Section: General Surgery

Original Research Article

TO STUDY THE ROLE OF SERUM BILIRUBIN, C-REACTIVE PROTEIN AND TOTAL LEUCOCYTE COUNT IN ASSESSMENT OF ACUTE APPENDICITIS AND ITS COMPLICATIONS

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ABSTRACT

Background: Acute appendicitis is one of the most common causes of acute abdominal pain that requires surgical intervention. Despite advances in diagnostic and imaging modalities early and accurate diagnosis remains a challenge. Biochemical markers such as total leukocyte count (TLC), C-reactive protein (CRP) and serum bilirubin are increasingly studied for their diagnostic value.

Materials and Methods: This prospective observational study was conducted over two years in the Department of Surgery at GMCH Chandigarh. Fifty patients clinically and on the basis of imaging diagnosed with acute appendicitis and undergoing appendectomy were included. Preoperative biochemical markers TLC, CRP, and serum bilirubin were assessed and compared with intraoperative findings and histopathological examination (HPE), the latter serving as the gold standard. Statistical analysis was done using SPSS version 23 and p value less than 0.05 was taken as statistically significant.

Results: We found a male predominance (72%) with a mean age of 35.86 years. HPE confirmed acute appendicitis in 66%, gangrenous in 24%, and perforated in 10%. Increased CRP was found to be associated with complicated appendicitis cases (p=0.045). Raised TLC count was also found to be associated with complicated appendicitis (p=0.045). Serum bilirubin was elevated in 56% however it did not show a statistically significant correlation with complicated appendicitis (p=0.569). Combined elevation of CRP and TLC improved sensitivity and specificity for predicting complicated appendicitis cases (p=0.004).

Conclusion: Elevated CRP and TLC levels are reliable indicators for acute and complicated appendicitis. While serum bilirubin showed limited independent predictive value it can complement other markers. Routine assessment of CRP and TLC in addition to clinical evaluation enhances early diagnosis of acute appendicitis.

Keywords: Appendicitis, CRP, Leucocytosis, Hyperbilirubinemia, Perforation.

INTRODUCTION

The vermiform appendix is a blind ending tubular structure at the base of the caecum, near the ileocecal valve where the taenia coli converge. Acute Appendicitis is a surgical cause of acute abdominal pain. Life time risk of acute appendicitis is 8.6% males and 6.7% for females.[1] Appendicitis commonly occurs in young adults (the highest incidence, approximately 40%, in 2nd decade of life i.e. 10–19 years and 70% of the subjects are less than 30 years old).[3] Various causes of acute appendicitis include appendicolith, appendiceal tumors (carcinoid tumor), intestinal parasites, hypertrophied lymphatic

tissue. Acute inflammation and local ischemia increase intramural and intraluminal pressure and cause lymphatic stasis. This leads to vascular compromise, perforation and abscess formation due to bacterial overload.[2]

Different diagnoses may mimic the signs and symptoms of acute appendicitis, as there are multiple causes leading to pain in right iliac fossa. Simple appendicitis can be difficult to differentiate from other causes of right iliac fossa pain that do not require surgery. These conditions include mesenteric adenitis in children, benign gynaecological disease such as ovarian cysts, Mittelschmerz or pelvic inflammatory disease in women or inflammatory bowel disease in young adults.[3]

Despite the advances in diagnostic medicine and therapeutics over the past forty years, the accurate diagnosis of appendicitis and pain in right iliac fossa remains a clinical challenge. Negative appendectomy rates at 10% -30% are not uncommon, and morbidity rates of negative appendectomy often parallel those of surgery for acute appendicitis. Surgical delay in a prompt management of the subjects with appendicitis (not with perforation, in particular), either due to delay in presentation (particularly in males with retrocaecal or retro-ileal position) or misjudgement, leads to dread complications like gangrenous changes and perforation of the appendix.[4]

Gangrene or perforation further leads to more complications like appendicular abscess formations, localized/generalized peritonitis, fecal fistula formation, intestinal obstruction due to adhesion formation, portal pyaemia, sepsis and sterility in women of child-bearing age (though recent studies denies it as a major risk factor) with overall increased morbidity and prolonged hospital stay.[10] The rate of perforation is reported to increase as the duration of symptoms increases. Therefore, early diagnosis and treatment are required to reduce morbidity mortality and other late complications. Various scoring systems have proved useful in predicting the accurate diagnosis of acute appendicitis in patients presenting with right iliac fossa pain but none of them ascertain the risk of appendiceal perforation using hyperbilirubinemia and CRP as predictors.[5]

