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Original Article

Assessing the Diagnostic Value of Serum Total Bilirubin in Appendicitis: A Prospective Cross-Sectional Study

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ABSTRACT

Background: Despite advancements in diagnostic methodologies, including advanced imaging techniques and laboratory markers, the medical community continues to search for more reliable, accessible, and cost-effective prognostic indicators. This study aims to investigate the predictive utility of serum total bilirubin in appendicitis. **Materials and Methods:** A prospective cross-sectional study was conducted with 100 subjects who were clinically suspected of presenting with acute appendicitis or appendicular perforation and were aged over 18 years. The routine biochemical test, complete blood count, liver function test, and ultrasonograph scan was performed on all the participants for pre operative diagnosis. The pre-operative diagnosis was compared with post-operative findings based on histopathology and operative records. The sensitivity and specificity of total bilirubin in detecting appendicitis severity were measured. **Results:** The average total bilirubin level in acute appendicitis was 0.74 ± 0.25 mg/dL, gangrenous appendix was 1.96 ± 20 mg/dL, and perforated appendix was 1.78 ± 0.35 mg/dL. When segregated based on total bilirubin cutoff value of 1.2 mg/dL, 81.15% of cases exhibited acute appendicitis, 16.6% manifested a gangrenous appendix, and 4% presented with a perforated appendix among individuals with a total bilirubin level <1.2 mg/dL. Conversely, 18.8% showcased acute appendicitis, 83.3% displayed a gangrenous appendix, and 96% exhibited a perforated appendix among subjects with a total bilirubin level >1.2 mg/dL. A statistically significant variance was detected between total bilirubin levels below 1.2 mg/dL and those exceeding 1.2 mg/dL among various appendicitis. The sensitivity and specificity of total bilirubin in detecting acute appendicitis were 86.7% and 65.9%, for gangrenous appendix 89.06% and 62.63%, and for perforated appendix 89.6% and 65.25%, respectively. **Conclusion:** The study concluded that A total bilirubin level below 1.2 mg/dL typically indicates acute appendicitis, while a level above 1.2 mg/dL is often associated with complicated appendicitis.

Keywords: Appendicitis, Gangrenous appendix, Perforated appendix, Total bilirubin, Prognostic indicator, Sensitivity, Specificity.

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INTRODUCTION

Acute appendicitis, a prevalent cause of abdominal surgical emergencies, presents a diagnostic conundrum that spans from mild, localized inflammation to severe, life-threatening complications such as perforation, abscess, and gangrene (Birnbaum *et al*., 2000). Timely identification of high-risk patients is crucial, impacting surgical timing and patient prognosis (Drake *et al*., 2016). Despite advancements in diagnostic methodologies, including advanced imaging techniques and laboratory markers, the medical community continues to search for more reliable, accessible, and cost-effective prognostic indicators (Farooqui *et al*., 2015). This ongoing pursuit underscores the need to enhance diagnostic accuracy and refine risk stratification in acute appendicitis management. By exploring novel biomarkers and integrating them into clinical practice, healthcare providers aim to improve patient outcomes, streamline treatment decisions, and optimize resource utilization in the management of acute appendicitis.

Serum bilirubin, a byproduct of heme breakdown, has long been recognized as a marker for liver function and hemolysis (Guerra Ruiz *et al*., 2021). However, emerging research indicates its involvement beyond these roles. Recent literature shown associations of serum bilirubin levels with systemic inflammation and ischemic damage, expanding its diagnostic significance (Ai *et al*., 2021). Elevated levels of serum bilirubin have shown correlations with the severity of appendiceal inflammation, suggesting its potential as a prognostic indicator for appendicitis

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complications (Nomura *et al.*, 2014). This association underscores the multifaceted nature of bilirubin, extending its utility from hepatobiliary diseases to systemic inflammatory conditions. However, those studies were limited in sample size and the nature of the study design. More comprehensive studies are needed to establish a strong correlation between serum bilirubin levels and appendicitis.

