensitivity

$$= \frac{a}{a + c} = \frac{69}{69 + 17} = 80.23\%$$

Therefore, sensitivity of bilirubin in predicting acute appendicitis and appendiceal perforation diagnosis was 80.23%.

Specificity

$$= \frac{d}{b + d} = \frac{2}{12 + 2} = 14.28\%$$

Therefore, specificity of bilirubin in predicting acute appendicitis and appendiceal perforation diagnosis was 14.28%

Positive predictive value

$$= \frac{a}{a + b} = \frac{69}{69 + 12} = 85.18\%$$

Therefore, Positive predictive value of bilirubin in predicting acute appendicitis and appendiceal perforation diagnosis was 85.18%

Negative predictive value

$$= \frac{d}{c + d} = \frac{2}{17 + 2} = 10.50\%$$

Therefore, Negative predictive value of bilirubin in predicting acute appendicitis and appendiceal perforation diagnosis was 10.50%.

Odds ratio:

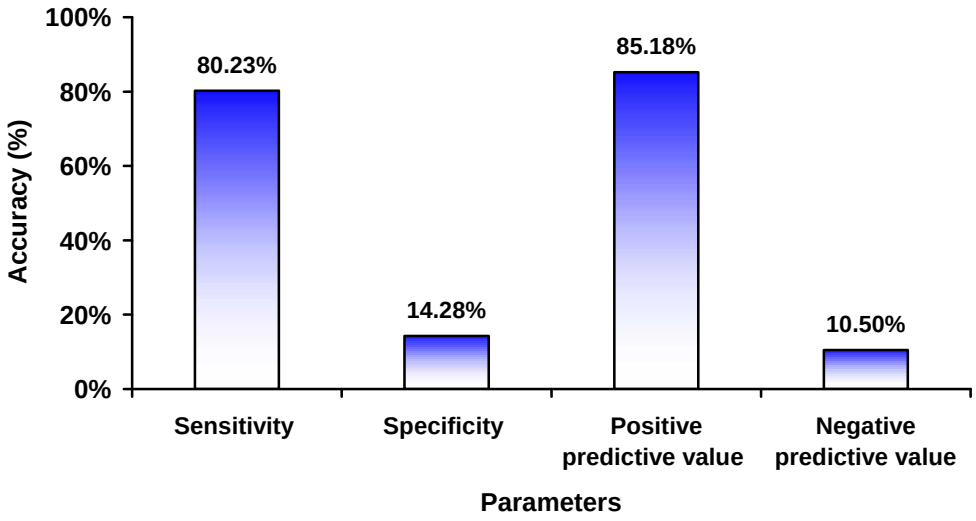
$$= \frac{ad}{bc} = \frac{69 \times 2}{17 \times 12} = 0.676$$

Therefore, Odds ratio is 0.676.

Table 15. Accuracy of serum bilirubin as a marker in predicting acute appendicitis and appendiceal perforation

	Accuracy
Sensitivity	80.23%
Specificity	14.28%
Positive predictive value	85.18%
Negative predictive value	10.5%
Odds ratio	0.676

Graph 14. Accuracy of serum bilirubin as a marker in predicting acute appendicitis and appendiceal perforation



The Sensitivity and Specificity of serum bilirubin as a marker in predicting acute appendicitis and appendiceal perforation was 80.23% and 14.28% respectively. Similarly the Positive predicative value and Negative predicative value for the same is 85.18% and 10.5% respectively. The Odds ratio was calculated to be 0.67.

DISCUSSION

Acute appendicitis is the most common cause of an 'acute abdomen' in young adults. Appendicectomy is the most frequently performed urgent abdominal operation and is often the first major procedure performed by a surgeon in training.[1] About 8% of people in Western countries have appendicitis at some time in their lifetime.[42]

The peak incidence of acute appendicitis is in the second and third decade of life.[74] It is relatively rare in infants, and becomes increasingly common in childhood and early adult life. The incidence of appendicitis is equal in males and females before puberty. In teenagers and young adults, the male – female ration increases to 3:2 at age 25.[1] The lifetime rate of appendicectomy is 12% for men and 25% for women, with approximately 7% of all people undergoing appendectomy for acute appendicitis during their lifetime.[43,44]

Obstruction of the lumen is believed to the major cause of acute appendicitis.[42] Faecoliths are the usual cause of obstruction. Less- common causes are hypertrophy of lymphoid tissue, tumors, intestinal parasites.[34] The bacteriology of normal appendix is similar to that of normal colon. The principal organism seen in normal appendix, in acute appendicitis, and in perforated appendicitis are *Escherichia Coli* and *Bacteroids fragilis*. However a wide variety of both facultative and anaerobic bacteria may be present.[34]

The diagnosis of acute appendicitis is essentially clinical; however, a decision to operate based on clinical suspicion alone can lead to the removal of a

normal appendix in 15 to 30% of cases. The premise that it is better to remove a normal appendix than to delay diagnosis does not stand up to close scrutiny, particularly in the elderly.[1] Hence, the diagnosis of Appendicitis still remains a dilemma in spite of the advances in various laboratory and radiological investigations.

A new tool to help in the diagnosis of acute appendicitis would thus be welcome.

