EVALUATION OF HYPERBILIRUBINEMIA IN PATIENTS WITH APPENDICULAR PATHOLOGY

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ABSTRACT Background: The physiological obstruction of the bile flow associated with appendicular pathology leads to hyperbilirubinemia, which can be used as a predictive factor of appendicular perforation. Objective/Aim: Primary objective of this study was 1. To evaluate hyper-bilirubinemia associated with appendicular pathology. Secondary objectives of this study were 2. To evaluate elevated bilirubin level as an additional diagnostic marker for severity of acute appendicitis. 3. To evaluate whether elevated bilirubin levels have a predictive potential for the diagnosis of appendicular perforation. Methods: The study was conducted in the department of Surgery, Central Referral Hospital, associated with Sikkim Manipal Institute of Medical Sciences, Gangtok. The study was designed as a prospective observational study spanning 12 months, with an enrolment of patients from 12.05.2016 to 11.05.2017. A total of 52 patients with a clinical diagnosis of features of acute appendicitis or appendicular perforation admitted to the surgical ward were included. Results: Patients with appendicular perforation (n=14) were divided based on the location of perforation: 9 (64.3%) had perforation at the apex, 2 (14.3%) at the base and 3 (21.4%) had perforation at the body of appendix. A patient who had perforation at the apex of the appendix had mean total bilirubin of 1.84 mg/dl, 7 (77.8%) of these patients had elevated total bilirubin while an equal number had elevated direct bilirubin. Two (22.2%) had normal total bilirubin and value. Patients with perforation at the base had mean total bilirubin of 1.11 mg/dl and direct bilirubin of 0.56 mg/dl. Though one had elevated total bilirubin, the other had a normal value. On the other hand, both had elevated direct bilirubin. The patients having perforation on the body of the appendix had mean total bilirubin and direct bilirubin of 1.51 mg/dl and 0.57 mg/dl, respectively. Both these patients had elevated total bilirubin and direct bilirubin. It was found that both total and direct bilirubin increased in cases having perforation of the appendix, but these elevations were not statistically significant. Conclusions: Based on the data obtained, the bilirubin levels cannot be used as an indicator for: a) Severity of acute appendicitis. b) appendicular perforation.

KEYWORDS acute appendicitis, appendicular perforation, hyperbilirubinemia

Introduction

Acute appendicitis comes under one of the most frequently encountered causes of acute abdomen 1,2. Experienced clinicians accurately diagnose appendicitis based on a combination of history, physical examination, and laboratory studies about 80% of the time 3. Delay in the diagnosis of Acute Appendicitis leads to perforation and peritonitis and increased mortality. Perforation ranges 50-90% in various series. To supplement the clinical diagnosis and to reduce the frequency of unnecessary appendectomy, the importance of laboratory investigations like white blood cells and C-reactive protein values have been stressed 4.
Ultrasonography as a diagnostic tool for appendicitis has been widely known and studied. However, there is no confirmatory laboratory marker for the preoperative diagnosis of acute appendicitis and appendicular perforation. Recently elevation in serum bilirubin was reported, but the importance of raised total bilirubin has not been stressed in appendicitis. Bacterial invasion in the appendix leads to the transmigration of bacteria and the release of pro-inflammatory cytokines such as tumour necrosis factor-alpha, interleukin-6, and cytokines. These reach the liver via the superior mesenteric vein and may produce inflammation, abscess, or liver dysfunction directly or indirectly by altering the hepatic blood flow.

Considering the above observation, it seemed pertinent to undertake the present study to better define case selection before offering surgery for appendicular pathology.

**Methods**

**Study Design**

The study was designed as a prospective observational study. The patients diagnosed with appendicitis and subsequently undergoing appendectomy in this setting were index cases. The same group was studied before, during and after appendectomy. The Institutional Ethics Committee (IEC) approval was obtained before commencing the study.

**Study Duration**

The study spanned 12 months, with an enrolment of patients from 12.05.2016 to 11.05.2017. A total of 52 patients with clinical diagnoses of appendicitis were studied based on the inclusion and exclusion criteria.

**Inclusion Criteria**

All Patients diagnosed with acute uncomplicated or complicated appendicitis clinically on admission and operation were included in the study.