Total leukocyte count (TLC), C-reactive protein (CRP) and serum bilirubin have been increasingly studied for their role in diagnosis of acute appendicitis and its complications. Elevated TLC is frequently observed in histologically confirmed cases of appendicitis whereas CRP is also reported to be significantly elevated in appendicitis and correlates with the severity of inflammation. Recent evidence supports the diagnostic utility of hyperbilirubinemia in cases of appendicitis particularly in cases of appendiceal perforation. Many studies have demonstrated a significant increase in the risk of perforated appendicitis in patients with total bilirubin levels >1.0 mg/dL. This isolated rise in bilirubin is thought to result from endotoxemia or bacteremia associated with appendiceal inflammation. When interpreted in with clinical findings, the combined assessment of TLC, CRP, and bilirubin enhances diagnostic accuracy and aids in assessing the risk of perforation in patients with suspected acute appendicitis.[6]

Through this study an attempt is made to suggest that pre-operative TLC, CRP and Serum bilirubin elevation may be used as diagnostic markers of Acute Appendicitis and its complications namely perforation and gangrenous appendicitis.

MATERIALS AND METHODS

This was a prospective observational study conducted in the department of surgery of GMCH Chandigarh. In this study cases of acute appendicitis undergoing open or laparoscopic appendectomy were included on the basis of a predefined inclusion and exclusion criteria. The duration of study was 2 years. Based on previous studies and using a 95% confidence interval with 90% power to detect a significant difference in biochemical marker levels (such as CRP and serum bilirubin) between patients with simple inflamed appendicitis and those with complicated (gangrenous/perforated) appendicitis, the minimum required sample size was calculated to be 45 patients. Taking into account possible exclusions and dropouts during the study period, a total of 50 patients were included in the final analysis. All patients were clinically evaluated for the diagnosis of acute appendicitis. Following a clinical diagnosis every patient underwent biochemical and radiological investigations. These investigations included total leukocyte count (TLC), differential leukocyte count (DLC), C-reactive protein (CRP), serum bilirubin and liver function tests (LFTs). Ultrasound abdomen was done in all cases. Computed tomography was done in cases where ultrasound report was ambiguous. The patients subsequently underwent surgery under either general or spinal anesthesia depending upon the general condition and ASA grade of patient. Operative approaches including McBurney incision, lower midline incision or laparoscopy. Intraoperative findings were analysed to assess for acute appendicitis or its complications such as perforation or gangrene. The final diagnosis was confirmed by postoperative histopathological examination (HPE). Cases were classified as either acutely inflamed or complicated by perforation or gangrene. The clinical, biochemical and operative data were then compared with the histopathological findings.

Data was compiled and statistically analyzed using SPSS software version 23.0. Descriptive statistics were used to analyse patient demographics and clinical variables. The diagnostic performance of biochemical markers (TLC, CRP, and serum bilirubin) in differentiating simple from complicated (perforated or gangrenous) appendicitis was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy. The Chi-square test or Fisher's

exact test was applied to assess the association between categorical variables. A p-value of <0.05 was considered statistically significant.

Inclusion Criteria

1. Patients presenting with clinical features consistent with acute appendicitis and undergoing appendectomy.
2. Age above 18 years.
3. Ready to give informed and written consent to be part of study.

Exclusion Criteria

1. Age less than 18 years.
2. Refusal to give informed consent to be part of study.
3. History of alcoholic liver disease or other liver pathologies associated with hyperbilirubinemia.
4. History of gastrointestinal malignancies or hepatopancreaticobiliary surgery.

RESULTS

Out of the 50 patients included in the study, 36 (72%) were males and 14 (28%) were females. There was a male predominance with male-to-female ratio of 2.57:1 (Figure 1).

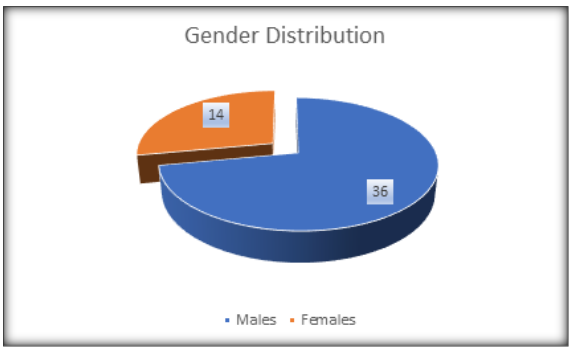

Figure 1: Gender Distribution of studied cases.

