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A Prospective Study to Asses the Role of Leucocyte and Hyperbi-lirubinaemia as a Predictive Factor for Appendicular Perforation

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Abstract

Background: Acute appendicitis, though a common surgical emergency, is often missed by young and inexperienced surgeons who need to know the importance of the age-old battery of investigative gadgets and laboratory tests along with the importance of estimation of total serum bilirubin to exclude the possibility of a rupture of the organ. *Objective:* Study To Assess the role of leucocyte and hyperbilirubinemia as a predictive factor for appendicular perforation in acute appendicitis. *Materials and Methods:* This study was performed on 125 patients who have been clinically diagnosed of having acute appendicitis and who were posted for appendectomy in Post-Graduate Department of General Surgery, in collaboration with the Department of Pathology and the Post-Graduate Department of Radiology, S.C.B. Medical College and Hospital, Cuttack, Odisha, India during the period from Jan 2013 to Dec 2014. *Results:* The sensitivity of TLC > 11 x 10^3 cells/µL and Total Bilirubin level > 1.1 mg/dL was found to be 21.43 and 89.29 respectively and Specificity was 2.33 and 53.49 for TLC and Bilirubin Level Respectively. *Conclusion:* The present day surgeons should emulate the findings of this piece of work to minimise the morbidity and mortality of acute appendicitis in future by just adding one more laboratory test i.e. total serum bilirubin; though the total serum bilirubin may not be very helpful for diagnosing the catarrhal and non-perforated appendicitis.

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Introduction

It is well established that when microbes invade the body, leucocytes 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. The most commonly used laboratory tests to support the diagnosis of appendicitis are white blood cell count (WBC) and C-reactive protein (CRP) [1,2,3], these markers have been studied together with other parameters in an effort to improve and predict the pre-operative diagnosis of perforated appendicitis, nonetheless only an elevated CRP, a prolonged period of symptoms evolution, and fever have been identified as useful markers of perforation [2,3].

Recently, it has been proposed that an elevated total Bilirubin level could be used as a specific marker for the prediction of perforated appendicitis [4,5]. The rationale for this proposal is based on the hepatic dysfunction occurring during bacterial sepsis secondary to Gram negative bacteria [6[, such as *Escherichia coli*, which is the main bacteria present in patients with appendicitis [7,8]. Consequently a low-grade hyperbilirubinaemia, often unnoticed in septic patients not presenting with clinically evident jaundice, is present in patients with Gram-negative infections [9].

Our purpose of this study was to compare the performance of Total Bilirubin versus other well known non-specific inflammatory markers (WBC and CRP), the time period of symptoms evolution from the onset of symptoms to surgery, and the systemic inflammatory response syndrome (SIRS), to suspect perforated appendicitis.

Objective

Role of Total Leucocyte Count Hyperbilirubinaemia as a Predictive Factor for Appendicular Perforation in Acute Appendicitis

Materials & methods

Source of data

This study was performed on 125 patients who have been clinically diagnosed of having acute appendicitis and who were posted for appendicectomy in Post-Graduate Department of General Surgery, in collaboration with the Department of Pathology and the Post-Graduate Department of Radiology, S.C.B. Medical College and Hospital, Cuttack, Odisha, India during the period from Jan 2013 to Dec 2014.

Method of Collecting Data

Sample size: 125 cases of Acute Appendicitis

Sampling method: Simple random sampling

Inclusion criteria

All patients, diagnosed clinically to have acute appendicitis, are subjected for appendicectomy in S.C.B. Medical College, Cuttack, Odisha, India.

Exclusion criteria

1. Patients with co-morbid conditions were not included in the study.
2. Patients who were managed conservatively were also excluded from the study.
3. Patients admitted for interval appendicectomy following recurrent appendicitis or appendicular mass previously treated conservatively, were also excluded.
4. Other diagnoses (peptic perforation, acute cholecystitis etc.) were also excluded by history, clinical examination and investigations.
5. Patients shall be excluded if they have documented liver disease, history of alcoholism, hemolytic disease, and other acquired or congenital biliary diseases.