**Exclusion Criteria**

1. Patients are documented to have a history of jaundice or liver disease.
2. Chronic alcoholism (that is, the intake of alcohol of &gt;40 g/day for men and &gt;20 g/day in women for 10 years.)
3. Haemolytic disease.
4. Acquired or congenital Hepato-biliary disease.
5. Patients with positive viral markers for hepatitis.
6. Patients with other hepato-biliary pathology, including malignancies.

**Date Collection Procedure**

After informed valid verbal consent from the patient, personal details of the patient and his illness were recorded, followed by clinical examination, imaging, and laboratory investigations (haematological, biochemical, imaging, histo-pathological and micro-biological).

In some patients, pre-operative appendicitis was diagnosed by clinical examination using a scoring system like ALVARADO score, Appendicitis inflammatory response score, and ultrasonography.

Intraoperative findings were noted, and post-operatively histopathological reports were collected. The patient was followed for any complications till their return to normal work.

**Data Analysis**

The data collected were tabulated and analysed by SPSS (statistical package for social sciences) software version 23.0 for Windows and Microsoft Excel 2013 with an inbuilt statistical analysis tool. Statistical aggregates like mean (average), median and mode were used to analyse numerical (scale) variables. Frequency distribution was used for non-numerical (nominal and ordinal) variables. Attempts were made to graphically represent the results as far as possible. Appropriate statistical methods were used to determine the differences between various comparisons.

**Results**

All patients presenting to the central referral hospital diagnosed with acute appendicitis and operated for this were included in the study and divided into three groups based on the final histopathological findings.

Group 1 consisted of all patients with normal appendix (N=5).
Group 2 consisted of uncomplicated acute appendicitis (N= 28) and
Group 3 consists of complicated acute appendicitis (GA+PA, N=19). This division has been followed throughout the study.

The mean age of patients in group 1 was 30.8 years, group 2 was 28.9 years, while in group 3, it was 26.7 years. None of these differences was statistically significant.

**Total Bilirubin**

Of the five patients in group 1, 3 patients (60%) had normal total bilirubin, and 2 patients (40%) had elevated total bilirubin. The mean total bilirubin was found to be 0.72 mg/dl. Group 2 had mean total bilirubin of 1.40 mg/dl, of which 14 (50%) had normal total bilirubin, and 14 patients (50%) had elevated total bilirubin. The mean total bilirubin of group 3 was 1.6 mg/dl in these 7 patients (36.8%) with normal total bilirubin and 12 (63.2%) patients with elevated total bilirubin.

**Direct Bilirubin**

Group 1 had mean direct bilirubin of 0.25 mg/dl. In these, 3 (60%) had normal direct bilirubin while 2 (40%) had elevated direct bilirubin.

Group 2 was having mean direct bilirubin of 0.48 mg/dl. Out of these, 6 (21.5%) had normal direct bilirubin, while 22 patients (78.5%) had elevated direct bilirubin. While group 3 were found with mean direct bilirubin of 0.63 mg/dl, out of these, only 1 (5.2%) had normal direct bilirubin. In contrast, 18 (94.8%) had elevated direct bilirubin.

**Comparison of Total bilirubin/Direct bilirubin with Appendicular perforation and its location**

Patients without appendicular perforation (n=38) had mean total bilirubin of 1.31 mg/dl. Of these, 17 patients (44.7%) had elevated total bilirubin, while 21 patients (55.3%) had normal total bilirubin. Mean direct bilirubin was found to be 0.47 mg/dl, of which 30 patients (78.9%) had elevated direct bilirubin, and 8 patients (21.1%) had normal direct bilirubin. Patients with appendicular perforation (n=14) were further divided based on the location of perforation: 9 (64.3%) had perforation at the apex, 2 (14.3%) at the base and 3 (21.4%) had perforation at the body of appendix.

