Investigating the Diagnostic Value of Serum Calprotectin Level in Patients With Acute Appendicitis

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Abstract- Appendicitis is one of the main causes of acute abdominal surgery; however, the accurate diagnosis of appendicitis has remained uncertain. This study aimed to investigate the serum calprotectin as a diagnostic indicator for acute appendicitis. This prospective study was conducted on 79 patients suspected of acute appendicitis who underwent an appendectomy and 70 healthy volunteers. The correlation of serum calprotectin level and histopathological results was investigated. Screening performance characteristics of calprotectin (CP) were calculated on patients suspected with acute appendicitis. The mean serum calprotectin level in the patients was 0.791±0.148 mg/dl with a minimum of 0.567 mg/dl and a maximum of 1.26 mg/dl. The serum calprotectin ranged from 0.10 mg/dl to 0.50 mg/l in the healthy group. The AUC of CP was 0.58 (95% CI: 0.43-0.73). At a 0.72 mg/dl cutoff value, CP had 70% (95% CI: 58-82) sensitivity and 50% (95% CI: 39-61) specificity. According to the main finding of our study, the accuracy and sensitivity of serum CP in the detection of patients with acute appendicitis is good, and it seems that it can be used beside clinical symptoms for the diagnosis of acute appendicitis.

Introduction

Acute appendicitis is the most common cause of the right lower quadrant abdominal pain and the most frequent acute surgical abdomen that requires urgent surgery (1). The mortality rate of this disease is 0.3%, which can increase up to 6.5% in perforated cases, 5.5% in the elderly, and 80% in neonates. Advanced bacterial peritonitis following the rupture of inflamed appendicitis can increase the mortality up to 80 to 100%, which emphasizes the potential vital risk of this frequent disease (2).

The risk of rupture in the first 24 hours of symptoms onset can be ignored. This risk will increase by up to 6% within 36 hours. Due to the complications associated with appendiceal perforation, its accurate and early diagnosis is important since, besides the complications of appendiceal perforation, the removal of normal appendices has an ethic, economic and legal limitations (3).

Clinical examinations and laboratory tests are commonly used in the diagnosis of appendicitis; the use of other techniques such as radiological methods have limitations (3). One of these techniques is a computed tomography (CT) scan, which has high sensitivity and specificity in the diagnosis of the disease and a decrease of negative appendectomy. The restriction of this method is the exposure to X-ray (4). Moreover, this method and other radiological techniques are not always available in emergency units or clinical institutions in developing countries (3).

Thuijls *et al*., showed that the ultra-sonography performed by a professional person could improve the diagnosis to some extent, but it cannot be used for the primary diagnosis because it requires a significant dilatation of the appendix. Particularly, in adults due to the presence of intra-abdominal fat and gastrointestinal gas, the detection of appendicitis is very difficult (5).

On the other hand, as the delay in the diagnosis and treatment can increase the perforation rate and consequently can cause morbidity and mortality, most surgeons prefer to perform appendectomy at the early

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stages. As a result, the frequency of normal appendices among patients suspected of acute appendicitis who undergo diagnostic laparotomy is around 5% to 40%. Though, the frequency of perforated appendicitis among patients suspected of acute appendicitis to get surgical treatment is around 5% to 30% (5,6). So, any mistake in the diagnosis of appendicitis can lead to the removal of the normal appendix, and any delay in the diagnosis of appendicitis can cause perforation of appendix and peritonitis (5,7). On the other hand, the current criteria are associated with a high rate of negative appendectomy; thereby, the use of serum factors such as calprotectin (CP) for the diagnosis of this disease has received more attention nowadays.

CP is a heterodimer protein that is formed from the connection of two cytosolic proteins MRP8 and MRP14, and it consists of 60% of proteins dissolved in neutrophils. As soon as neutrophils are activated, it is released, which has antibacterial and cytokine-like effects (8). Immediately after neutrophil activation, e.g., in patients with inflammatory bowel disease, neutrophilic CP is released, which can be quickly identified in the feces (7-10). As this test is easy and inexpensive, it is possible to use it together with other tests if other studies also support such results (5).