This study aims to investigate the prognostic utility of serum bilirubin in the context of appendicitis. The study has two primary objectives: firstly, to quantify serum bilirubin levels in patients diagnosed with acute appendicitis, thereby establishing a foundational correlation with the disease; and secondly, to assess the potential of serum bilirubin levels as predictive markers for appendicitis complications. Through comprehensive data analysis and comparison with existing diagnostic criteria, we seek to emphasize the predictive significance of serum bilirubin, which could potentially transform the clinical approach to early detection and management of high-risk appendicitis cases.

MATERIALS AND METHODS

Study design and setting

After obtaining the ethical clearance from Institutional Ethics Committee, Sapthagiri Institute of Medical Sciences and Research Center (SIMS & RC) with clearance no. SIMS &RC/IEC/02/2021, a prospective cross-sectional study was conducted at the Department of General Surgery, SIMS & RC over an 18-months period spanning from March 2021 to September 2022.

Study sample

The sample size was determined using the formula $n = (Z_{\alpha/2})^2 pq/d^2$, with a precision error of 10%. Considering a prevalence of 50%, the sample size was estimated to be 100.

Study subjects

The study encompassed participants who, upon admission, were clinically suspected of presenting with acute appendicitis or appendicular perforation and were aged over 18 years. Exclusion criteria included individuals with chronic liver disease, chronic alcoholism, hemolytic disease, or a history of cholestasis-inducing drug consumption. Moreover, patients diagnosed with cholelithiasis or malignancies of the hepatobiliary system were also precluded from participation in the study.

Study procedure

Patients exhibiting classic symptoms of appendicitis, including low-grade fever, localized abdominal pain, muscular guarding, and rebound tenderness, were thoroughly informed about the study and obtained informed consent to participate. Once enrolled, these individuals underwent comprehensive physical examinations to assess for signs indicative of appendicitis, including McBurney's sign, Blumberg's sign, the pointing sign, Rovsing's sign, psoas sign, Cope's (obturator) sign, Sherren's triangle, Baldwin's test for a retrocecal appendix, and shifting tenderness (Alvarado score). Additionally, a series of biochemical tests were performed, encompassing a Complete Blood Count (CBC), liver function tests, Hepatitis B surface Antigen (HbsAg) seropositivity, and urinalysis. Diagnostic imaging techniques, including plain radiography, and Ultrasonography (USG), were also employed to further evaluate the condition. Detailed history including demographic was taken and recorded in the predesigned data collection form. Patients were scheduled to undergo emergent laparotomy or laparoscopy. The individuals were subsequently sorted into three consecutive categories based on their appendix histopathology and operative records. These categories consisted of patients diagnosed with acute appendicitis, gangrenous appendicitis, and perforated appendicitis.

Statistical analysis

The collected data was input into Microsoft Excel for organization and subsequently analyzed through SPSS statistical software, version 22. To summarize the data, we used basic descriptive statistics, including frequency counts, percentages, averages, and measures of variability. For comparing groups and examining the relationships between variables, we applied inferential statistical methods, such as the student *t*-test, Spearman's rank correlation, and either the Chi-Square test or Fisher's exact test, depending on the data distribution. We determined statistical significance by a *p*-value of less than 0.05. Additionally, to explore the association between bilirubin levels and the outcomes of appendicitis, Pearson's correlation coefficient was used.

RESULTS

In the study, a notably higher percentage of male subjects, accounting for 69% of the overall cohort, was noted in comparison to female participants, who made up 31% of the sample. The mean age of the subjects was recorded at 29.8±12.13 years, with no statistically significant difference observed between the mean ages of the male and female subgroups (28.81±10.9 vs 32±14.45, respectively, $p > 0.05$). In the study, no statistically significant variations were noted in laboratory parameter values between male and female subjects, with the exception of bilirubin levels ($p > 0.05$). There were statistically significant differences observed in the average total bilirubin, direct bilirubin, and indirect bilirubin levels between males and females ($p < 0.05$) (Table 1).