A patient with perforation at the apex of the appendix had mean total bilirubin of 1.84 mg/dl, 7 (77.8%) of these patients had elevated total bilirubin, while an equal number had elevated...
Table 1

| Significance of difference in mean (Student T-test) | P Value |
|---------------------------------------------------|---------|
|                                                   | Total bilirubin | Direct bilirubin |
| Absent vs Perforation                             | 0.149    | 0.088   |
| Absent vs apex                                    | 0.053    | 0.081   |
| Absent vs base                                    | 0.728    | 0.692   |
| Absent vs body                                    | 0.667    | 0.578   |
| Apex vs base                                      | 0.306    | 0.691   |
| Apex vs body                                      | 0.552    | 0.652   |
| Base vs body                                      | 0.138    | 0.911   |

Table 2

| MANTRELS Out of 10 | Total bilirubin (No. of patients) | Mean Total bilirubin (mg/dl) | Direct bilirubin (No. of patients) | Mean direct bilirubin (mg/dl) |
|--------------------|-----------------------------------|-----------------------------|-----------------------------------|-------------------------------|
|                    | Normal | Raised | Normal | Raised | Normal | Raised | Normal | Raised | Normal | Raised |
| <7                 | 12     | 7       | 1.32   | 6       | 13     | 0.79   |
| ≥7                 | 12     | 21      | 1.44   | 4       | 29     | 0.80   |

Table 3

| Author               | Year | Place            | Mean Total bilirubin | Min Total bilirubin | Max Total bilirubin | P value   |
|----------------------|------|------------------|----------------------|---------------------|---------------------|-----------|
| Y.R. Hong et al.      | 2012 | Korea            | 0.85                 | 0.50                | 4.3                 | <0.0001   |
| Veerabhada et al.     | 2017 | Bengaluru        | 1.6                  | 0.8                 | 2.4                 | <0.05     |
| P. Chaudhary et al.   | 2013 | RML, Delhi       | 1.9                  | 0.6                 | 5.0                 | <0.05     |
| Sand et al           | 2009 | Holland          | NA                   | NA                  | NA                  | <0.05     |
| Estrada et al        | 2007 | Los Angeles      | NA                   | NA                  | NA                  | =0.031    |
| Current Study        | 2017 | CRH, Gangtok     | 1.4                  | 0.18                | 3.84                | >0.05     |

Table 4

| Age group (years)   | Male | Female | Percentage |
|---------------------|------|--------|------------|
| 1-15                | 9    | 3      | 23.1       |
| 16-30               | 10   | 8      | 34.6       |
| 31-45               | 6    | 9      | 28.8       |
| 46-60               | 1    | 4      | 9.6        |
| More than 60 years  | 2    | 0      | 3.8        |

Table 5

| Histopathology | Total Bilirubin (No. of patients) | Mean total bilirubin (mg/dl) |
|----------------|----------------------------------|-----------------------------|
|                | Normal | Raised | Normal | Raised | Normal | Raised |
| Group 1        | 3      | 2       | 0.72    |
| Group 2        | 14     | 14      | 1.40    |
| Group 3        | 7      | 12      | 1.58    |
Table 6

| Significance of difference in mean of total bilirubin (Student T-test) |
|---------------------------------------------------------------|
| Group 1 Vs group 2 | Group 1 Vs group 3 | Group 2 Vs group 3 |
| P Value | 0.87 | 0.022 | 0.442 |

Table 7

| Histopathology | Direct bilirubin (Number of patients) | Mean direct bilirubin (mg/dl) |
|----------------|--------------------------------------|-------------------------------|
|                | <0.2 | >0.2 |                               |
| Group 1        | 3    | 2    | 0.26                          |
| Group 2        | 6    | 22   | 0.48                          |
| Group 3        | 1    | 18   | 0.63                          |

Table 8

| Significance of difference in mean of direct bilirubin (Student T-test) |
|---------------------------------------------------------------|
| Group 1 Vs group 2 | Group 1 Vs group 3 | Group 2 Vs group 3 |
| P Value | 0.205 | 0.018 | 0.154 |

Table 9

| Perforation | Total Bilirubin (No. of patients) | Mean total bilirubin (mg/dl) | Direct bilirubin (No. of patients) | Mean Direct bilirubin (mg/dl) |
|-------------|----------------------------------|-----------------------------|-----------------------------------|------------------------------|
|             | Normal | Raised | Normal | Raised | Normal | Raised | 0.65 |
| Absent      | 21     | 17     | 1.31   |        | 8      | 30     |     |
| Apex        | 2      | 7      | 1.84   | 1.67   | 2      | 7      | 0.70 |
| Base        | 1      | 1      | 1.11   | 1.67   | 0      | 2      | 0.56 |
| Body        | 0      | 3      | 1.51   | 1.67   | 0      | 3      | 0.57 |