The age of patients ranged from 18 to 65 years, with a mean age of 35.86 ± 13.44 years. The majority of patients (32%) were in the 31–40-year age group, followed by 24% in the 21–30-year group and 20% in the 51–60-year group. Patients ≤20 years accounted for 16% of the cohort, while those aged 41–50 years constituted 8%. On gross intraoperative examination, acute appendicitis was observed in 33 patients (66%), gangrenous appendicitis in 12 (24%), and perforated appendicitis in 5 cases (10%) (Table 1).

Table 1: Age distribution and type of appendicitis in studied cases

Age and Type of Appendicitis	Age(years)	No. of patients	Percentage(%)
Age Distribution	≤ 20	8	16
	21-30	12	24
	31-40	16	32
	41-50	4	8
	51-60	10	20
	Total	50	100
	Minimum- maximum	18 - 65	
	Mean ± SD	35.86 ± 13.44	
Type of appendicitis	Acute appendicitis	33	66
	Gangrenous appendicitis	12	24
	Perforated appendix	5	10
	Total	50	100

C-reactive protein (CRP) was elevated in 94% of patients overall and showed a statistically significant increase in complicated appendicitis compared to acute cases (100% vs 88%, p = 0.045). The mean CRP level was markedly higher in the complicated group (319.32 ± 179.97 mg/L) than in the acute group (115.54 ± 114.06 mg/L). Total leukocyte count (TLC) was raised in 62% of patients, with a significantly higher prevalence in complicated appendicitis (80% vs 44%, p = 0.045). The mean TLC was also higher in the complicated group (12.84 ± 4.93 ×10⁹/L) compared to the acute group (10.40 ± 4.48 ×10⁹/L). Serum bilirubin, although elevated in 56% of the cohort, did not demonstrate a statistically significant difference between acute and complicated appendicitis (p > 0.05) (Table 2).

Table 2: CRP, TLC and Bilirubin in Acute Vs complicated Appendicitis

CRP, TLC and Bilirubin in Acute Vs complicated Appendicitis		No. of patients	Raised	%	Min.	Max.	Mean	Std. deviation	Median	p-value
C-Reactive Protein	Acute appendicitis	25	22	88%	2.8	425	115.54	114.06	167	0.045S
	Complicated appendicitis	25	25	100%	12	678	319.32	179.97	288	
	Total	50	47	94%	2.8	678	217.43	181.19	173	
Total Leukocyte Count	Acute appendicitis	25	11	44%	3.7	20.9	10.40	4.48	8.80	0.045S
	Complicated appendicitis	25	20	80%	2.9	20.1	12.84	4.93	12.5	

	Total	50	31	62%	2.9	20.9	11.62	4.82	12.05	
Serum Bilirubin	Acute appendicitis	25	13	52%	0.2	4.4	1.14	0.90	1.101	> 0.05
	Complicated appendicitis	25	15	60%	0.2	3.2	1.39	0.89	1.40	
	Total	50	28	56%	0.2	4.4	1.272	0.8985	1.1	

Serum bilirubin levels were elevated (>1.0 mg/dL) in 28 cases (56%) and within the normal range (0.2–1.0 mg/dL) in 22 cases (44%). The overall mean serum bilirubin was 1.27 ± 0.89 mg/dL, with values ranging from 0.2 to 4.4 mg/dL (Table 3).

Table 3: Serum Bilirubin levels in studied cases

Bilirubin(mg/dl)	No. of patients	Percentage (%)
Normal	22	44.0
Raised	28	56.0
Minimum - maximum	0.2 - 4.4	
Mean ± SD	1.27 ± 0.89	

The relationship between serum bilirubin levels and intraoperative findings was evaluated by comparing patients with gangrenous or perforated appendicitis to those with uncomplicated inflammation. Among the 25 patients with gangrenous or perforated appendicitis, 60% had elevated bilirubin levels (>1.0 mg/dL), whereas 52% of patients with inflamed appendicitis also exhibited elevated bilirubin. Conversely, normal bilirubin values (0.2–1.0 mg/dL) were found in 40% and 48% of the respective groups. The difference between the two groups was not statistically significant (p = 0.569), indicating limited discriminatory value of serum bilirubin in predicting complicated appendicitis (Table 4).