Serum Bilirubin level elevation will help in the accuracy of clinical diagnosis of acute appendicitis and more importantly help in foreseeing and preventing impeding complications of acute appendicitis.

This study was taken up with this thought – that is it possible to add serum bilirubin as a new laboratory marker to aid in the diagnosis of acute appendicitis and if so, does it have the credibility to help us foresee an impending complication of acute appendicitis?

Importance of hyperbilirubinemia and its association in acute appendicitis has being postulated recently. There are only a few case reports in the available literature that describe the finding of hyperbilirubinemia in patients of acute appendicitis.[55] It is hypothesized that an association exists between hyperbilirubinemia and acute appenditics and its complications.[55]

The present study was undertaken to study the relationship between hyperbilirubinemia and acute appendicitis and to evaluate its credibility as a diagnostic marker for acute appendicitis and also, to evaluate whether elevated

bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

This study was conducted in the Department of General Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum over a period from January 2010 to December 2010 on 100 patients with clinical diagnosis of Acute appendicitis and Appendiceal perforation.

In the present study of the 100 patients enrolled for the study, 45 patients (45%) were males while the remaining 55 patients (55%) were females.The mean age in our study population (100 patients) was 23.6 years ± 6.6 years. (range, 17.0 – 30.2 years). This is consistent with the quoted incidence of Appendicitis in the literature where it is most frequently seen in patients in their second through fourth decades of life.[43,44] The average age group in females 24.1 ± 6.97 years (range, 17.13 – 31.07 years) was slightly higher than males 22.9 ± 6.27 years (range, 16.63 – 29.17 years).

Hyperbilirubinemia (> 1.0 mg/dL) in our study was found in 81 patients (81%) of all the 100 patients (n=100) enrolled in the study, while 19 patients (19%) had normal bilirubin levels (≤ 1.0 mg/dL). Estrada et al[55] had found hyperbilirubinemia in 59 (38%) of 157 patients studied with acute appendicitis.

The mean total serum bilirubin of all 100 patients was 1.5 ± 0.8 mg/dL (range, 0.7 – 2.3 mg/dL), which was above the normal range (≤ 1.0 mg/dL) considered for the study, hence indicating the occurrence of hyperbilirubinemia. The mean of Direct bilirubin was 0.9±0.8 mg/dL (range, 0.1-1.7 mg/dL) while that of Indirect bilirubin was 0.4±0.2 mg/dL (range, 0.2 – 0.6 mg/dL). Our

finding was consistent with hyperbilirubinemia found in a study conducted by Khan S,[15] who found average level of serum bilirubin in his study population to be 2.38 mg/dL.

All patients were found to have SGOT and SGPT within the normal range, thus excluding any associated liver pathology (Exclusion criteria). The mean SGOT and SGPT were 26.5 ± 11.3 U/L (range, 15.2-37.6 U/L) and 25.5 ± 10.0 U/L (range, 15.5 – 35.5 U/L). The mean ALP values were 76.0 ± 24.2 U/L (range, 51.8 -100.2 U/L).

In our study population of 100 patients, 86 patients (86%) were diagnosed as acute appendicitis pre-operatively while 14 patients (14%) were diagnosed with Appendiceal perforation. The diagnosis was confirmed post-operatively by histopathological reports (HPR) and those differing from the pre-operative diagnosis were excluded from the study.

Amongst the patients diagnosed with Acute appendicitis pre-operatively (n=86), 69 patients (80.23%) were found to have elevated bilirubin (>1.0 mg/dL) while only 17 patients (19.26%) had normal bilirubin levels (≤1.0 mg/dL). In patients diagnosed with Appendiceal perforation (n=14), 12 patients (85.71%) had bilirubin elevated (>1.0 mg/dL), while only 2 patients (14.28%) had normal levels (>1.0 mg/dL). Thus, Hyperbilirubinemia was found in most of the patients diagnosed with acute appendicitis (80.23%) or appendiceal perforation (85.71%).

The total leukocyte count was found elevated in just 31 patients (31%) of the total 100 patients. The mean of TLC count in all patients was 9800.5±3675.9

/mm^3 (range, 6124.6 - 13376.4/mm^3), in which the highest percentage constituted Neutrophils with 71.4% followed by 23.7% by Lymphocytes.

On Ultrasonography, 49 patients (49%) were diagnosed as acute appendicits while 51 patients (51%) were reported as normal ultrasonographic findings. None however were diagnosed as Appendiceal perforation on ultrasonography. Ultrasonography per-se was not helpful as a useful investigation for appendicitis or appendiceal perforation in our study as none of the USG findings reported Appendiceal perforation, hence belief that the diagnosis of appendicitis still remains essentially clinical, still hold true.

The mean bilirubin levels in patients diagnosed with acute appendicitis was 1.33 ±0.40 mg/dL (range, 1.29 – 1.73 mg/dL) while in patients diagnosed with Appendiceal perforation was 3.11±1.10 mg/dL (range, 2.01 – 4.21 mg/dL). Hence, we see that patients with appendiceal perforation had nearly three times more levels of bilirubin as compared to that of acute appendicitis. So we infer that, patients with features suggestive of appendicitis with three times their normal range of bilirubin, are more susceptible of having appendiceal perforation than those with normal or slightly elevated total serum bilirubin.

