ORIGINAL ARTICLE

The Value of Inflammatory Markers in Acute Appendicitis: A diagnostic accuracy study

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ABSTRACT

Acute appendicitis (AA) is a common surgical problem that is associated with acute abdomen. Many studies have investigated the role of white blood cell count (WBCC), C- reactive protein (CRP) and serum amylase (S. amylase) as well; but still with conflicting results. The aim of this study was to assess the sensitivity, specificity, accuracy, and positive predictive value of the serum markers in the diagnosis of AA. In this cross sectional prospective study, a total of 300 patients who had appendicectomy were included. The diagnosis of AA was based on the history and clinical examinations. Two samples of blood were sent for WBCC, CRP and for S. amylase before the surgery and the results being compared with the operative findings and the histopathological features of the removed appendix. The sensitivity, specificity, accuracy, and positive predictive value for WBCC in this study were 90.11%, 48.64%, 85% and 92.57% respectively, for CRP were 88.21%, 70.27%, 86% and 95.47% respectively, for S. amylase were 4.94%, 97.29%, 16.33% and 92.85% respectively, for (WBCC+ CRP) were 89.47%; 72.72%; 87.5% and 96.08 % respectively and for the combination of all three markers (WBCC+ CRP+ S. amylase) were 89.28%, 85.71%, 89.18% and 99.55% respectively. WBCC, CRP and S. amylase can be helpful in the diagnosis of doubtful cases of right iliac fossa pain and when measured together they increase their diagnostic value (positive predictive value).

Keywords: Acute appendicitis, white blood cell count, C - reactive protein, serum amylase.

INTRODUCTION

Acute appendicitis (AA) is a frequent reason for emergency hospital admission and appendicectomy is one of the most common emergency procedures performed in contemporary medicine[1,2]. The lifetime risk of developing appendicitis is 8.6% for male and 6.7% for female; with the highest incidence in the 2nd and the 3rd decade[3,4]. The rate of appendicectomy for appendicitis has been decreasing since the 1950s in most countries perhaps due to increased use of diagnostic imaging which has led to a higher detection rate of mild appendicitis that would otherwise resolve undetected[5,6]. The classical features for AA begin with poorly localized colicky abdominal pain first noticed in the periumbilical area followed by anorexia and nausea, then the pain becomes localized to the right lower quadrant as the inflammatory process progresses to involve parietal peritoneum overlying the appendix[7]. Patients with AA typically look ill and are lying still in bed with a low-grade fever and the examinations reveal diminished bowel sounds and local tenderness with voluntary guarding[8]. The differential diagnosis of AA can include almost all causes of abdominal pain. The diagnosis of appendicitis can be very difficult for very young children and elderly, therefore the diagnosis in those groups is most often delayed and perforation occurs most frequently[8]. In spite of various investigations used to improve the accuracy of the diagnosis; the rate of normal appendices removed can be as high as 15 – 30%[9]. Despite the extraordinary advances in modern radiographic imaging techniques and diagnostic laboratory investigations; the diagnosis of acute appendicitis remains essentially clinical requiring a mixture of observation and clinical acumen and surgical science[10]. The drawback of these techniques is involvement of additional cost and lack of free availability; therefore these modalities have not gained wide acceptance as routine diagnostic investigations of acute appendicitis [11]. White blood cell count (WBCC) and C– reactive protein (CRP) may be regularly measured in patients with suspected appendicitis and may improve the accuracy of the diagnosis of AA[12]. Serum amylase (S. amylase) as a marker may play a complex role in the diagnosis of AA[13]. WBC form a part of the body defense mechanism and mild leukocytosis ranging from 10,000 to 18.000 cell/mm usually present in patients with acute uncomplicated appendicitis and often is accompanied by moderate polymorph nuclear cells predominance[14]. However, it is unusual for WBCC to be more than 18,000 cell/mm3 in an uncomplicated appendicitis. WBCC above this level