Clinical diagnosis of acute appendicitis was done in the Post-Graduate Department of Surgery, based on symptoms of migratory pain, nausea and vomiting, anorexia, fever and signs of peritoneal inflammation like right iliac fossa tenderness, rebound tenderness and guarding. Once acute appendicitis was suspected, each patient was subjected to routine investigations as per the hospital protocol. Urine microscopy was performed in all cases. Elderly patients were subjected to further investigations as part of pre-anaesthetic work up including X-ray chest, ECG etc.

Serum Bilirubin (Total and Direct), CRP, Total leukocyte count and differential count were done in all cases. WBC count of more than 10000 cells/ mm^3 was considered positive and neutrophil count of more than 75% was considered positive. Ultrasonography of abdomen was done in most of the cases to confirm diagnosis and rule out other causes of pain abdomen. Total serum bilirubin more than 1.1 mg/dL was considered to be positive. No special preparation of the patient was required prior to sample collection by approved techniques. When there was delay, the sample was stored at 2-8^{0}C. Maximum period of storage was 72 hours.

Patients with strong suspicion of acute appendicitis were advised appendicectomy. After obtaining consent, patient was operated, and the appendicectomy specimen was sent for histopathological examination. The histopathology report was considered as the final diagnosis.

The histopathologically positive cases among hyperbilirubinaemia positive group were considered true positives. The histopathologically negative cases in the same group were considered as false positives. The histopathologically positive cases among hyperbilirubinaemia negative group were considered false negatives. The histopathologically negative cases in the same group were considered as true negatives. Similarly CRP, WBC, differential count, USG were also classified as true and false positives, and true and false negatives after correlating it with HPE reports.

The patients were meticulously monitored in the post-operative period for any complications. All patients were followed up in the outpatient department every 2 weekly for a period of 8 weeks. During follow-up, they were enquired about the result of the operation and examined to detect the

occurrence of late complications. The case study was done as per a proforma which is detailed below. The hospital ethical committee clearance was obtained prior to undertaking the study.

Results

The present study was performed on 125 patients who have been clinically diagnosed as a case of acute appendicitis and who were posted for appendicectomy in the Post-Graduate Department of General Surgery, SCB Medical College and Hospital, Cuttack during the period from Jan 2013 to Dec 2014.

The age of the patients ranged from 4 years to maximum of 70 years, with a mean age of 30.76 ± 14.09 years. The maximum number of patients with acute appendicitis presented in the age group of 21-40 years (40%) where as patients with appendicular perforation presented mostly in age group of 21-30 years (7.2%).

In the present study, females are affected slightly more in acute appendicitis than males (M:F = 1:1.15) while males predominate in appendicular perforation (M:F = 3:1).

Among clinical signs, right iliac fossa tenderness was seen in all cases (100%); rebound tenderness was seen in 46.4% cases, guarding/rigidity was seen in 27.1% of cases. Other peritoneal signs like Rovsing's sign were elicited in 4 cases and Psoas sign only in 1 case.

In the present study, 91.2% (114 cases) were histopathologically found to be positive and 11 cases were negative on histopathology for various forms of acute appendicitis. Therefore the rate of negative appendicectomy in the present study is only 8.8% (Table 1).

Out of 114 cases of acute appendicitis, 90 (78.94%) had elevated total WBC count, rest 24 (21.06%) patients had normal WBC count. The sensitivity of the test was found to be 78.95%; specificity 54.55%; Positive Predictive value 94.74% and Negative Predictive Value 20%. The test is statistically significant as the p value is 0.013. (Table 2).

The sensitivity of the test in predicting perforation among the acute appendicitis patients was found to be 21.43%; specificity 2.33%; Positive Predictive value 6.67% and Negative Predictive Value 8.33%. The test is statistically significant as the *P* value is < 0.0001.(Table 3).