Table 4: Relationship between serum bilirubin levels and intraoperative findings

Bilirubin(mg/dl)	N	Intra-operative finding				p-value
		Gangrenous/Perforated (n-25)		Inflamed (n-25)		
		F	%	F	%	
>1.0	28	15	60.0	13	52.0	0.569NS
0.2 - 1.0	22	10	40.0	12	48.0	

The diagnostic performance of serum bilirubin in identifying complicated appendicitis (gangrenous or perforated) was assessed using standard statistical indices. Serum bilirubin >1.0 mg/dL yielded a sensitivity of 60.0% (95% CI: 38.67%–78.87%) and specificity of 48.0% (95% CI: 27.80%–68.69%). The positive predictive value was 53.57%, while the negative predictive value was 54.55%. Overall diagnostic accuracy was 54.0%. These findings indicate that while elevated bilirubin was moderately sensitive, it lacked specificity and had limited utility as an independent predictor of complicated appendicitis (Table 5).

Table 5: Diagnostic performance of serum bilirubin in identifying complicated appendicitis

True Positive	15	
False Positive	13	
False Negative	10	
True Negative	12	
Statistic	Value	95% CI
Sensitivity	60.00%	38.67% to 78.87%
Specificity	48.00%	27.80% to 68.69%
Disease prevalence (*)	50.00%	35.53% to 64.47%
Positive Predictive Value (*)	53.57%	41.31% to 65.42%
Negative Predictive Value (*)	54.55%	38.99% to 69.26%
Accuracy (*)	54.00%	39.32% to 68.19%

When both CRP and TLC were elevated, 80% of patients had gangrenous or perforated appendicitis, compared to only 40% with uncomplicated inflammation. Conversely, when both markers were within normal limits, 60% of patients had only inflamed appendicitis, and just 20% had complicated disease. This association was statistically significant (p = 0.004), indicating that the concurrent elevation of CRP and TLC significantly increases the likelihood of complicated appendicitis (Table 6).

Table 6: Diagnostic performance of CRP and TLC in identifying complicated appendicitis

CRP and TLC		Intra-operative finding				p-value
		Gangrenous/Perforated (n-25)		Inflamed (n-25)		
	N	F	%	F	%	
Both raised	30	20	80	10	40	0.004[S]

Both not raised	20	5	20	15	60	

Liver transaminases were assessed to evaluate potential hepatic involvement in appendicitis. SGOT levels were elevated (>40 IU/L) in 26% (13/50) of patients, with a mean value of 36.26 ± 15.22 IU/L across the cohort. SGPT was elevated (>35 IU/L) in 16% (8/50), with a mean value of 27.22 ± 11.58 IU/L. The majority of patients had transaminase values within normal ranges, and no significant correlation was observed between elevated liver enzymes and the severity of appendicitis (Table 7).

Table 7: Liver Transaminases in cases of Appendicitis

	No. of patients	%	Minimum - maximum	Mean ± SD
SGOT(IU/L)				
Normal(5-40)	37	74	13 - 79	36.26 ± 15.22
Raised(>40.0)	13	26		
SGPT(IU/L)				
Normal(5-35)	42	84	Oct-65	27.22 ± 11.58
Raised(>35.0)	8	16		

DISCUSSION

Surgeons have grappled with the difficulty of making a reliable preoperative diagnosis of acute appendicitis for more than a century—so much so that Ravitch et al,[7] observed that the only way to have 100% diagnostic accuracy in all cases of acute appendicitis is to wait till they all perforate. This stark remark underscores the urgency of finding simple, affordable, and dependable in addition to clinical judgment in cases of appendicitis. While White et al,[8] have shown that "active observation" can cut the negative appendectomy rate from 15% to 1.9%, it is often impractical in a busy emergency setting. A range of techniques clinical scoring systems, ultrasound (USG), colour Doppler, laparoscopy, and peritoneal aspiration cytology have been explored with mixed success. Given these difficulties in accurately diagnosing cases of appendicitis our prospective study evaluated three biochemical markers (total leukocyte count [TLC], C-reactive protein [CRP], and serum bilirubin) alongside clinical assessment and USG against histopathology as the gold standard.