Table 10

| Author | Year | Place          | Mean | Min | Max | P Value |
|--------|------|----------------|------|-----|-----|---------|
| P. Chaudhary et al. | 2013 | RML, New Delhi | 0.74 | 0.1 | 2.1 | <0.05  |
| Veerabhadra et al. | 2017 | Bengaluru     | 1.1  | 0.4 | 1.8 | <0.05  |
| Dipen Patel et al. | 2013 | S.S.G. Hospital, Baroda | 0.7 | 0.4 | 1.0 | NA     |
| Current Study     | 2017 | CRH, Gangtok  | 0.52 | 0.1 | 1.82| >0.05  |

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direct bilirubin. Two (22.2%) had normal total bilirubin and direct bilirubin value. Patients with perforation at the base had mean total bilirubin of 1.11 mg/dl and direct bilirubin of 0.56 mg/dl. Though one had elevated total bilirubin, the other had a normal value. On the other hand, both had elevated direct bilirubin.

The patients having perforation on the body of the appendix had mean total bilirubin and direct bilirubin of 1.51 mg/dl and 0.57 mg/dl, respectively. Both these patients had elevated total bilirubin and direct bilirubin. It was found that both total and direct bilirubin increased in cases having perforation of the appendix, but these elevations were not statistically significant. On further scrutinizing, it was evident that both total bilirubin and direct bilirubin increased as the perforation location changed from base to body to apex. Still, the statistical analysis failed to demonstrate any signs of the observed difference.

Nevertheless, a crosstab comparison between direct bilirubin and the site of perforation of the appendix revealed that direct bilirubin value rises in patients with perforation at the apex. This was found to be statistically significant (P value of 0.020, Chi-square test). Thus, it can be concluded that perforation at the apex leads to a rise in direct bilirubin. On applying independent samples Mann Whitney U test between direct bilirubin and different categories of perforation, it was found that direct bilirubin distribution is not the same across the different categories of perforation. (P value=0.040, Mann-Whitney U test)

**MANTRELS Score**

Based on the MANTRELS Score, patients were divided as MANTRELS score ≥ 7 (n= 33, 63.5%), MANTRELS score < 7 (n=19, 36.5%). The overall mean of the MANTRELS score was 7.1

**Comparison of MANTRELS score and Total bilirubin/Direct bilirubin**

Patients with MANTRELS score ≥ 7 had mean total bilirubin of 1.44 mg/dl. 21 patients (63.6%) had elevated total bilirubin. In comparison, 12 patients (36.4%) had normal total bilirubin, and the mean direct bilirubin was found to be 0.80 mg/dl; out of these, 29 patients (36.4%) had elevated direct bilirubin while 4 patients (12.1%) had normal direct bilirubin.

Patients with a MANTRELS score < 7 had mean total bilirubin of 1.32 mg/dl and direct bilirubin of 0.79 mg/dl. When total bilirubin was compared, 7 (36.8%) patients had elevated total bilirubin, while 12 (63.2%) had a normal range of total bilirubin. For direct bilirubin, 13 patients (68.4%) had elevated direct bilirubin, while 6 (31.6%) had normal direct bilirubin values.

The difference in mean total bilirubin and direct bilirubin was statistically insignificant.

Furthermore, the correlation coefficient between MANTRELS score Vs total bilirubin and MANTRELS score Vs direct bilirubin was low at 0.125 and 0.037 with no statistical significance.

**Discussion**

In this study, we compared the total bilirubin levels between patients with normal appendix and those with appendicitis and between patients with a normal appendix and those with complicated appendicitis.

Analysis of the data obtained revealed that the total bilirubin rises in all patients of appendicitis irrespective of the presence or absence of complications (Normal vs. Appendicitis: P value =0.039, Student T-test; Normal vs. Complicated appendicitis: P value = 0.022, Student T-test).

No such comparison was evaluated in the previous studies mentioned below. These studies evaluated the significance of the rise in total bilirubin levels in uncomplicated appendicitis. The values in complicated appendicitis were found to be significant. However, our study does not corroborate this, as no significant difference was found between the rise in total bilirubin levels between uncomplicated and complicated appendicitis (P value = 0.442, Student T test).