Table 1: Histopathological Findings of Appendicitis

Acute appendicitis	86 (68.8%)
Perforated appendix	18 (14.4%)
Gangrenous appendicitis	10 (8%)
Total	114 (91.2%)

Table 2: Sensitivity, Specificity, Positive and Negative Predictive Value of TLC in all forms of Acute Appendicitis (n = 125)

TLC	Histopathology			
	Acute Appendicitis	Normal	Total	
Elevated (>11 x 103 cells/µL)	90	5	95	Sensitivity = 78.95% Specificity = 54.55% PPV = 94.74% NPV = 20% Chi Square Value = 6.1679 Degrees of freedom = 1 p-value = 0.013
Normal	24	6	30	
Total	114	11	125	

PPV = Positive Predictive Value; NPV = Negative Predictive Value

Table 3: Sensitivity, Specificity, Positive and Negative Predictive Value of TLC in differentiating Gangrenous / Perforated from Non-perforated Appendicitis (n = 114)

TLC	Histopathology			
	Perforated /Gangrenous Appendicitis	Non-perforated Appendicitis	Total	
Elevated (>11 x 103 cells/µL)	6	84	90	Sensitivity = 21.43% Specificity = 2.33% PPV = 6.67% NPV = 8.33% Chi-square value = 73.8822 Degrees of freedom = 1 p-value = < 0.0001
Normal	22	2	24	
Total	28	86	114	

PPV = Positive Predictive Value; NPV = Negative Predictive Value

Table 4: Sensitivity, Specificity, Positive and Negative Predictive Value of Total Serum Bilirubin in all forms of Acute Appendicitis (n = 125)

Total Serum Bilirubin	Histopathology			
	Acute Appendicitis	Normal	Total	
Elevated (> 1.1 mg/dL)	65	2	67	Sensitivity = 57.02% Specificity = 81.82% PPV = 97.01% NPV = 15.52% Chi Square value = 6.0837 Degrees of freedom = 1 Two-tailed P-value = < 0.0136
Normal	49	9	58	
Total	114	11	125	

Table 5: Sensitivity, Specificity, Positive and Negative Predictive Value of Total Serum Bilirubin in differentiating Gangrenous / Perforated from Non-perforated Appendicitis (n = 114)

Total Serum Bilirubin	Histopathology			
	Perforated /Gangrenous Appendicitis	Non-perforated Appendicitis	Total	
Elevated (> 11 x 103 cells/µL)	25	40	65	Sensitivity = 89.29% Specificity = 53.49% PPV = 38.46% NPV = 93.88% Chi Square value = 15.7693 Degrees of freedom = 1 Two-tailed p-value = < 0.0001
Normal	3	46	49	
Total	28	86	114	

Table 6: Total Serum Bilurubin and Total Leucocyte Count in type of Appendix

Type of Appendix	Total Serum Bilirubin		Total Leukocyte Count	
	< 1.1 mg/dL No (%)	> 1.1 mg/dL No (%)	< 11 x 103 cells/µL No (%)	> 11 x 103 cells/µL No (%)
Acute Appendicitis	46 (36.8%)	40 (32%)	2 (1.6%)	84 (67.2%)
Gangrenous Appendix	2 (1.6%)	8(6.4%)	8 (6.4%)	2 (1.6%)
Perforated Appendix	1 (0.8%)	17 (3.6%)	14 (11.2%)	4 (3.2%)
Normal Appendix	9 (7.2%)	2 (1.6%)	6 (4.8%)	5 (4%)
Total	58	67	30	95

Table 7: Comparison between Total Serum Bilirubin and Total Leukocyte Count as laboratory markers of Appendicular Perforation

Laboratory value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
TLC > 11 x 103 cells/µL	21.43	2.33	6.67	8.33
Total Bilirubin > 1.1 mg/dL	89.29	53.49	38.46	93.88

Out of 114 Acute Appendicitis cases elevated Serum Bilirubin was seen in 65 cases which had sensitivity of 57.02%, Specificity of 81.82%, Positive Predictive value of 97.01% and Negative predictive value of 15.52%. The two tailed p value was also found to be statistically Significant (Table 4).