In this study, we investigated the diagnostic value of CP in patients with acute appendicitis

Materials and Methods

This diagnostic accuracy study was conducted on patients with acute abdominal pain who were referred to the emergency department of Imam Reza Hospital of Mashhad from October 2017 to October 2018. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences and written informed consent was taken from each patient

Participant

All patients clinically suspected for acute appendicitis with a high likelihood (Alvardo Score ≥7) were included in this study (11). Patients who were pregnant, had a history of systemic or gastrointestinal disease (such as diabetes, hypertension, cancer, sickle cell anemia, IBD and etc.) or had a concurrent infectious disease and had a history of recent abdominal trauma were excluded from the study. Patients with a history of chronic consumption of steroidal and non-steroidal anti-inflammatory drugs and those patients who had consumed antibiotics before hospitalization were also excluded. Finally, after the aforementioned consideration, 79 patients were included

in this study. We also included 70 healthy volunteers (matched for sex and age with the cases) as controls.

Procedure Suspected patients were primarily detected at the emergency triage unit, and then was examined by a surgeon. Alvarado score (12) was recorded for all patients, and an appendectomy was carried based on the routine surgery department protocol. Blood samples for laboratory tests were collected from all patients on admission before appendectomy. Serum samples were sent to the laboratory for measuring serum CP level using CP Human, ELISA kit.

After an appendectomy, a histopathological study was conducted on all samples by a pathologist unaware of biomarkers. The results were categorized as G1 (cases without any histopathologic findings suggestive of appendicitis), G2 (reactive follicular hyperplasia or chronic appendicitis), G3 (cases with histopathologic signs of acute appendicitis with intact appendical mucosa and a mild to moderate infiltration of the inflammatory cells), G4 (macroscopic or microscopic findings of perforation or perforated mucosa accompanied by a strong pan-mural infiltration) and G5 (necrotizing appendicitis).

Reference test

The surgical histopathology finding was used as the confirmative test for the diagnosis of acute appendicitis.

Data collection

Demographic information (age, sex), duration of symptoms and systemic inflammatory response syndrome (SIRS) criteria (temperature, heart rate, respiratory rate), white blood cell count (WBC), CP serum level, patients' Alvarado score, as well as the results of surgical histopathology of the appendix were recorded for all participants using a pre-design checklist. There were no missing data. All data were collected prospectively by a trained pathology resident.
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Statistical analysis

Bootstrap re-sampling method was carried out to obtain 95% bias-corrected confidence intervals for each area under the ROC curve (AUC) due to the small sample size of our study (13). Youden index was used for the cutoff value of the biomarkers for the acute appendicitis diagnosis (14). Receiver operator characteristic (ROC) curves were drawn to calculate the diagnostic accuracy of the studied CP serum biomarker with a 95% confidence interval (CI). Statistical tests (*Chi*-square, Fisher's exact, logistic regression and independent t-test tests and Mann Whitey U test (nonparametric independent-paired

comparison)) were used for analysis. Differences were assumed significant at a level of *P*<0.05. Statistical analysis was performed using SPSS Ver. 20. software and data were presented as mean±standard deviation or frequency (%). Furthermore, the relationship of variables was assessed by a logistic regression method.

Results

Baseline characteristics Study population included 79 patients suspected of acute appendicitis (43 men and 36 women) and 70 healthy volunteers (40 men and 30 women) as the control group. 19 (24 %) patients were pathologically categorized into the non-acute group, and 61(76%) patients were confirmed as acute appendicitis based on surgical histopathology. The baseline characteristics of studied patients are listed in Table 1. Suspected to AA and control groups were similar regarding mean age (26.32±10.67 vs. 28.97±9.63; *P*=0.115) and male to female ratio (34/27 vs. 34:36; *P*=0.319).

The mean serum CP level in the patients was 0.791±0.148 mg/dl with a minimum of 0.567 mg/dl and a maximum of 1.26 mg/dl. The serum CP ranged from 0.10 mg/dl to 0.50 mg/dl in the healthy group. The differences between the CP level of patients and healthy control

statistically was significant (*P*<0.05. The histopathologic report is summarized in Table 1. Group 4 had the largest sample size (n=47), and Group 5 had the smallest sample size (n=3). None of the cases were included in the G1.