In the study, based on preoperative USG scan we observed 91% of acute appendicitis and 9% of perforated appendix. The post operative observation shown 69% of acute appendicitis and 6% of gangrenous appendix and 25% of perforated appendix (Table 1). The average total bilirubin level in acute appendicitis was 0.74 ± 0.25 mg/dL, gangrenous appendix was 1.96 ± 20 mg/dl, and perforated appendix was 1.78 ± 0.35 mg/dL. When segregated based on total bilirubin cutoff value of 1.2 mg/dL,

Table 1: Distribution of demographic details, biochemical data, preoperative, and postoperative findings by gender.

Characters		f (%)	Male	Female	*p* value
Gender	Male	69 (69%)			
	Female	31 (31%)			
Age group	<30 years	27(27%)	16(23.2%)	11(35.5%)	
	30-40 years	57(57%)	42(60.9%)	15(48.4%)	
	> 40 years	16(16%)	11(15.9%)	5(16.1%)	
	Average age (Mean ± SD)	29.8±12.13	28.81±10.9	32±14.45	0.226 [x]
TLC (cells/mm^3)	<4000	2(2%)	1(1.4%)	1(3.2%)	0.624 [y]
	4000-11000	48(48%)	32(46.4%)	16(51.6%)	
	>110000	50(50%)	36(52.2%)	14(45.2%)	
	Average (Mean ± SD)	12365.29±4216.85	12229.38±3995.02	12667.81±4729.34	0.633 [x]
Neutrophil (%)	<40	1(1%)	0(0%)	1(3.2%)	0.25 [y]
	40-70	46(46%)	34(49.3%)	12(38.7%)	
	>70	53(53%)	35(50.7%)	18(58.1%)	
	Average (Mean ± SD)	73±14.11	71.9±13.78	75.45±14.76	0.246 [x]
Lymphocyte	<40	3(3%)	2(2.9%)	1(3.2%)	1 [y]
	40 - 60	97(97%)	67(97.1%)	30(96.8%)	
	Average (Mean ± SD)	16.66±11.29	17.48±11.39	14.84±11.04	0.282 [x]
Eosinophils	<3	20(20%)	9(13%)	11(35.5%)	0.14 [y]
	(1-7)	75(75%)	55(79.7%)	20(64.5%)	
	>7	5(5%)	5(7.2%)	0(0%)	
	Average (Mean ± SD)	1.83±2.27	1.14±2.57	1.13±1.18	0.38 [x]
Monocytes	(04-10)	89(89%)	59(85.5%)	30(96.8%)	0.165 [y]
	>10	11(11%)	10(14.5%)	1(3.2%)	
	Average (Mean ± SD)	4.57±2.27	5.28±2.06	5±1.93	1 [x]
Total bilirubin	<1.2	42(42%)	36(52.2%)	6(19.4%)	0.004 [y]
	≥1.2	58(58%)	33(47.8%)	25(80.6%)	
	Average (Mean ± SD)	1.3±0.72	1.44±0.75	0.99±0.53	0.003 [x]
Direct bilirubin	<0.2	67(67%)	44(63.8%)	23(74.2%)	0.427 [y]
	>0.2	33(33%)	25(36.2%)	8(25.8%)	
	Average (Mean ± SD)	0.26±0.18	0.29±0.2	0.21±0.1	0.041 [x]
Indirect bilirubin	<0.8	43(43%)	44(63.8%)	23(74.2%)	0.165 [y]
	>0.8	57(57%)	25(36.2%)	8(25.8%)	
	Average (Mean ± SD)	1.05±0.66	1.17±0.7	0.78±0.5	0.007 [x]

