Original article:

Pre-operative serum total bilirubin level as an indicator marker of perforated appendicitis.

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Abstract

Objectives*:** Acute appendicitis is one of the most common surgical emergencies. Perforated appendicitis can result in increased morbidity and mortality. Thus, early identification of perforation can reduce the impact on patient and improve outcome. Apart from total white cell count (TWCC), serum total bilirubin (SB) has been shown to indicate perforation in appendicitis. The aim of this study was to assess the usefulness of serum total bilirubin (SB) as the preoperative indicator for perforated appendicitis. ***Materials and Methods*:** This was a retrospective study of all appendicectomies cases done in Hospital Universiti Sains Malaysia (HUSM) over a one-year period in 2014. Only patients with histopathologically confirmed appendicitis with available liver function tests and total white blood cell count preoperatively were included in the study. These appendicitis cases were further divided into perforated and non-perforated based on histopathological reports obtained. ***Results: A total of 245 patients had appendicectomy done in 2014 between January to December 2014. Out of 110 from these 245 patients met the inclusion and exclusion criteria. SB was significantly higher ($p<0.001$) in patients with perforated appendix compared to non-perforated. ***Conclusion:*** In conclusion, this study showed that preoperative SB is a useful laboratory parameter for perforated appendicitis to improve clinical decision-making by the clinician.

Keywords: perforated appendicitis; hyperbilirubinemia; serum total bilirubin; indicator marker.

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Introduction

Acute appendicitis is one of the most common acute surgical conditions that require urgent management of emergency surgery[1]. However, it has been a diagnostic challenge for surgeons as appendicitis may not present with classical symptoms and signs. Most of appendicitis cases are uncomplicated. A delay in the management of appendicitis may lead to various complications such as perforation, abdominal abscess and peritonitis in which will cause an increase in morbidity and increase in hospital stay[2].

Ultrasonography and computed tomography (CT)

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scan have been used in cases suspected of appendicitis. Even though an early diagnosis of the perforated appendix by this method gave accuracy up to 85 to 99%[3], this method of radiological investigations can be very costly and time-consuming. There is much real interest in finding ways to diagnose and estimate the extent of acute appendicitis besides radiological examinations. The current interest is looking at serum markers for perforated appendicitis. Investigation supported by raised inflammatory or biochemical markers such as C-reactive protein (CRP) and total white cell count (TWCC) are currently being used for the management of acute appendicitis especially in identifying perforated appendicitis. Apart from CRP and TWCC, many studies have shown that hyperbilirubinemia could be used as an indicator of perforation in appendicitis[4,5,6].

Studies have shown the association between elevated SB levels with multiple infectious diseases[1,7,8]. Elevated SB is commonly found in the patient with severe intra-abdominal infection. The pathogenesis of this is due to septicaemia causing haemolysis of red blood cells. Elevated SB is also thought to be due to the release of inflammatory cytokines causing a large variety of abnormalities in bilirubin processing which include decreased bilirubin uptake, deficient intrahepatic processing and intrahepatic cholestasis[1,9]. The aim of this study was to assess the usefulness of preoperative SB as a useful parameter in the early diagnosis of perforated appendicitis in order to improve clinical decision making by clinician and improve patient prognosis.

Materials And Methods

This was a retrospective study of all appendicectomies done in Hospital Universiti Sains Malaysia (HUSM) between January to December 2014.Patients were identified from the laboratory information system (LIS) of histopathological reports for those who underwent an appendicectomy. Based on the sample size calculation, the minimum sample required for our study was 112 subjects.

Sample size was calculated using sample size calculation for two independent mean. With Type I error of 5%, Type II error of 20% (80% power of study), population standard deviation of serum bilirubin level from the previous study of 19.61 and detectable difference of 11 (*N.D 'Souza et al, 2013),*the sample size required was 84. After adding to 10% anticipated dropout rate, the number of patients required for this study was 112.