Sand et al[69] in his study found the mean bilirubin levels in patients with appendiceal perforation to be significantly higher than those with a non-perforated appendicitis.

The Direct bilirubin and indirect bilirubin in patients diagnosed with acute appendicitis was 0.75±0.40 mg/dL and 2.36±1.00 mg/dL respectively. Similarly, direct bilirubin and indirect bilirubin in patients diagnosed with

Appendiceal perforation was 0.43±0.20 mg/dL and 0.74±0.30 mg/dL respectively.

The Sensitivity, Specificity, Positive predictive value, Negative predictive value and Odds ratio was calculated from a 2x2 table. Sensitivity and Specificity of bilirubin in predicting acute appendicitis and appendiceal perforation diagnosis was 80.23% and 14.28% respectively. Similarly Positive predictive value and Negative predicative value of bilirubin in predicting acute appendicitis and appendiceal perforation diagnosis was 14.28% and 10.50% respectively. The Odd's ratio was calculated to be 0.67.

The sensitivity in our study was more than that by Sand et al[69] in which, he found the sensitivity and specificity in his study of hyperbilirubinemia for predicting appendiceal perforation to be 70% and 86.0% respectively.

CONCLUSION

Finding of the persent study suggest;

- Serum bilirubin levels appears to be a promising new laboratory marker for diagnosing acute appendicitis, however diagnosis of appendicitis remains essentially still - clinical. Its levels come out to be a credible *aid* in diagnosis of acute appendicitis and would be helpful investigation in decision making.

- Patients with clinical sings and symptoms of appendicitis and with hyperbilirubinemia three times the normal range should be identified as having a higher probability of appendiceal perforation suggesting, serum bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

SUMMARY

Acute appendicitis is the most common cause of an 'acute abdomen' in young adults. Diagnosis of Appendicitis still remains a dilemma in spite of the advances in various laboratory and radiological investigations. Importance of hyperbilirubinemia or elevated Serum Bilirubin and its association in acute appendicitis has being postulated recently. It is hypothesized that an association exists between hyperbilirubinemia and acute appenditics and its complications

The present study was undertaken to assess relationship between hyperbilirubinemia and acute appendicitis and to evaluate its credibility as a diagnostic marker for acute appendicitis and also, to see whether elevated bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

The present one year cross sectional study was conducted in the Department of Surgery, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum during the period of January 2010 to December 2010. A total of 100 patients with clinical diagnosis of acute appendicitis or appendiceal perforation were studied. The serum bilirubin and LFTs were carried out in all the patients.

In this study, female (55%) outnumbered males (45%) and overall the mean age was 23.6 ± 6.6 years. Mean total bilirubin was noted as 1.5 ± 0.8 mg/dL (range, 0.7 – 2.3 mg/dL) while direct bilirubin was 0.9 ± 0.8 mg/dL (0.1-1.7 mg/dL). The mean SGOT and SGPT were 26.5 ± 11.3 U/L (range, 15.2-37.6

U/L) and 25.5 ± 10.0 U/L (range, 15.5 – 35.5 U/L). The mean ALP values were 76.0 ± 24.2 U/L (range, 51.8 -100.2 U/L).

Normal bilirubin values were seen in 19% patients while, 81% had raised bilirubin levels (Hyperbilirubinemia). Of 86 patients with acute appendicitis, 80.23% had raised bilirubin levels, while 19.76% had normal levels. 14 patients diagnosed as Appendiceal perforation, 12 patients (85.71%) had raised bilirubin levels, while the remaining 02 patients (14.28%) had normal levels. The total leukocyte count was less than 11,000/mm^3 in 69% patients while, 31% patients had counts above 11,000/mm^3.

Of the 100 patients, 86% were diagnosed as acute appendicitis while 14% were diagnosed with appendiceal perforation. On Ultrasonography, 49% patients were diagnosed with acute appendicits while 51% had normal findings. On ultrasonography, none of the patient was diagnosed with appendiceal perforation. Post-operatively 86% were confirmed as acute appendicitis while 14% were diagnosed with appendiceal perforation.

The mean bilirubin levels in patients diagnosed with acute appendicitis was 1.33±0.40 mg/dL (range, 1.29 – 1.73 mg/dL) while in patients diagnosed with appendiceal perforation was 3.11±1.10 mg/dL (range, 2.01 – 4.21 mg/dL). The Direct bilirubin and Indirect bilirubin in patients diagnosed with acute appendicitis was 0.75±0.40 mg/dL and 2.36±1.00 mg/dL respectively. The Direct bilirubin and Indirect bilirubin in patients diagnosed with appendiceal perforation was 0.43±0.20 mg/dL and 0.74±0.30 mg/dL respectively.

69 patients (80.23%) of the total patients diagnosed with acute appendicitis (n=86) were found to have elevated bilirubin levels while 17 patients (19.76%) had normal bilirubin levels. Similarly, 12 patients (85.71%) of the total patients diagnosed with Appendiceal perforation (n=14) were found to have elevated bilirubin levels while 02 patients (14.28%) had normal bilirubin levels. The Sensitivity and Specificity of serum bilirubin as a marker in predicting acute appendicitis and appendiceal perforation was 80.23% and 14.28% respectively. Similarly the Positive predicative value and Negative predicative value for the same was 85.18% and 10.5% respectively with odds ratio 0.67.