Characters		f (%)	Male	Female	*p* value
SGOT (IU/L)	<31	89 (89%)	60 (87%)	29(93.5%)	0.527 [y]
	>31	11 (11%)	9 (13%)	2(6.5%)	
	Average (Mean ± SD)	32.51±30.83	32.32±16.06	32.94±50.52	0.927 [x]
SGPT (IU/L)	<31	92 (92%)	63 (91.3%)	29 (93.5%)	1 [y]
	>31	8 (8%)	6 (8.7%)	2 (6.5%)	
	Average (Mean ± SD)	29.16±25.66	31.71±21.85	23.48±32.27	0.139 [x]
ALT (IU/L)	<105	43 (43%)	26 (37.7%)	17 (54.8%)	0.16 [y]
	>105	57 (57%)	43 (62.3%)	14 (45.2%)	
	Average age (Mean ± SD)	137.9±72.36	147.58±77.78	149.23±32.45	0.1[x]
Pre-operative (USG)	Acute appendicitis	91 (91%)	57 (82.6%)	28 (90.3%)	0.167 [y]
	Perforated appendicitis	9 (9%)	8 (11.6%)	1 (3.2%)	
Post-operative	Acute appendicitis	69 (69%)	43 (62.3%)	26 (83.9%)	0.006 [y]
	Gangrenous appendix	6 (6%)	3 (4.3%)	3 (9.7%)	
	Perforated appendix	25 (25%)	23 (33.3%)	2 (6.5%)	

[x] is the Student T & [y] is the Chi-Square test.

Table 2: Average and standard deviation of serum total bilirubin levels across various types of appendicitis, segregated based on a cutoff value of 1.2 mg/dL.

Post-operative diagnosis	Total bilirubin (mg/dL)			*p* value
	Mean ± S.D.	<1.2	≥1.2	
Acute appendicitis	0.74 ± 0.25	56 (81.15%)	13 (18.8%)	0.001
Gangrenous appendix	1.96 ± 20	1 (16.6%)	5 (83.3%)	
Perforated appendix	1.78 ± 0.35	1 (4%)	24 (96%)	

81.15% of cases exhibited acute appendicitis, 16.6% manifested a gangrenous appendix, and 4% presented with a perforated appendix among individuals with a total bilirubin level <1.2 mg/dL. Conversely, 18.8% showcased acute appendicitis, 83.3% displayed a gangrenous appendix, and 96% exhibited a perforated appendix among subjects with a total bilirubin level >1.2 mg/dL. A statistically significant variance was detected between total bilirubin levels below 1.2 mg/dL and those exceeding 1.2 mg/dL in the context of acute appendicitis, gangrenous appendix, and perforated appendix ($p < 0.05$) (Table 2 and Figure 1).

In the context of acute appendicitis, the dataset reveals 13 True Positives (TP) and 56 True Negatives (TN), indicating accurate diagnoses of both positive and negative cases, respectively. However, the analysis identifies 29 False Positives (FP) and 2 False Negative (FN), suggesting some diagnostic inaccuracies primarily in false positive identifications. The resulting Sensitivity was calculated at 86.7%, and Specificity was 65.9%, indicating a relatively high capability to correctly identify cases of acute appendicitis, though with potential for specificity improvement. The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were found to be 30.35% and 96.6%, respectively, with the high NPV indicating a reliable exclusion of acute appendicitis in negative cases. The overall diagnostic Accuracy stood at 69%, with the correlation between bilirubin levels and acute appendicitis achieving statistical significance ($p < 0.001$) (Table 3).

For gangrenous appendicitis, our findings revealed a perfect diagnostic outcome for non-gangrenous conditions, with 6 TPs and 94 TNs, and no FN cases, alongside an FP count of 0. These outcomes translated to an NPV of 97.24% and an unprecedented diagnostic accuracy of 100%. Sensitivity and Specificity were reported at 89.06% and 62.63%, respectively, with a PPV of

Table 3: Evaluating the diagnostic performance of total bilirubin in appendicitis variants.