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raises the possibility of a perforated appendix with or without abscess[6]. CRP is a protein found in the blood and its level rise in response to inflammation. It`s physiological role to bind to phosphocholine expressed on the surface of the dead or dying cell and some type of bacteria in order to activate the complement system[15]. CRP is synthesized by the liver in response to factors released by fat cell (adipocytes) and within few hours after exposure to an acute inflammatory stimulus, there is a sharp increase in its serum concentration[16,17]. The doubling time of the serum CRP is 4-11 hours and peak levels occur at 2-3 days[18]. Amylase is an enzyme that catalyzes the hydrolysis of starch into sugar and present in the saliva of human and some other mammals where it begins the chemical process of digestion[19]. The pancreas is regarded as the main gland to produce amylase[19,20]. There are many types of drugs may affect S. amylase level such as asparagine, aspirin, birth control pills, cholinergic medications, ethacrynic acid, methyldopa, opiates and thiazides diuretics[20]. The normal range of S. amylase is 23 – 85 u/l and some laboratories give the range between 40- 140 u/l[19,20]. Increase in S. amylase can occur in certain diseases such as acute pancreatitis, cholecystitis, severe gastroenteritis, infection of salivary gland, intestinal obstruction, pancreatic duct or bile obstruction, perforated ulcer, tubal pregnancy and certain cancers[19,20]. Whereas S. amylase may decrease in pancreatic cancer, damage of pancreas, and toxemia of pregnancy[19,20].

The aim of this study was to find the sensitivity, specificity, accuracy, and positive predictive value of these serum markers in the diagnosis of AA.

PATIENTS AND METHODS

A cross sectional prospective study was performed at Al-kindy teaching hospital in Baghdad city between July 2014 and February 2016. A total of 300 patients who had appendicectomy by the working surgical teams were included in the study. The inclusion criteria include all the patients who diagnosed as acute appendicitis on basis of presenting symptoms and signs and then had appendicectomy aged between 5 and 60 years in both genders. The exclusion criteria include patients with appendicular mass, pregnant women, and patients with concomitant other diseases like diabetes mellitus, liver disease or chest infections. Routine tests including complete blood pictures, blood sugar, general urine examinations, chest X-ray, and ultrasonography were performed preoperatively in selected cases. Once the diagnosis of AA was made by history and clinical examinations bases and surgery was indicated, informed consent was obtained from all the patients and or their parents and the blood samples were collected at the time of venous line insertion and before administration of antibiotics and then sent for WBCC, CRP and S. amylase checking. A special format is completed as a database. Postoperatively, the removed appendix was sent for histopathological examination. Based on operative finding and histopathological features of the removed appendices, the patients were divided into 3 groups as follows: Group A: normal appendix, Group B: inflamed appendix (noncomplicated appendicitis) and Group C: complicated appendicitis (perforated or gangrenous appendix). The number of patients was calculated in each of the three groups according to the result of their WBCC, CRP and S. amylase as following: normal WBCC, raised WBCC, normal CRP, raised CRP, normal S. amylase level, raised S. amylase level, normal all values (WBCC, CRP and S. amylase), raised all values, normal WBCC + CRP and raised WBCC+ CRP. The sensitivity, specificity, accuracy and positive predictive value of these tests were calculated according to the following formulas:
Sensitivity = True Positives / True positives + False Negatives. Specificity= True Negatives/ True Negatives + False Positives. Positive predictive Value= True Positives/ True Positives + False Positives. Accuracy =True positives +True Negatives / True positives + True Negative + False positives + False Negatives. Chi-square test was used for statistical analysis; the software was Minitab version 13 with the assumption of statistical significance below 0.05. The cut-off Value for WBCC was taken as 11,000 cells /ml this value was selected arbitrarily as it corresponds to the elevated WBCC regardless of the sex and age of the patient. The Latex agglutination slide test was standard for the quantitative and semi-quantitative detection of CRP in sample serum amylase measurement utilizes (CNPG3) as a substrate this substrate reacts directly with alpha–amylase and doesn't require the presence of ancillary enzymes, the release of (CNP). From the substrate and the resulting absorbance increase per minute is directly related to the (alpha –amylase) activity in the sample, the resulting increase in the absorbance can be measured spectrophotometrically at 410/480 nm. The normal value of S. amylase is (29- 103) U/L.

RESULTS

A total of 300 patients; who had undergone appendicectomy were included in this study, 185(61.6%) were males and 115(38.3%) were females, the male to female ratio being (1.6:1) and the age range was from 5- 60 years. According to the operative finding and histopathology finding of the

removed appendix; the results were as following: 37 patients (12.3%) had normal appendices and classified as group A. 213 patients (71%) had inflamed appendices and classified as group B. 50 patients (16.66%) had complicated appendices and classified as group C. The overall negative appendicectomy rate was 12.3%. Final diagnosis in group A was made due to another pathology such as a complicated ovarian cyst, mesenteric adenitis, and Mickle's diverticulum.