Serum bilirubin levels appears to be a promising new laboratory marker for diagnosing acute appendicitis, however diagnosis of appendicitis is essentially still - clinical. Patients with clinical sings and symptoms of appendicitis and with hyperbilirubinemia three times the normal range should be identified as having a higher probability of appendiceal perforation suggesting, serum bilirubin levels have a predictive potential for the diagnosis of appendiceal perforation.

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ANNEXURE I – CONSENT FORM

Dear Mr/Mrs/Dr_____, you are kindly requested to participate in a research study tilted "Evaluation of Hyperbilirubinemia as a new diagnostic tool for Acute Appendicitis and, also as a predictor of Appendiceal Perforation" conducted by Dr. **** *****, a post graduate student in M.S. General Surgery in Jawaharlal Nehru Medical College, Belgaum.

You have been requested to participate in this as you fit into the laid out criteria for a study 'subject'/ participant. The important elements of this study have been explained below in a question-answer format to help clear your queries/doubts.

During the study you will be asked some questions and you are supposed to answer to the best of your knowledge. Your participation in this research is voluntary. Your decision whether or not to participate in the study will not affect your treatment in any form during your hospital stay. If you decide to participate you are free to withdraw at any time.

TITLE OF THE STUDY:

"Evaluation of Hyperbilirubinemia as a new diagnostic tool for Acute Appendicitis and, also as a predictor of Appendiceal Perforation"

OBJECTIVE/PURPOSE OF THE STUDY:

Acute appendicitis is one of the most common acute intra abdominal affections seen in surgical departments even today[1]. The diagnosis of Appendicitis still remains a dilemma in spite of the advances in various laboratory and radiological investigations.

A new tool to help in the diagnosis of acute appendicitis would thus be welcome.

The purpose of the study is to find out if it is possible to add Sr. Bilirubin as a new laboratory marker to aid in the diagnosis of acute appendicitis and if so,

does it have the credibility to help us foresee an impending complication of acute appendicitis.

Why am I being asked to participate in this research?

You are being asked to participate in this research as you find all the criteria laid by the author of the study as 'subjects'

Who is being recruited or selected?

Patients being selected are -

i. All patients diagnosed as Acute Appendicitis clinically on admission.
ii. All patients diagnosed as Appendiceal Perforation clinically on admission

Who are the investigators? Who is funding the study?

The investigator/author of this study is Dr. ***** ******, a postgraduate student in Dept. of General Surgery, Jawaharlal Nehru Medical College. The study is self funded by the author the study.

PROCEDURES:

What procedures are involved?

Procedure(s) involved would be a prick on the skin to collect blood sample.

Will they cause pain or discomfort?

It may cause slight/minimal pain

Will they cause any temporary or lasting problems to me?

None

How many times I need to undergo the procedure?

Ideally only one prick would be involved, however two or more pricks may be needed if vein is not accessible.

RISKS AND BENEFITS:

What are the potential risks and discomforts?

There are no potential risks involved with the procedure. However, if there is h/o any bleeding disorder, then the duration on bleed from the prick site would be a little longer than usual, but not to an extent where it could be lethal to the patient.

Are the benefits to taking part in this research?

None

ALTERNATIVES:

What other options are there?

What happens if I decline participation? You would be simply be excluded from the study and all your details shall be kept confidential.

Will I be told about new information that may affect my decision to participate?

Yes, you would be informed as when their is any information that may affect your desicion to participate in this study.

WITHDRAWING / REMOVAL FROM THE STUDY:

Can I withdraw from the study any time I want?

You would be always have the voluntary will to withdraw from participation from the study anytime during the study.

Will I be penalized for that in anyway?

No.

Can I be removed from the study?

Yes, you can be removed from the study anytime as the author wishes to, if at any stage the author concludes that you may not continue to be an appropriate 'subject' for the study.

PRIVACY AND CONFIDENTIALITY:

What about privacy and confidentiality?

All data collected or disclosed by you during the course of participation of study, will be kept fully confidential.

Will my identity be disclosed?

No. If however during the course it becomes necessary for the progress of the course to disclose the identity, it would be done so only after your informed & written consent.

FINANCIAL INCENTIVES FOR PARTICIPATION:

What are the costs for participating in this research? e.g., for services, etc

No additional costs shall be incurred upon you for the purpose of this study.

Will be reimbursed for any of my expenses for participation in this research?

N.A

CONTACT DETAILS:

Whom should I contact if I need my clarification or help at any time during the study period?

You shall be free to contact the below mentioned name & addresses anytime during the study period for any clarification or help as you may desire for.

Chairman
College Ethical Dissertation
& Research Committee
Jawaharlal Nehru Medical College
Nehru Nagar, KLE Hospital Road
Belgaum 590 010

Dr. *** ******
Professor of Surgery
Department of Surgery
Jawaharlal Nehru Medical College
Nehru Nagar, KLE Hospital Road
Belgaum – 590 010

Dr. **** ******
(Post Graduate Student)
Department of Surgery
Jawaharlal Nehru Medical College
Nehru Nagar, KLE Hospital Road
Belgaum 590 010
Mobile No.: +91 9194009895

Will they be available all the time?