We also recorded the Alvarado score for all the patients; The possibility of appendicitis was considered to be low in 20 patients with Alvardo score of 5-6 and to be highly likely in patients with score ≥7 (59 patients).

Relationships

There was no relationship between serum CP level and Alvarado scores (r=0.07, *P*=0.5), pathology grade (r=0.14, *P*=0.21), WBC count (r=0.19, *P*=0.08), and neutrophil count (r= 0.03, *P*=0.74).

Screening performance characteristics of evaluated parameters

The area under the curve (AUC) of CP were 0.58 (95% CI: 0.43-0.73). At a 0.72 mg/dl cutoff value, CP had 70 (95 % CI: 58-82) sensitivity, 50 (95 % CI: 39-61) specificity, 0.72(95% CI: 0.66-0.79) Positive predictive value, 0.21 (CI 95%:0.17-0.35) negative predictive value, 1.4 (95% CI: 1.12-1.68) positive likelihood ratio, and 0.6 (95% CI: 0.46-0.75) negative likelihood ratio. Table 2 and figure 1 shows the screening performance characteristics of WBC, neutrophil count, and Alvarado score for comparison with CP.

Table1. Baseline Characteristics of patients suspected of acute appendicitis (AA) (n=79)

Baseline Characteristics		Suspected to AA
Age (year) Mean±SD		26.32±10.67
Sex Male/Female ratio		43:36
Temperature (C) Mean±SD		37.22± 0.63
WBC (10^3 mm^3) Mean±SD		13.28±4.1
Neutrophil (%) Mean±SD		76.05±16
Symptoms n (%)	Shifting pain	62 (77.5)
	Nausea-vomit	67 (83.8)
	Anorexia	69 (86.3)
Calprotectin (CP) (µ) Mean±SD		0.791±0.148
Alvarado-score n (%)	<4	1 (1.3)
	5-6	20 (25)
	7-8	26 (32.5)
	9-10	33 (41.3)
Pathology score n (%)	1	-
	2	19
	3	10
	4	47
	5	3

Table 2. Screening performance characteristics of CP (mg/dl), white blood cell count (× 10³ mm3), neutrophil count, and Alvarado score in the detection of patients with acute appendicitis

Variable	Cut off	Sensitivity (95 % CI)	Specificity (95 % CI)	AUC (95 % CI)
Calprotectin (CP)	.72	70 (58 - 82)	50 (39 - 61)	0.58 (0.43-0.73)
WBC count	10	85 (72-98)	45(32-58)	0.70 (0.56-0.83)
Neutrophil	75	72 (60-84)	69(52-86)	0.71 (0.59-0.85)
Alvarado Score	7	95(91-99)	22(16-28)	0.66 (0.53-0.85)

All measures were presented with a 95% confidence interval. AUC: area under the ROC curve
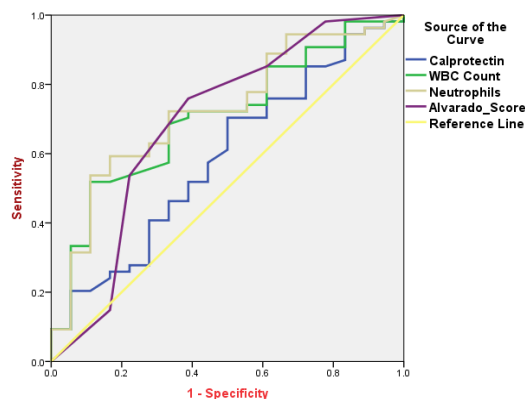

Figure 1. The area under the ROC curve of Calprotectin (CP), white blood cell (WBC) count, neutrophil count, and Alvarado score in the detection of patients with acute appendicitis

Discussion

Acute appendicitis is the most frequent cause of acute surgical abdomen (1), and its diagnosis is based on history and physical examination (15,16).Accurate diagnosis of acute appendicitis has been known as a big challenge in patients with suspected appendicitis. To prevent the morbidity of perforated patients with acute appendicitis, a correct early diagnosis is essential. it also decreases the unnecessary appendectomy for misdiagnosed patients (17). To reach this goal, many clinical investigations have been tried to identify gold biomarkers. The results of previous studies have presented CRP and WBC as the most accurate clinical biomarkers for the diagnosis of acute appendicitis. meanwhile, they do not have this ability to discriminate against all true patients among suspicious subjects (18,19).