Only patients with histopathological confirmed appendicitis with available liver function tests and total white blood cell count preoperatively were included in the study. Patients with documented haemolytic or liver disease, history of alcoholism and biliary disease associated with hyperbilirubinemia were excluded. These appendicitis cases were further divided into perforated and non-perforated based on the histopathological reports obtained. Perforated cases were defined as those that had documented histological diagnosis of perforation that is rupture of the appendiceal wall to the serosa. Non-perforated appendices were defined as all operation with the finding of acute appendicitis, which did not have a perforation noticed on the histopathological report. Serum bilirubin assay was performed on Architect C8000 analyser using Diazo method. The principle of this method was based on solubilized bilirubin forms a red colored compound (diazo-bilirubin) with diazotized sulphanilic acid. Total white cell count was analysed by flow cytometry using Sysmex analyzer. We defined the laboratory findings as normal or elevated depending on the reference range set by our hospital. Normal reference range for total SB is 7-17 μmol/L and normal reference range for TWCC is 4-11.0 $x10^9$/L.

Statistical analyses were carried out using the SPSS® statistical package, version 22.0. Baseline characteristics of study participants were compared between the perforated group and non-perforated group using independent sample t-test (for age, TWCC and SB) and chi-square test (for gender). Linear association between TWCC and SB was determined using Pearson Correlation analysis. The level of significance was set at $P < 0.05$ for all the statistical tests above. In this study, SB was used as an indicator to differentiate between perforated and non-perforated appendicitis, thus sensitivity, specificity, positive-predictive values (PPV) and negative- predictive values (NPV) were carried out using the SPSS®. The correctly classified percentage, positive and negative likelihood (LR^+, LR^-) ratio was calculated by STATA version 11.

Ethical Clearance: The study has been approved by the Human Research Ethic Review Committee, Universiti Sains Malaysia (USM).

Results

A total of 245 patients undergone appendicectomy between January to December 2014, and 110 patients out of the total met the inclusion and exclusion criteria. The mean age (SD) for the 110 patients was 28 (16.6) years range from 2 to 81 years old. There was no significant difference in age of patient between two groups ($p > 0.05$). Seventy-five patients

(68%) had evidence of perforated appendicitis and 35 patients (32%) had non- perforated appendicitis on final the histopathological report. Total serum bilirubin ranged from 3 to 57 µmol/L.

Hyperbilirubinemia (> 17 µmol/L) was found in 42 (38%) patients. Out of this 33 (78%) patient had perforated appendicitis. The mean (SD) of TWCC in all patients was 14.6 (5.52) $x10^9$/L and 73 (66.4 %) patients were found to have elevated TWCC.

Analysis on different variables was done comparing the two groups of perforated and non-perforated appendicitis (Table 1). There was no significant difference in gender as shown in table 2. Analysis between TWCC and SB showed no significant correlation (r=0.09, p=0.305, n=110).

Table 1: Gender distribution between perforated and non-perforated appendicitis

Sex	n (%)		χ^2 (df)	P-value
	Perforated (n=75)	Non-perforated (n=35)		
Female	41	14	2.05 (1)	0.15
Male	34	21		

Table 2: Comparison of different variables in the study group between perforated and non-perforated appendicitis

Variables	Mean (SD)		Mean difference (95% CI)	t-statistics (df)	P-value
	Perforated (n=75)	Non-perforated (n=35)			
Age	29.20(17.64)	25.11(13.81)	-2.62, 10.79	1.21 (108)	0.230
Total white count	15.11(5.63)	13.51(5.17)	-0.63, 3.83	1.42(108)	0.158
Total bilirubin	17.59(9.05)	10.94(6.21)	3.29, 9.98	3.93(108)	<0.001

Table 3: Distribution of serum bilirubin between perforated and non-perforated appendicitis

Serum Bilirubin	Perforated appendicitis (75)	Non- perforated Appendicitis (35)
>17mmol/L	33	9
<17 mmol/L	42	26

Table 4: Sensitivity, specificity, positive and negative likelihood ratio and correctly classified percentage for serum total bilirubin as a marker of perforation in acute appendicitis based on histopathological findings.