Among 50 appendectomy patients—all confirmed histologically—the male predominance was striking (36 M, 14 F; 2.5:1). This male predominance was also reported by Lewis et al,[9] who reported a 20% negative appendectomy rate overall and nearly 40% in women. Most patients in our cohort were aged 31–40 years (mean 35 years), consistent with prior observations that acute appendicitis peaks in the second to fourth decades.

Fever was relatively uncommon at presentation only 6% exhibited a temperature above normal—supporting Rivera-Chavaz et al10 report of generally mild fevers in appendicitis. In contrast Anderson et al,[11] found that absence of fever occurs in 14–28% of cases. Some studies documented high fevers (>38 °C) in up to 74% of complicated presentations. Tachycardia was noted in 58% of our cases, paralleling Sertali et al,[12] observation that heart rate increases rapidly after 24 h of symptoms, correlating with fever and systemic inflammation.

USG proved highly sensitive in our study identifying a non-compressible, dilated appendiceal structure in all 50 patients with 35 complicated and 15 uncomplicated cases correctly classified. This aligns with Matthew Fields et al,[13] meta-analysis reporting USG sensitivity and specificity of 91% and 97%, respectively. Yet Orr et al,[14] highlighted a significant false-negative rate in patients with classic signs and false positives in low-probability cases, reminding us that operator dependency and patient factors can limit USG's reliability.

A raised CRP (>5 mg/L) was seen in 94% of our patients (range 2.8–678 mg/L; mean 217.4 mg/L), with a median of 173 mg/L and a significant p value of 0.045. Elevated CRP supported the surgeon's clinical impression, consistent with Raja et al15 who linked very high CRP levels (>100 mg/L) to perforation, and Gurleyik et al16 who noted a CRP sensitivity of 96.6%. However, Salem et al,[17] and Davies et al18 failed to establish a clear CRP threshold for operative versus non-operative management, and Ramroa et al19 questioned CRP's diagnostic value despite its usefulness in ruling out disease.

Abnormal TLC (>10 × 10⁹/L) occurred in 62% of our cases (mean 11.62 × 10⁹/L, median 12.05 × 10⁹/L; p = 0.045). This echoes Al-Gaithy et al20 demonstration that elevated TLC and neutrophil counts correlate with both inflamed and complicated appendicitis, and Gronroos et al,[21] finding that TLC rises early, whereas CRP lags until perforation. Yet Cardell et al,[22] warned that WBC counts have poor sensitivity and specificity and Er et al remind us that normal TLC does not exclude appendicitis.

Hyperbilirubinaemia was present in 56% of our patients (mean 1.27 mg/dL; median 1.1 mg/dL), with sensitivity and specificity of 60% and 48% for complicated cases—though this did not reach significance (p = 0.569). Panagiotopoulou et al,[23] similarly found bilirubin sensitivity and specificity of 65% and 68%, but with only a 17% positive predictive value and 95% negative predictive value for perforation. Yet smaller prospective studies by Cheekuri et al,[24] and D'souza et al,[25] reported sensitivities up to 94% and specificities up to 100%,

suggesting that in carefully selected cohorts, bilirubin may offer added value.

When CRP and TLC were both elevated, sensitivity rose to 80% and specificity to 60% (accuracy 70%; p = 0.004), broadly consistent with Dnyanmote et al findings of 90.2% sensitivity and 78.6% specificity. Marginal elevations of SGOT and SGPT were seen in 26% and 16% of cases. Most studies report only minimal liver enzyme changes confirming that transaminase elevations offer little diagnostic utility in appendicitis.

CONCLUSION

This study demonstrated that in addition to clinical evaluation elevated Total Leukocyte Count (TLC ≥10×10⁹/L) and C-reactive Protein (CRP ≥5 mg/L) levels are useful indicators in the diagnosis of acute appendicitis and its complications. These markers show good sensitivity (80%) and specificity (60%) with statistically significant results. Moreover, isolated hyperbilirubinaemia was not found to reliably predict complicated appendicitis such as perforation or gangrene. However, hyperbilirubinaemia could still have served as a helpful adjunct in guiding early surgical intervention. The combination of classical clinical signs such as migratory pain and peritoneal irritation along with raised TLC and CRP can facilitate prompt surgical decision-making and help avoid unnecessary delays in cases where urgent appendectomy is indicated.

Conflict of Interest: None

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