Yes.

Will I be given the contact numbers & addresses of these persons?

Yes. If desired by you, contact numbers and addresses will be provided to you separately.

AUTHORIZATION TO PUBLISH RESULTS:

How will the results of the study be used?

The results of the study may be used to publish an article.

CONSENT STATEMENT:

I the undersigned Mr/Mrs/Dr_____ do hereby give consent for my participation in this research study after being explained in-depth about the important elements of this study in own my vernacular language.

I give this consent voluntarily in my sound mind knowing very well the risks involved and been given enough time to clear my doubts and other queries to participate as a 'subject' in this study. I do hereby also give consent for publication of this article in any media / journal and have no objections whatsoever.

Did you read the contents or were the contents read out to you?

Tick one as appropriate:

- ☐ Read the contents by myself
- ☐ The contents were read out to me in English/Marathi/Kannada/Vernacular language

Were you informed that participation is voluntary?

Tick one as appropriate:

- ☐ Yes
- ☐ No

Did you have adequate time to clarify any doubts about the study or your rights as a study participant?

Tick one as appropriate:

- ☐ Yes
- ☐ No

Signature or left thumb print of participant or legally authorized representative

Participant' name_____ Signature_____

Experimenter's name_____ Signature_____

Witness' name_____ Signature_____

Guardian's name_____ Signature_____

Date_____

(If the participant are Minors (under 18), the parents sign the form, rather than the participants)

ANNEXURE II – PROFOMA

The proposed proforma / questionnaire to be used for data collection for the study tilted "Evaluation of hyperbilirubinemia as a new diagnostic tool for acute appendicitis and, also as a predictor of appendiceal perforation" is as:

Personal Details:		
	Name:	Age / Sex:
	Address:	
	Educational Status:	Occupation:
	IP No.	Date of Admission:
	Ward:	Date of Discharge:
Chief Complaints:		
Past history: any p/h/o Jaundice or Liver disease ☐ Yes ☐ No Hemolytic disease ☐ Yes ☐ No Acquired or congenital biliary disease ☐ Yes ☐ No any other significant p/h :		

Personal history: any personal h/o Chronic alcoholism ☐ Yes ☐ No	
Family history (if significant) :	
General examination:	
	Built : Pulse : BP : Respiratory rate : Jaundice : Pallor / Clubbing / Cyanosis / Lymphadenopathy
Systemic Examination:	
	CNS: CVS: RS: P/A:
Clinical Diagnosis:	

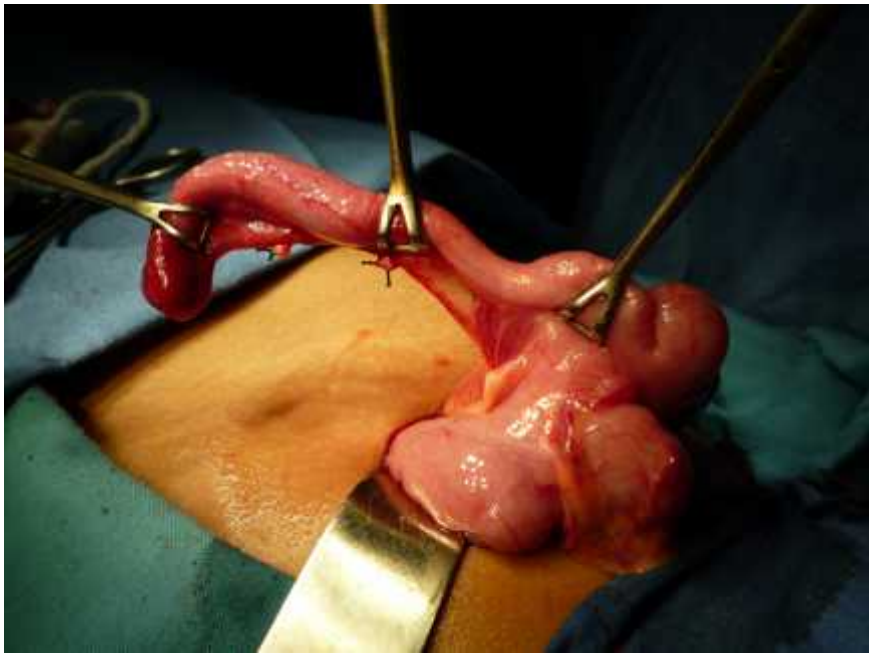
Investigations:			
	Serological Investigations		Normal Range
	1.	Total Bilirubin: .....mg/dl	0.3 – 1.0 mg/dl
	2.	Indirect Bilirubin: .....mg/dl	0.1 – 0.3 mg/dl
	3.	SGPT: .....U/L	0 – 35 U/L
	4.	SGOT: .....U/L	0 – 35 U/L
	5.	ALP: .....U/L	30 -120 U/L
	USG – Abdomen (if done):		
	Histopathology report (post-operatively):		

ANNEXURE III – PHOTOGRAPHS

Photograph 1. Semi-Auto Clinical Chemistry Analyser machine (ERBA Chem-5 Plus) used for LFT

