Original Article
Evaluation of CK-MB as a diagnostic biomarker in acute appendicitis

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Abstract: Objective: Negative appendectomy (normal histopathology) occurs in 5-42% of patients undergoing surgery with a preliminary diagnosis of acute appendicitis. Although accurate anamnesis and physical examination are an essential component of diagnosis, inexpensive, effective, and easily implemented markers of appendicitis are necessary to reduce morbidity associated with this condition. We investigated the use of common laboratory parameters as markers in the diagnosis of acute appendicitis. Materials-methods: The study followed a case-control design. There were 100 patients in the control group and 100 patients with the acute appendicitis in study group. White blood cell count (WBC), bilirubin, mean platelet volume (MPV), and creatine kinase (CK)/creatine kinase MB (CK-MB) values were compared between the two groups. Results: The mean CK-MB value was 18.0 u/l (27.5-47.8) and 15.0 u/l (10.3-19.0) in study and the control groups, respectively. Using an optimal CK-MB cut-off value of 17.5 u/l in ROC analysis, the diagnostic sensitivity was 81% and the specificity was 70%. The diagnostic value of CK-MB to show acute appendicitis was less than the diagnostic value of WBC, which had sensitivity of 93% and specificity of 73% at 9500 u/l cut-off value. Bilirubin had a sensitivity of 79% and specificity of 40% using a cut-off value of 0.5 mg/dl. There was no significant difference between groups in CK and MPV values (P = 0.803 and P = 0.172, respectively). Conclusion: The aim of this study was to evaluate the diagnostic value of CK-MB in the diagnosis of acute appendicitis. We determined that CK-MB can be a valuable diagnostic parameter when used in conjunction with WBC and bilirubin.

Keywords: Acute appendicitis, creatine kinase mb, sensitivity, specificity

Introduction

Acute appendicitis (AA) is among the most common indications for emergency abdominal surgery worldwide [1, 2]. The individual lifetime risk of developing AA is approximately 8% for people living in Western societies [3]. Although AA is usually diagnosed clinically, clinical symptoms and findings are not always typical, establishing a definitive diagnosis can be difficult [4, 5]. The clinical diagnosis ratio of AA is approximately 85% [1].

Immediate and accurate diagnosis is crucial. Unnecessary delays between the onset of symptoms and initiation of surgical treatment increases the risk of appendiceal perforation and associated morbidity and mortality [6]. Perforated AA occurs in 13% to 37% of all cases of appendicitis in adults [7]. On the other hand, the proportion of patients undergoing appendectomy who ultimately are found to have nor-

mal histopathological ranges from 5% to 42%. The morbidity is a significant concern for patients undergo surgery due to inaccurate diagnosis of AA [8, 9].

Advanced imaging methods such as ultrasonography, computed tomography, and magnetic resonance imaging are promising techniques for the diagnosis of AA, but are not available for all patients. Therefore new methods are necessary to distinguish AA and non-specific abdominal pain to reduce the rate of negative appendectomy. Ideal methods for diagnosis of AA are inexpensive, practical and can be administered and interpreted rapidly [10].

Although a careful clinical history, physical examination, and commonly used laboratory parameters such as white blood count (WBC), neutrophil percentage, and C-reactive protein (CRP) value are extremely important for the diagnosis of AA, these techniques are not used

Table 1. Demographic data and laboratory findings of study and control groups

	Study group (AA) (n = 100)	Control group (n = 100)	P
Age (Mean ± SD)	33.6±15.8	36.9±13.9	[1]0.119
Gender (M/F)	55/45	69/31	[2]0.058
	Median (IQR)	Median (IQR)	[3]p
BMI	28.5 (23.0-36.0)	30.5 (23.0-38.0)	0.429
CK (u/l)	99.50 (66.5-156.5)	95.5 (74.0-132.0)	0.803
MPV (fl)	$8.3 (7.2-9.5)×10^3$	$8.1 (6.7-9.2)×10^3$	0.172
WBC (u/l)	$15.6 (12.0-18.2)×10^3$	$7.7 (6.7-8.7)×10^3$	< 0.0001
CK-MB (u/l)	18.0 (27.5-47.8)	15.0 (10.3-19.0)	< 0.0001
Total bilirubin (mg/dl)	0.8 (0.5-1.1)	0.5 (0.4-0.8)	< 0.0001