Correlation of total bilirubin with	Observation					Correlation					*p* value
	TP	FP	FN	TN	Total	Se	Sp	PPV	NPV	Accuracy	
Acute appendicitis	13	29	2	56	100	86.7	65.9	30.35	96.6	69.00	<0.001
Gangrenous appendix	6	0	0	94	100	89.06	62.63	40.9	97.24	100	<0.001
Perforated appendix	44	1	0	56	100	89.6	65.26	57.1	98.11	98.00	<0.001

True Positive (TP), False Positive (FP), False Negative (FN), and True Negative (TN) assessments. Derived statistical measures include Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Accuracy.

40.9%. The exemplary NPV and Accuracy metrics underscore the high diagnostic reliability for gangrenous appendicitis, with a statistically significant correlation ($p < 0.001$) with serum bilirubin levels confirmed (Table 3).

For the perforated appendix category, the dataset yields 44 TPs and 56 TNs, with no FNs and a singular FP, indicating high diagnostic precision. This category achieved the highest Sensitivity 89.6% and a Specificity of 65.26%, alongside PPV and NPV values of 57.1% and 98.11%, respectively. Such outcomes denote a particularly strong predictive capability of bilirubin levels for perforated appendix cases, with an Accuracy of 98% and a p value of <0.001, affirming the statistical significance of these findings (Table 3).

DISCUSSION

Our study investigated the correlation between serum total bilirubin levels and the severity of appendicitis, categorizing cases into acute, gangrenous, and perforated appendicitis. The significant correlations observed between bilirubin levels and the various appendicitis categories highlight the potential utility of bilirubin as a diagnostic biomarker. In comparison to similar studies in the literature, our findings align with existing evidence while offering novel insights into the diagnostic efficacy of bilirubin across different appendicitis severities.

The diagnostic accuracy observed in our study for gangrenous appendicitis, with a sensitivity of 89.06% and specificity of 62.63%. Furthermore, our investigation into perforated appendicitis revealed a strong predictive value of bilirubin levels, with a sensitivity of 89.6% and specificity of 65.26% (Table 3). These findings corroborate previous studies such as D'Souza *et al.*, 2013; Muller *et al.*, 2015; Giordano *et al.*, 2013; Silva *et al.*, 2016; Sand *et al.*, 2009; and Chaudary *et al.*, 2013, which have demonstrated the diagnostic efficacy of bilirubin in the detection of gangrenous appendicitis and perforated appendicitis. These investigations elucidate that the specificity, sensitivity, PPV, and NPV of hyperbilirubinemia in distinguishing between simple and perforated appendicitis ranged between 70-82%, 48-70%, 18-47%, and 92-93% (D'Souza *et al.*, 2013; Muller *et al.*, 2015; Giordano *et al.*,2013; Silva *et al.*, 2016; and Sand *et al.*, 2009), respectively. The diagnostic accuracy observed in our study for acute appendicitis, with a sensitivity of 86.7% and specificity of 65.9%. However, it has a high chance of false positive results. Notably, our study further substantiates these findings by providing a comprehensive evaluation of bilirubin's diagnostic performance across multiple appendicitis categories, thereby enhancing the clinical applicability of these results.

In the investigation concerning USG findings, 91% of cases displayed indications of appendicitis without evidence of perforation, whereas the remaining 9% manifested appendicitis with perforation. Following surgical intervention, 49 patients were confirmed to have acute appendicitis, 6 exhibited gangrenous appendices, and post-operative analysis revealed perforation in 46 cases. This raises concerns regarding the efficacy of USG in detecting complicated appendicitis preoperatively, as more complex presentations were observed post-surgery. Babington *et al.*, 2022, reported that solely relying on ultrasound for diagnostic purposes is insufficient. Hosseini *et al.*, 2018 and Crichton *et al.*, 1988, both advocate prudence regarding the extensive application of ultrasound, citing its limited sensitivity and susceptibility to diagnostic errors. However, USG plays a significant role in the diagnosis of appendicitis that merits attention and should not be disregarded. The study also demonstrates a notable relation between total bilirubin levels exceeding 1.2 mg/dL and complicated appendicitis, while levels below 1.2 mg/dL were markedly more prevalent in cases of acute appendicitis ($p < 0.001$) (Table 2). This finding suggests that the incorporation of total bilirubin levels in conjunction with USG can serve as a diagnostic tool for distinguishing between simple and complicated appendicitis.