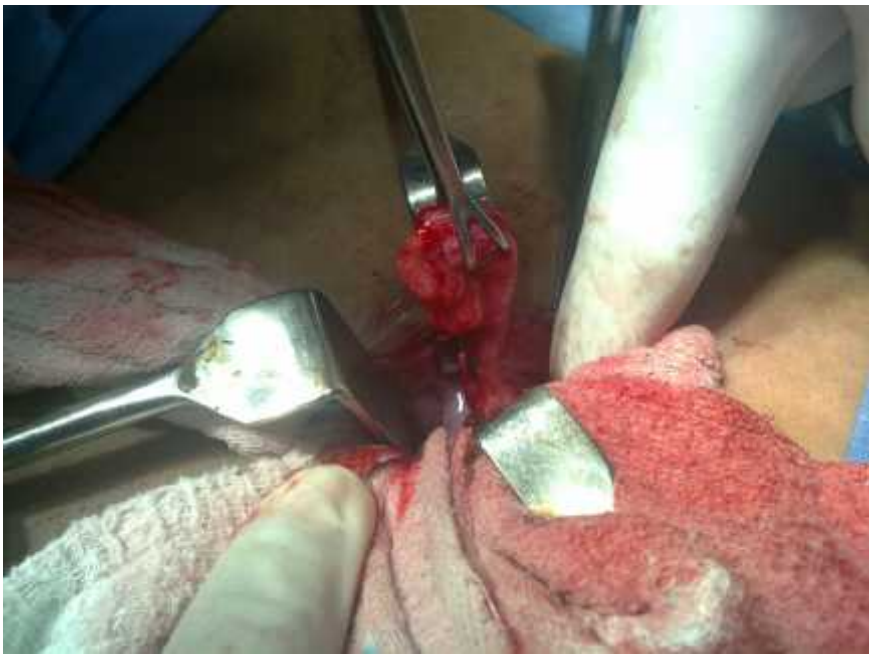
Photograph 2. Diagnostic Reagents used for determination of LFTs

Photograph 3. Acute appendicitis



Photograph 4. Acute appendicitis

Photograph 5. Appendiceal perforation



Photograph 6. Appendiceal perforation

ANNEXURE IV - MASTER CHART

Serial Number	In Patient (IP) Number	Gender	Age (Years)	Liver function tests						TLC (/mm^3)	DLC				Clinical Diagnosis on Admission (Pre-Operative)	Ultra - Sonography findings	Post-Operative Diagnosis
				Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)		Neutrophil	Lymphocyte	Monocytes	Eosinophils			
1	348929	M	28	0.9	0.3	0.6	42	55	79	10250	78	20	1	1	AA	AA	AA
2	348923	M	21	1.8	1.3	0.5	22	35	100	8300	58	28	10	4	AA	N	AA
3	350220	F	20	1.1	0.7	0.4	18	23	65	8600	64	30	5	1	AA	N	AA
4	350416	F	26	1.4	1.2	0.2	32	25	111	15400	90	10	0	0	AA	N	AA
5	350592	M	20	1.3	0.5	0.8	26	39	91	4500	69	27	4	1	AA	AA	AA
6	350679	F	18	1.7	0.2	0.5	19	25	56	6900	63	32	5	1	AA	AA	AA
7	351772	F	19	0.9	0.3	0.6	34	46	120	14300	89	7	4	0	AA	N	AA
8	350077	F	28	1.1	0.4	0.7	22	43	83	8200	75	20	10	2	AA	N	AA
9	350790	M	21	0.8	0.2	0.6	91	26	100	9900	85	7	6	2	AA	AA	AA
10	351950	F	12	1.2	0.1	0.4	16	26	56	12200	57	40	3	1	AA	N	AA
11	352340	F	35	1.4	0.2	0.5	35	12	93	6700	63	29	8	2	AA	AA	AA
12	352594	M	20	0.9	0.2	0.7	46	22	106	9300	87	12	1	0	AA	N	AA
13	353203	F	14	1.2	0.8	0.4	12	24	110	10900	64	28	6	1	AA	N	AA
14	354685	M	24	1.4	0.2	0.2	34	12	76	8600	66	21	5	2	AA	N	AA
15	355083	F	26	1.5	1.1	0.4	38	12	117	12300	78	13	6	2	AA	AA	AA
16	355290	M	30	1.4	1.0	0.4	10	35	22	6300	58	32	2	0	AA	N	AA
17	355390	M	22	4.4	3.4	1.0	25	39	54	10500	86	12	1	1	AP	AA	AP
18	352277	F	24	0.8	0.2	0.6	34	29	92	2300	58	32	5	3	AA	N	AA
19	352267	M	14	1.1	0.7	0.4	34	26	78	8600	57	42	3	1	AA	AA	AA
20	353030	M	21	0.9	0.3	0.6	18	16	45	14100	90	8	2	0	AA	AA	AA
21	353500	F	20	0.9	0.2	0.7	32	11	77	7400	59	37	4	0	AA	AA	AA
22	356071	F	21	1.6	1.2	0.4	29	17	74	14200	85	13	2	0	AA	N	AA
23	355857	F	25	1.7	1.3	0.4	25	25	29	6900	71	29	4	1	AA	N	AA
24	356374	M	15	1.3	0.1	0.2	38	29	81	8100	54	44	1	1	AA	AA	AA
25	357696	M	18	0.9	0.3	0.6	24	23	100	9900	58	40	2	0	AA	N	AA
26	357947	F	25	3.2	2.5	0.7	14	15	59	15500	80	18	2	0	AP	AA	AP
27	358261	F	15	1.0	0.3	0.7	29	31	94	8200	50	48	2	1	AA	N	AA
28	358894	M	23	1.4	1.2	0.2	33	11	72	9900	80	18	3	0	AA	N	AA
29	359512	F	27	1.3	0.9	0.4	21	25	88	11200	80	20	0	0	AA	AA	AA
30	356302	F	32	1.1	0.3	0.3	12	34	59	12100	80	18	2	0	AA	N	AA
31	356908	F	28	0.9	0.4	0.5	69	37	110	3400	67	22	6	2	AA	AA	AA
32	358064	F	12	1.2	0.9	0.3	33	13	88	13800	84	16	3	1	AA	N	AA
33	358463	M	35	0.8	0.4	0.4	18	23	79	9900	69	30	1	0	AA	N	AP
34	359341	F	32	1.7	1.5	0.2	12	32	109	11000	69	26	3	0	AA	N	AA
35	363970	F	17	1.5	1.3	0.2	12	7	67	6900	80	18	2	0	AA	AA	AA