In our study, serum CP level was obviously higher in the study group than the control, and CP levels increased with increasing the injury severity. These results are in accordance with other studies (5,20-22). In Kharbanda et al., study, in the control group, individuals with perforated appendicitis had higher levels of serum CP than those with unperforated appendicitis (21). In our research, CP levels increased by the increase in the

severity of pathological groups. Meanwhile, another study found no significant relationship between CP levels and appendicitis (1).

Cikot *et al.,* showed that plasma CP levels are increased in patients with acute appendicitis and may be used to differentiate uncomplicated from complicated acute appendicitis (23). It has been shown that CP may be useful as a marker of inflammatory disease activity and could, therefore, be implicated in the diagnosis and treatment of a variety of inflammatory and other pathological conditions in pediatric patients (24). Ambe *et al*., argue that high activity of CP could be proven within the lumen of appendix specimens following an appendectomy. They demonstrated that in patients with suspected appendicitis, the high luminal accumulation of CP-carrying cells could be used to study the expression of CP in stool as a new diagnostic aid (25). In meta-analysis research, Andersson showed that clinical evaluation of inflammatory markers is helpful in diagnosing appendicitis (26). Currently, the serum factors such as CP have received great attention for appendicitis diagnosis (5,27).

Another study showed that CP level increases in the severe phase of inflammatory response. This increment is sometimes accompanied by the increment of other factors such as C-reactive protein and Erythrocyte sedimentation

rate (27-29). Nevertheless, Jangjoo *et al.*, showed that the measurement of CRP levels is not an ideal diagnostic tool for ruling out or determination of acute appendicitis (30).

We evaluated the correlation of CP with the clinical criterion, including the Alvarado score, WBC, and neutrophil count. Meanwhile, there was no significant relationship between serum CP levels and other clinical parameters (WBC, neutrophil count, and Alvarado score).

Patients with acute appendicitis were separated from non-appendicitis by pathology score. The results of the ROC curve analysis showed that neutrophil count and WBC had the best accuracy tests for diagnosis of acute appendicitis so that the AUC result for the neutrophil count and WBC were 0.71 and 0.7 respectively. Neutrophil count at a cutoff of 75 showed 72% sensitivity and 69% specificity, and the sensitivity and specificity at a defined cutoff of 10×103mm3 were 85% and 45%, respectively. These results are similar to those reported by Thuijls *et al.*, (5). In Kharbanda *et al.*, study, WBC level higher than 8.85×109/L had a specificity of 42% and sensitivity of 100% compared to CP in the diagnosis of appendicitis (21). Whereas in Schellekens *et al.*, study, WBC level higher than 7.5×109/L had a sensitivity of 97% in the diagnosis of acute appendicitis (22). The accuracy of the test for CP was 0.58, and the sensitivity and specificity of CP were 70% and 50%, respectively. AUC shows the accuracy of the test, and AUC of less than 0.5 is not statistically appropriate for the diagnosis of disease. Since the AUC of CP is more than 0.5, the serum level of CP is considered as an appropriate diagnostic factor.

In previous studies, serum CP level had high sensitivity and low specificity in the diagnosis of acute appendicitis; however, the WBC level had much higher specificity compared to CP level for this purpose (21,22). Meanwhile, in the present study, CP sensitivity in the diagnosis of appendices was 70%, whereas sensitivity and specificity of WBC were 85% and 45%, respectively.

In summary, serum CP level significantly increases in patients with acute appendicitis. Based on the finding of the present study, the overall accuracy and sensitivity of serum CP in the detection of patients with acute appendicitis is good, and it seems that it could be considered as a screening tool along with clinical symptoms for the diagnosis of acute appendicitis. Also, performing further researches on a larger statistical population and on other samples (such as luminal secretion) are recommended.

Acknowledgments

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