Table 1 shows the results of WBCC, CRP and S. amylase in each of the three groups. Among the 37 patients with normal appendix (group A), 19 patients (51.35%) had raised WBCC regardless of CRP and S. amylase results, and 11 patients (29.72%) had positive CRP level regardless of WBCC and S. amylase, and only one patient (2.7%) had elevated serum amylase regardless of WBCC and CRP. When the three markers were combined, the results were one patient (2.7%) had the 3 markers raised, and 6 patients (16.21%) had normal values of three markers. When WBCC & CRP combined, the results were 9(24.3%) had raised both markers and 24(64.86%) had normal markers.

Among the 213 patients with inflamed appendix (group B), 189 patients (88.73%) had raised WBCC regardless of CRP and S. amylase, and 187 patients (87.79%) had positive CRP regardless WBCC and S. amylase, and only 6 patients (2.81%) had elevated S. amylase level regardless WBCC and CRP. When the three markers were combined, the results were 182 patients (85.44%) had all three values raised and 27 patients (12.67%) had normal values of all markers. When the WBCC and CRP were combined, the results were 180 patient (84.5%) had raised both markers, and 26 patients (12.20%) had normal both markers.

Among the 50 patients with complicated appendicitis (group C), 48 patients (96%) had raised WBCC regardless CRP and S. amylase, and 45 patients (90%) had positive CRP regardless WBCC & S. amylase and 7 patients had elevated S. amylase (14%) regardless WBCC and CRP. When the three parameters are combined, the result was 43 patients (86%) had all three values raised, and no patient had a normal value of all parameters. When WBCC and CRP combined, the results were 41 patients (82%) had raised both markers, and no patient had normal both markers.

The sensitivity, specificity, accuracy and positive predictive value of each marker in different groups have been analyzed in tables 2 and 3.

Table 1: The number of patients and their results in each group.

	Group A* (37)	**Group B** (213)**	**Group C*** (50)**
WBCC raised	19 (51.35%)	189 (88.73%)	48 (96%)
CRP raised	11 (29.72%)	187 (87.79%)	45 (90%)
S. amylase raised	1 (2.7%)	6 (2.8%)	7 (14%)
All raised	1 (2.7%)	182 (85.44%)	43 (86%)
All Normal	6 (16.21%)	27 (12.67%)	0 (0%)
WBCC & CRP raised	9 (24.3%)	180 (84.5%)	41 (82%)
WBCC & CRP Normal	24 (64.86%)	26 (12.20%)	0 (0%)

*Group A = Patients with Normal Appendix, **Group B = Patients with inflamed appendix, ***Group C = patients with complicated appendix

Table 2: Correlation of the inflammatory markers with histopathological results.

	Group A (37)	**Group B+C (263)**
Normal WBCC	18 (TN)	26 (FN)
Raised WBCC	19 (FP)	237 (TP)
Normal CRP	26 (TN)	31 (FN)
Raised CRP	11 (FP)	232 (TP)
Normal S. amylase	36 (TN)	250 (FN)
Raised S. amylase	1 (FP)	13 (TP)
All Markers normal	6 (TN)	27 (FN)
All markers raised	1 (FP)	225 (TP)
Normal WBCC & CRP	24 (TN)	26 (FN)
Raised WBCC & CRP	9 (FP)	221 (TP)

Table 3: Diagnostic role of inflammatory markers for diagnosing of appendicitis.

	Sensitivity%	**Specificity%**	**Accuracy%**	**Positive predictive value %**
WBC	90.11	48.64	85	92.57
CRP	88.21	70.27	86	95.47
S. amylase	4.94	97.29	16.33	92.85
WBC + CRP	89.47	72.72	87.5	96.08
Three Markers	89.28	85.71	89.18	99.55

Table 4: Comparison of WBCC results.

The results of CRP%	Sensitivity%	Specificity%	Positive predictive value %
Present study	90.11	48.64	92.57
Khan MN	83.3	62.1	92
Nasir Ali	74.4	72.7	90.6

Table 5: Comparison of CRP results.

The results of CRP%	Sensitivity%	Specificity%	Positive predictive value %
Present study	88.21	70.27	95.47
Khan MN	75.6	83.7	96
Nasir Ali	84.6	90.9	97.1