ANNEXURE IV - MASTER CHART

Serial Number	In Patient (IP) Number	Gender	Age (Years)	Liver function tests						TLC (/mm^3)	DLC				Clinical Diagnosis on Admission (Pre-Operative)	Ultra - Sonography findings	Post-Operative Diagnosis
				Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)		Neutrophil	Lymphocyte	Monocytes	Eosinophils			
36	360691	M	25	1.3	1.0	0.3	24	26	100	8100	60	35	3	2	AA	N	AA
37	362430	M	28	0.9	0.4	0.5	22	22	71	10200	90	5	3	1	AA	AA	AA
38	361653	F	25	3.1	2.7	0.4	35	23	85	16700	89	10	1	0	AP	AA	AP
39	361654	F	25	0.7	0.3	0.4	10	10	45	7100	65	30	3	2	AA	AA	AA
40	362787	F	23	1.5	0.3	0.6	27	38	102	7600	70	20	5	4	AA	AA	AA
41	361074	F	35	1.4	0.9	0.5	31	38	82	8500	77	20	3	0	AA	N	AA
42	361063	M	24	1.1	0.7	0.4	30	19	93	15500	62	36	2	0	AA	N	AA
43	361356	M	12	1.3	0.3	0.2	23	43	90	7200	65	25	4	4	AA	AA	AA
44	363447	M	27	1.7	1.2	0.5	34	31	65	12500	87	10	2	1	AA	AA	AA
45	361887	M	28	1.2	0.3	0.6	25	14	67	12400	56	28	4	10	AA	N	AA
46	360380	M	24	1.1	0.6	0.5	23	23	94	9500	60	30	10	0	AA	N	AA
47	360582	M	18	1.4	1.1	0.3	34	12	81	12700	86	8	4	0	AA	AA	AA
48	363284	F	27	1.6	1.2	0.4	19	22	71	10400	60	40	0	0	AA	N	AA
49	363544	F	26	4.2	3.8	0.4	22	12	80	12500	64	26	6	0	AP	AA	AP
50	360915	M	35	1.3	0.9	0.4	34	12	96	4400	76	12	4	2	AA	AA	AA
51	362154	M	18	1.1	0.8	0.3	22	34	30	10000	68	30	1	1	AA	AA	AA
52	365972	M	28	3.4	2.5	0.9	24	28	34	16700	65	25	8	2	AP	AA	AP
53	366940	M	12	2.8	2.1	0.7	26	33	68	13300	74	15	4	1	AA	N	AP
54	365523	F	35	2.0	1.4	0.5	12	15	110	9866	88	11	1	0	AA	N	AA
55	366381	M	10	1.1	0.4	0.5	34	31	54	11300	85	10	4	1	AA	AA	AA
56	367312	F	27	1.7	1.2	0.5	31	12	90	10250	65	30	5	0	AA	AA	AA
57	365697	F	18	0.9	0.4	0.5	18	34	44	14000	80	18	1	1	AA	AA	AP
58	366957	M	23	2.3	1.5	0.8	24	32	100	4800	55	40	3	2	AA	AA	AA
59	364579	F	32	1.1	0.6	0.5	27	22	65	9800	45	50	5	0	AA	N	AA
60	366841	F	30	1.4	0.8	0.6	22	32	56	12000	88	10	1	1	AA	N	AA
61	366789	M	26	1.0	0.3	0.7	25	34	70	2300	80	20	0	0	AA	AA	AA
62	366922	F	28	1.4	1.0	0.4	32	14	56	15400	76	22	3	1	AA	N	AA
63	368322	M	22	1.6	1.4	0.2	38	27	86	5000	60	38	2	0	AA	N	AA
64	368695	M	19	4.2	3.2	1.0	21	33	45	11200	77	13	7	3	AP	AA	AP
65	368833	F	21	1.1	0.6	0.2	32	13	55	8400	78	20	1	1	AA	AA	AA
66	369441	F	36	1.1	0.9	0.2	12	33	80	12800	50	40	9	1	AA	N	AA
67	371717	F	35	2.1	1.5	0.6	35	25	90	7500	80	20	0	0	AA	AA	AA
68	372324	F	28	0.7	0.5	0.2	28	32	80	3500	65	30	5	0	AA	AA	AA
69	368220	M	25	3.4	2.6	0.8	29	21	33	18000	70	28	1	1	AA	AA	AP
70	369284	F	17	1.4	0.9	0.5	26	24	65	9800	80	18	1	1	AA	N	AA