While comparing direct bilirubin with a histopathological examination, the minimum, maximum and mean of direct bilirubin in the current study was comparable with the mentioned previous studies. When the site of perforation was compared with the total bilirubin and direct bilirubin levels, it was found that the direct bilirubin value rises in patients with a perforated appendix irrespective of the site of perforation. A crosstab analysis between the direct bilirubin levels and the site of perforation revealed that the rise in direct bilirubin levels was statistically significant only in patients who were found to have a perforation at the apex of the appendix when compared with a perforation at other sites (P value = 0.020, Chi-square test). Hence a rise in direct bilirubin levels can be used as an indicator of perforation at the apex of the appendix.

On applying Independent samples Mann Whitney U test between direct bilirubin and different categories of perforation, it was found that the distribution of direct bilirubin is not the same across the different categories of perforation. (P value=0.040, Mann-Whitney U test)

While reviewing available literature, no other studies were found where Intra-operative findings during appendectomy were compared with Total or Direct bilirubin.

When the difference in mean direct bilirubin of the patients with uncomplicated and complicated appendicitis was compared, it was found to be significant in other studies, unlike in the current study, which was not significant.

However, it was statistically significant when normal appendix compared with complicated appendicitis indicating that a rise in direct bilirubin in patients with suspected appendicitis points toward the presence of complications. (P=0.018, Student T-test)

On applying independent samples Kruskal-Wallis test between direct bilirubin and histopathological examination, it was found that the distribution of direct bilirubin is not the same across categories of histopathological examination with statistical significance. (P=0.006, independent samples Kruskal-Wallis test)

**Conclusion**

The following conclusions can be drawn from this study:

- The primary objective of this study is to establish a correlation between high bilirubin levels and appendicular pathology.
  - Direct bilirubin levels were found to be elevated in patients with complicated appendicitis.
  - Total bilirubin levels rise in all cases of appendicitis irrespective of a complex pathology.
- The secondary objective of this study was also fulfilled. Based on the data obtained, the bilirubin levels cannot be used as an indicator for a) Severity of acute appendicitis or b) appendicular perforation.
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Declarations

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Nil.

Conflict of interest
There are no conflicts of interest.

Ethical approval
SMIMS INSTITUTION ETHICS COMMITTEE (Ref No: SMIMS/IEC/C/414/16-022)

References
1. O’Connell PR, “The Vermiform Appendix “. In: Williams NS, Bulstrode CJK, O’Connell PR(Ed.) Bailey and Love’s-Short practice of surgery, 25 ed. London: Arnold: 2008; p. 1204-1208

2. Smink DS, Soybel DI, “Appendix and Appendectomy” . In: Zinner MJ, Stanley W (eds) Maingot’s abdominal operations, 11th ed. Ashley: Mcgraw Hill; 2007. P. 589612.

3. John Maa. “The Appendix.” In Townsend CM, Beauchamp RD, EversBM, Mattox KL, eds. Sabiston Textbook of surgery, 18th edition, Philadelphia, Pa: Saunders Elsevier; 2008. P. 1333-1347

4. Gronroos JM, Gronroos P. A Fertile-aged woman with right lower abdominal pain but unelevated leucocyte count and C- Reactive protein: acute appendicitis is very unlikely . Langenbecks Arch surgery 1999; 384: 437-440.

5. Jeffrey RB, Laing FC, Lewis FR. Acute Appendicitis: High Resolution real-time US findings, Radiology 1987; 163: 11-4.

6. Khan S. Evaluation of Hyperbilirubinemia in acute inflammation of Appendix: A Prospective study of 45 cases. KUMJ 2006; 4(5) 15:281-9.

7. Hong YR, Chung C-W, Kim JW, et al. Hyperbilirubinemia Is a Significant Indicator for the Severity of Acute Appendicitis. Journal of the Korean Society of Coloproctology. 2012,28(5):247-252. doi:10.3393/jksc.2012.28.5.247

8. Radha krishna, Veerabhadraratil, S. M.; Patil, Rajeshkar S. Evaluation of hyperbilirubinemia as an innovative diagnostic marker for acute appendicitis and its role in the prediction of appendicular perforation.International Surgery Journal, [S.l.], v. 4, n. 5, p. 1662-1666, apr. 2017. ISSN 2349-2902

9. Chaudhary P, Kumar A, Saxena N, Biswal UC. Hyperbilirubinemia as a predictor of gangrenous/perforated appendicitis: a prospective study. Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology. 2013,26(4):325-331.