Out of the 28 cases of perforated Appendicitis 25 cases had elevated serum Bilurubin level. The Sensitivity was 89.29% and Specificity was 53.49% with PPV and NPV value of 38.46% and 93.88% respectively (Table 5).

Out of 125 cases in our study 58 cases had total serum bilirubin level less than 1.1 mg/dl and 68 of them had more than 1.1 mg/dl. 30 cases had Total leucocyte count less than $11*10^3$ cells/ul (Table 6).

The sensitivity of TLC > 11 x 10^3 cells/µL and Total Bilurubin level > 1.1 mg/dL was found to be 21.43 and 89.29 respectively and Specificity was 2.33 and 53.49 for TLC and Bilurubin Level Respectively (Table 7).

Discussion

Hyperbilirubinemia may be due to haemolysis, hepatocellular damage or cholestasis (intra and extrahepatic out flow obstruction). The biochemical picture of elevated serum bilirubin in haemolysis is unconjugated (indirect) type whereas it is of mixed type in hepatocellular and cholestatic diseases (conjugated and unconjugated) [10].

In the present study, the overall picture of LFT suggests elevation in conjugated and unconjugated Serum bilirubin (mixed type of hyperbilirubinaemia) in most of the patients (86.6%). But at the same time, there was no elevation or minimal elevation (<100U/L) in ALT and AST in most of the cases (99.0% and 100.0% respectively). Similarly, ALP was either within normal limit or minimal to moderately elevated in all cases (100.0%).

The above observations suggest that there is no damage but dysfunction of hepatocytes. The

dysfunction is either derangement in permeability of the hepatocytes to bilirubin or depressed function of ductule enzyme (Na^+-K^+-ATPase) leading to cholestasis, regurgitation and mixed type of hyperbilirubinaemia.

The present results are similar to the experimental study of Whiting et al., in which they have demonstrated depressed excretion of bile in canaliculi [13]. This finding is further supported by demonstrating inspissated bile in dilated, proliferated and periportal ductules in the histopathological study of liver [14].

The hepatocellular dysfunction/damage in sepsis may be either due to bacteria, its toxin or cytokines. The agent reaches from inflamed gut via portal vein or lymphatic with the process of transmigration or translocation. Bacterial translocation is the process by which bacteria moves across the mucosal barrier and invades the host. This phenomenon can occur in small percentage of healthy persons. The process seems to be accelerated with starvation and injury.

This has been proved by demonstrating bacteria in portal blood in 30% with non-inflammatory bowel disease [15]. But these patients do not develop any disease because hepatic clearance of portal bacteria is very efficient and it is a very common event in healthy persons. So, human liver remains sterile in most of the circumstances. Hence to develop any liver disease, adequate amount of bacteria as well as a vulnerable liver is needed. Bacterial involvement in hepatic dysfunction or damage has been proved by various direct, indirect, clinical and experimental studies.

Indirect evidences of bacterial translocation from inflamed gastro-intestinal tract or peritonitis to liver via portal vein and development of hepatitis, pyogenic liver abscess was observed by Fitz [16] and Dieulfoy [11] in their clinical studies. They have demonstrated two classical findings. Firstly; simultaneous inflammation of the intestine (e.g. appendix), peritoneum and development of pyogenic liver abscesses, Secondly; bacteriological similarities of the gastrointestinal tract and pyogenic liver abscesses. These bacteria commonly reach the liver from intra-abdominal organs, usually from the most common organ involved in inflammation i.e. appendix. So he coined the term le foie appendiculaire in describing multiple hepatic abscesses subsequent to perforated appendicitis with pylephlebitis.