ANNEXURE IV - MASTER CHART

Serial Number	In Patient (IP) Number	Gender	Age (Years)	Liver function tests						TLC (/mm^3)	DLC				Clinical Diagnosis on Admission (Pre-Operative)	Ultra - Sonography findings	Post-Operative Diagnosis
				Total Bilirubin (mg/dL)	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)		Neutrophil	Lymphocyte	Monocytes	Eosinophils			
71	369559	F	15	1.9	1.3	0.6	34	34	98	11000	60	30	8	2	AA	AA	AA
72	369975	F	35	1.8	1.2	0.6	40	29	87	6700	65	35	0	0	AA	N	AA
73	370472	M	24	1.2	1.0	0.2	25	35	65	9900	88	10	2	0	AA	N	AA
74	370414	M	28	1.2	0.6	0.6	19	21	47	6750	80	16	3	1	AA	N	AA
75	370608	M	20	1.3	0.9	0.4	33	24	88	12000	78	20	2	0	AA	N	AA
76	371214	M	20	1.5	0.6	0.2	20	13	24	6700	60	38	1	1	AA	N	AA
77	371138	F	17	1.6	1.0	0.6	35	34	65	3400	50	46	4	0	AA	N	AA
78	371148	F	32	1.1	0.6	0.3	24	13	54	9500	88	10	1	1	AA	AA	AA
79	372879	M	14	2.9	2.2	0.6	34	26	55	8900	65	30	4	0	AP	AA	AP
80	372835	F	22	1.6	1.0	0.6	22	21	86	10500	65	30	3	2	AA	N	AA
81	373311	M	28	2.0	1.2	0.8	16	34	120	18450	70	22	6	2	AA	N	AA
82	372454	F	11	1.9	1.3	0.6	34	28	45	5600	66	30	4	0	AA	N	AA
83	372686	F	27	3.5	2.7	0.8	33	55	78	22540	78	21	1	0	AP	AA	AP
84	373874	M	18	1.5	1.2	0.3	23	34	67	7500	60	30	8	2	AA	N	AA
85	373913	M	30	1.1	0.6	0.3	12	23	56	9000	75	20	2	3	AA	AA	AA
86	373844	M	35	1.3	0.9	0.4	21	34	46	8000	67	30	3	0	AA	AA	AA
87	374364	F	21	1.4	1.0	0.4	14	14	76	7600	78	22	0	0	AA	N	AA
88	374427	M	23	0.8	0.6	0.2	32	21	64	9050	50	40	10	0	AA	AA	AA
89	374571	F	30	1.7	0.7	0.2	34	34	76	8500	80	18	2	0	AP	AA	AA
90	373122	M	25	0.6	0.5	0.1	34	23	36	10500	76	20	3	1	AA	AA	AA
91	374356	F	23	1.4	0.3	0.2	22	32	24	7000	55	35	8	2	AA	N	AA
92	375251	F	30	2.9	2.1	0.8	25	32	88	13100	68	30	0	2	AA	AA	AP
93	375905	F	23	3.9	2.5	1.4	26	13	132	9900	80	10	6	4	AA	AA	AP
94	376711	F	12	2.3	1.6	0.7	21	19	100	14500	78	20	1	1	AA	N	AA
95	376609	F	28	1.5	1.2	0.3	24	12	78	4300	67	20	3	0	AA	N	AA
96	376960	M	15	1.2	0.2	0.2	12	22	89	6400	83	10	3	1	AA	N	AA
97	377074	F	17	1.8	1.4	0.4	10	10	113	13200	90	10	0	0	AA	N	AA
98	377086	F	12	0.9	0.3	0.6	23	21	67	4400	80	12	6	0	AA	AA	AA
99	373482	F	28	1.3	0.1	0.2	24	32	99	8000	67	23	3	1	AA	AA	AA
100	373830	M	35	1.6	0.2	0.4	12	34	110	9400	80	12	6	2	AA	N	AA

ANNEXURE IV – KEY TO MASTER CHART

AA	-	Acute appendicitis
ALP	-	Alkaline phosphatase
AP	-	Appendiceal perforation
dL	-	Deciliters
DLC	-	Differential leukocyte count
F	-	Female
M	-	Male
mg	-	Milligrams
mm	-	Millimeters
N	-	Normal
SGOT	-	Serum glutamic oxaloacetic transaminase
SGPT	-	Serum glutamic pyruvic transaminase
TLC	-	Total leukocyte count