While our study reinforces the diagnostic value of bilirubin in identifying appendicitis, it is essential to acknowledge certain limitations. One limitation lies in the single centre study with limited sample size study, which may introduce biases and confounding factors. Additionally, our study focused solely on the correlation between bilirubin levels and appendicitis severity, without considering other potential biomarkers or clinical parameters that could enhance diagnostic accuracy.

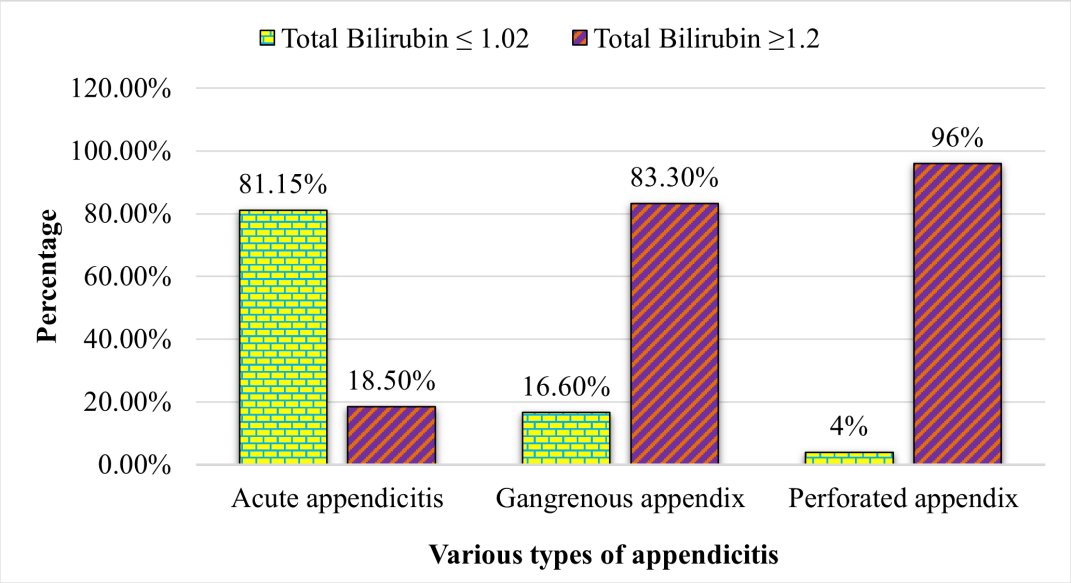

Figure 1: Serum total bilirubin levels across various types of appendicitis, segregated based on a cutoff value of 1.2 mg/dL.

CONCLUSION

The investigation revealed significant correlations of total bilirubin among acute, gangrenous, and perforated appendicitis categories. A total bilirubin level below 1.2 mg/dL typically indicates acute appendicitis, while a level above 1.2 mg/dL is often associated with complicated appendicitis. Integrating total bilirubin levels alongside other biomarkers or imaging techniques could function as a diagnostic tool for discerning between simple and complicated appendicitis. Future research endeavors should focus on validating these observations and investigating the potential synergistic impacts of bilirubin in conjunction with other clinical parameters to further refine diagnostic accuracy.

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ABBREVIATIONS

SIMS & RC: Sapthagiri Institute of Medical Sciences and Research Center; **CBC:** Complete Blood Count; **HBsAg:** Hepatitis B Surface Antigen; **USG:** Ultrasonography; **TP:** True Positives; **FP:** False Positives; **FN:** False Negatives; **TN:** True Negatives; **Se:** Sensitivity; **Sp:** Specificity; **PPV:** Positive Predictive Value; **NPV:** Negative Predictive Value.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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