Cut-off value SB (µmol/L)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Correctly Classified (%)	LR^+	LR^-
≥11	86.7	54.3	78.7	54.3	76.4	1.90	0.25
≥12	80.0	68.6	83.1	53.3	76.4	2.54	0.29
≥13	74.7	71.4	83.3	50.0	73.6	2.61	0.35
≥14	64.0	71.4	80.8	43.1	66.4	2.24	0.50
≥15	57.3	74.3	80.4	40.6	62.7	2.23	0.57
≥16	50.7	74.3	78.0	37.7	58.2	1.97	0.66
≥17	44.0	74.3	75.0	35.1	53.6	1.71	0.75
≥18	38.7	85.7	82.1	36.6	53.6	2.71	0.72

Discussion

Bilirubin is commonly ordered for the assessment of jaundice. However it is not known to be a marker for appendicitis. Recent studies have found bilirubin to be a potential marker for acute appendicitis[2,4,12]. The underlying mechanism for hyperbilirubinemia is thought to be due to compromised appendix wall integrity that leads to translocation of bacteria and endotoxin into the portal system. This will disrupt the excretion of bilirubin into bile canaliculi[9].Serum total bilirubin increases as the infection become more severe[6,12]. Pro-inflammatory cytokine and nitric oxide also play a role in triggering intrahepatic cholestasis[13]

There are no single clinical or laboratory testable to accurately predict acute appendicitis or perforated appendicitis[4]. Thus, a combination of complete history, clinical examination, laboratory results and radiological investigations are used to make the provisional diagnosis and plan for appropriate management. Those with atypical symptoms or signs need a period of observation and re-evaluation. However, this approach could result in unnecessarily prolonging patients' hospital stays and delaying their definitive treatment.

The main finding of our study indicates that, SB was significantly higher ($p<0.001$) in patients with perforated appendicitis compared to non-perforated. This finding is parallel with previous studies done in 2013[4,14].This study shows the odds of appendiceal perforation which are two times higher (odds ratio 2.27) for patients with hyperbilirubinemia compared to those with normal bilirubin levels. This finding is similar with a retrospective study conducted by USC Medical Center, Los Angeles that found patients with gangrene and perforation were significantly more likely to have hyperbilirubinemia than those with acute appendicitis.

Formerly, TWCC has been used as a parameter to indicate perforation in appendicitis[14]. In appendicitis, the early inflammatory response causes an increase in the neutrophil count without general increase in the TWCC. As time progresses, the TWCC will increase as the invasion of bacteria sets in[15]. However, TWCC has been found to be a non-specific marker as it can be elevated in many inflammatory conditions[16]. One study showed that in patients with other causes of right lower quadrant pain, the elevation of TWCC is up to 70 percent[17]. Furthermore, many studies have shown it is not specific for the diagnosis of appendicitis[18,19,20].

Many studies looked at the level of 17 µmol/L as the cut-off value to predict perforated appendix[23,25]. In view of the multiple findings on sensitivity and specificity, hyperbilirubinemia gave some value as a predictor of perforated appendix. Researchers suggested that bilirubin level should be assessed together with clinical sign and symptoms[24]. In our study, we found that at the level of 17 µmol/L, the sensitivity and specificity were 44%,74.3% with PPV 75% and NPV 35.1% in contrast to the study done by Atahan *et al* which showed a good NPV of 97% for perforated appendicitis.

There are potential limitations to this study. Since it was a retrospective study, it is susceptible to confounding factors. There was the possibility of selection bias as not all patients with appendicitis seen at our hospital during this period had liver function test done on admission. Therefore, a prospective research study which includes liver function test and inflammatory marker such as CRP upon admission should be conducted to improve the ability of laboratory marker to predict a perforation in suspected appendicitis cases. Apart from that, further extended research on newer biomarker, such as serum Pro-calcitonin may improve accuracy of predicting an appendix perforation.

Conclusion

In conclusion, the assessment of preoperative total SB is useful to be a new promising laboratory parameter that can identify patients who are more likely to have perforated appendicitis.Pre-operative SB should be used together with clinical findings and other routine laboratory tests to definitively manage patients with acute appendicitis in early setting.

Conflict of interest

All the authors declare no conflict of interest

Authors' Contribution:

Data gathering and idea owner of this study: Kassim NK, Jelani AM, Salwani T

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