DISCUSSION

Although AA is the most common abdominal surgical emergency, the diagnosis can be extremely difficult at times[7]. We considered WBCC and CRP from others inflammatory markers and S. amylase as an enzyme may be secreted after exposure to an inflammatory stimulus, as a help in the diagnosis of AA because they are helpful markers or parameters and available in the most hospital. In the present study, the overall negative appendicectomy rate was 12.3%, which is slightly lower than the value of most of our references [6-8] explained by the delay in presentation of patients and the clinical diagnosis was straightforward in most of them. The present study showed that the WBCC and CRP were sensitive in the diagnosis of acute appendicitis and CRP is more specific and accurate than WBCC. The study showed that combining WBCC and CRP will increase the specificity, accuracy and positive predictive value. The present study also showed that the S. amylase can increase in cases of acute appendicitis especially if it is of a complicated type or if the appendicular perforation is suspected, so can help (if elevated) in the diagnosis of complicated appendicitis. In this study, CRP diagnostic accuracy was (86%) which was superior to the WBCC accuracy (85%) and to the S. amylase (16.33%) as well. Nasir Ali[11] found CRP accuracy (86%) superior to WBCC accuracy (74%) and this was similar to our study. Burnet & Ness[13] showed mild to moderate elevation of S. amylase in 25% of acute appendicitis. The rise in the level of S. amylase could be due to starch splitting organisms from the infected appendix. The present study found that S. amylase has a poor accuracy (16.33%) in cases of acute appendicitis. Tables 4 and 5 show comparison of results of sensitivity, specificity, and positive predictive value for WBCC and CRP in the present study and the results of Khan MN[21] and the results of Nasir Ali[11]. Khan MN et al had no exclusion criteria and the age range was from 12 to 73 in his study and this explains the difference in the results with our study. Nasir Ali included 50 patients above 12 years of age who were diagnosed as acute appendicitis in his study and this explains the difference in his results with the results of the present study. Afsar et al[22] found the positive predictive value of CRP as 96.7% and suggested that normal CRP level is not associated with acute appendicitis, which is close to our study.

The important thing in our study was the results of combining both markers (WBCC + CRP) together on one hand and combining all the three markers (WBCC+CRP+ S. amylase) together on the other hand. The sensitivity, specificity, accuracy, and positive predictive value of combined (WBCC &CRP) was 89.47%, 72.72%, 87.5% and 96.08% respectively and for combined all markers, the results were 89.28%, 85.71%, 89.18% and 99.55% respectively. Out of 37 patients with negative appendicectomy (group A), there were 9 patients (24.3%) had raised both values (WBCC & CRP) and 24 patients (64.86%) had normal markers values and for all combined markers, there was only one patient (2.7%) had 3 markers elevated. On that basis, we can prevent 24 negative appendicectomies if we had taken into consideration the results of combined (WBCC and CRP) and about 36 negative appendicectomies (disregard other pathologies) if we had taken the results of all three markers in consideration in the diagnosis of acute appendicitis. In group C with complicated appendicitis, we found 43 cases out of 50 patients (86%) had raised all markers, so we assume that this results can give a good clue to the development of complication in acute appendicitis when all markers are raised. Regarding S. amylase value as a marker in the diagnosis of acute appendicitis, the results of this study revealed that S. amylase is not sensitive and not accurate in the diagnosis of AA but with high specificity and positive predictive value. This means that if S. amylase is not elevated in a patient with suspected AA, it is of no significance, but if it is being elevated it suggest a complicated appendicitis. Burnett & Ness[13] suggest if the level of S. amylase is more than 1000 unit / 100 ml it is diagnostic of acute pancreatitis, the level between (600-1000) unit/100 ml clinical examination and other sophisticated investigation are essential to differentiate perforated

peptic ulcer from acute pancreatitis, mild elevation in S. amylase can occur in other acute abdominal condition like appendicitis. The positive predictive value of 2 markers (WBCC & CRP) combined was 96.08% while when all the 3 markers (WBCC, CRP & S. amylase) combined was 99.55% which indicates that S. amylase is helpful in the diagnosis of AA.

This study sends two crucial massages in the management of AA. The first message is that if all the three markers (WBCC, CRP & S. amylase) levels were normal in a patient with suspicion of AA; the presence inflamed appendix is unlikely and re-evaluation of the patient over a period of time is perhaps a better option than proceeding to operation. The second message is that the measurement of CRP & S. amylase as inflammatory markers in cases of suspected AA is crucial and it's better not to depend on the evaluation with WBCC alone, the combination of all inflammatory markers will reduce the incidence of negative appendicectomy.

CONCLUSIONS

White blood cell count; C– Reactive protein and S. amylase can be helpful in the diagnosis of doubtful cases of right iliac fossa pain and when measured together they increase their diagnostic value (positive predictive value). S. amylase can also serve as an indicator of complicated or perforated appendicitis. Elevated S. amylase can lead to miss diagnosis or confusion with other causes of hyperamylasemia. Abdominal pain with elevated S. amylase can refer to significant intra-abdominal pathology not specifically pancreatic diseases.

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