Similarly, Oschner and Debakey [12] also provided classical treatises on pyogenic infection. These authors revised personal experiences and world literature, and emphasized its pathogenesis and clinical presentation. Bacteria isolated in pyogenic liver abscesses were similar to the bacteria involved in acute inflammation of the gut (i.e. acute appendicitis) and peritonitis. These agents were E. coli, Streptococcus fecalis, Klebsiella and Proteus vulgaris. Commonly the infection was of mixed type [12,17,18]. So, it was concluded from indirect evidences that pyogenic liver abscesses developed from the bacteria actually transmigrated from inflamed gut.

Direct evidences of bacterial translocation from inflamed organ was observed in clinical and experimental studies. Recently in one study, blood samples from superior mesenteric vein in acute appendicitis showed bacteria in 38% of patients [19]. Similarly, it has also been observed in experimental study done on Gonobiotic mouse model, that showed micro-organism moving from gut into lymphatics [20]. These finding suggest that bacteria may transmigrate and produce portal bacteraemia, hepatocellular dysfunction or pyogenic liver abscess. The hepatocellular dysfunction in majority of the cases cannot be explained only with the 38% of positive culture of bacteria from superior mesenteric blood. So there may be role of some other substances in the development of the disease.

The role of other substances have been demonstrated very recently in five experimental studies on rats. It has been shown that the liver dysfunction is caused by cytokines released from gut due to injury and/or inflammation. In this study, rats were subjected to intra-abdominal sepsis from caecal ligation and puncture and following observations were made.

- Small intestine is an important source of adrenomedullin release during polymicrobial sepsis [21].
- Norepinephrine-induced hepatocellular dysfunction in early sepsis, mediated by activation of alpha-2 adreno-receptor [22].
- TNF produces hepatocellular dysfunction despite of normal cardiac output and hepatic microcirculation [23].
- Hepatic extraction of indocyanine green is depressed early in sepsis despite increased hepatic blood flow and increase in cardiac output [23].

So, it is concluded that hepatocellular function is depressed during early stage of sepsis despite the increased cardiac output and hepatic blood flow and decrease peripheral resistance. The depression of hepatocellular function in early hyperdynamic stage of sepsis does not appear to be due to reduction

in hepatic perfusion but is associated with elevated levels of circulating pro-inflammatory cytokines such as TNF and interleukin-6. This observation is further duplicated by administration of recombinant murine TNF–alpha at a dose that does not reduce cardiac output and hepatic perfusion but produces hepatocellular dysfunction and increases IL-6 [24]. Thus up-regulation of TNF-alpha and/or IL-6 may be responsible for producing hepatocellular dysfunction during early hyperdynamic stage of sepsis [13].

The present findings can be applied in practical situations in that a patient with a suspected acute appendicitis who is male and has a WBC count > 12 × 10^3 cells/µl should be considered as potentially having acute appendicitis. In addition, a patient with symptoms of acute appendicitis lasting > 48 h, who also has a WBC count > 12 × 10^3 cells/µl and hyperbilirubinaemia > 1 mg/dl should be considered as a potential case of perforated appendicitis.

Conclusion

The present study clearly demonstrated the high specificity and sensitivity which may be used as a marker for early diagnosis of appendicular perforation and its immediate management to prevent all sorts of possible complications of perforation including its fatalities in both the extremes of age.

The message is clearly pronounced that the triad of laboratory tests (Differential Count, Total Leukocyte Count and Total Serum Bilirubin), imaging gadgets like Ultrasonography, Computed tomography and Magnetic Resonance Imaging and lastly and reliably coupled with the clinical evaluation findings would clinch the diagnosis of both uncomplicated and complicated appendicitis.

The present day surgeons should emulate the findings of this piece of work to minimise the morbidity and mortality of acute appendicitis in future by just adding one more laboratory test i.e. total serum bilirubin; though the total serum bilirubin may not be very helpful for diagnosing the catarrhal and non-perforated appendicitis.

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