SD: Standard error, IQR: Interquartile range, [1]Unpaired t test, [2]Fisher's exact test, [3]Mann Whitney test.

routinely. Many biochemical markers of AA have been proposed, although none have received widespread adoption [11, 12].

In this case-control study, WBC, mean platelet volume (MPV), bilirubin, creatinine kinase (CK), and creatine kinase MB (CK-MB) values were compared between a group of patients with confirmed AA and group of patients undergoing elective herniography.

Materials-methods

This study followed a retrospective case-control design. The local ethics committee reviewed and approved all study protocols.

Patients with a preliminary diagnosis of AA who were admitted to our clinic were examined consecutively. Exclusion criteria included: perforated appendicitis, absence of histopathological evidence for AA, age less than 15 years old, presence of chronic diseases such as diabetes and ischemic heart disease. All participants provided informed consent. A total of 100 patients (55 male and 45 female) with a histopathologic diagnosis of AA were included in the study group. The control group included 100 patients (69 male and 31 female) matched in age and gender with no diagnosis of chronic disease, age greater than 15 years old, and who underwent inguinal hernia repair. Demographic findings like age, gender, weight and height were recorded.

Preoperative WBC (u/l), MPV (fl), CK (u/l), CK-MB (u/l), and bilirubin (mg/dl) levels were examined in all study participants. Blood tests were taken 4-36 hours before the surgical procedure and followed at least three hours of fasting.

Statistical analysis

IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) software was used for all statistical analyses of data obtained in this study. Mean and standard deviation are used to represent continuous variables, and categorical variables are reported as number and percentage. Normally distributed variables were assessed using a *t*-test for paired groups and the ANOVA test for tests involving more than two groups. The Kruskal Wallis test was used for between-groups comparisons of non-normally distributed parametric data, and the Mann Whitney U test was used for individual comparisons. Chi-square and Fisher's Exact tests were used for the comparison of qualitative data. Binary logistic regression analysis was conducted using the acute appendicitis condition as a dependent variable and using WBC, MPV, CK, CK-MB, and bilirubin as independent variables. ROC analysis was conducted to find diagnostic cut-off values of significant results. We introduced P ≤ 0.05 for the rejection of null hypothesis. Confidence interval (CI) was established at 95%.

Results

There were 100 patients in study group with histopathological diagnosis of acute appendicitis after appendectomy, and 100 patients in control group. There was no difference between the study and control groups in terms of demographic characteristics (P = 0.119 for age, P = 0.058 for gender). Body mass index (BMI) of patients were calculated and no difference was detected between groups (P = 0.429).

There was no difference between the groups in terms of CK and MVP values (P = 0.803 and P = 0.172, respectively). WBC, CK-MB, and bilirubin values were higher in the study group compared to the control group (P < 0.0001, **Table 1**). After these primary analyses, significant parameters were used for the analysis in logistic regression.

Table 2. Binary Logistic regression analysis

| | B | SE | Wald | P | OR | 95% CI for OR | | Nagelkerke r² (%) |
						Lower	Upper	
WBC	-0.781	0.145	30.634	< 0.0001	0.458	0.347	0.604	86.2
CK-MB	-0.117	0.038	9.632	0.002	0.890	0.827	0.958	
Bilirubin	-2.006	0.888	5.108	0.024	0.134	0.024	0.766	

SE: Standard error, OR: Odds ratio. Independent variables of age, gender, CK, MPV, WBC, CKMB, bilirubin were assessed in multivariate analysis with acute appendicitis condition as dependent variable.
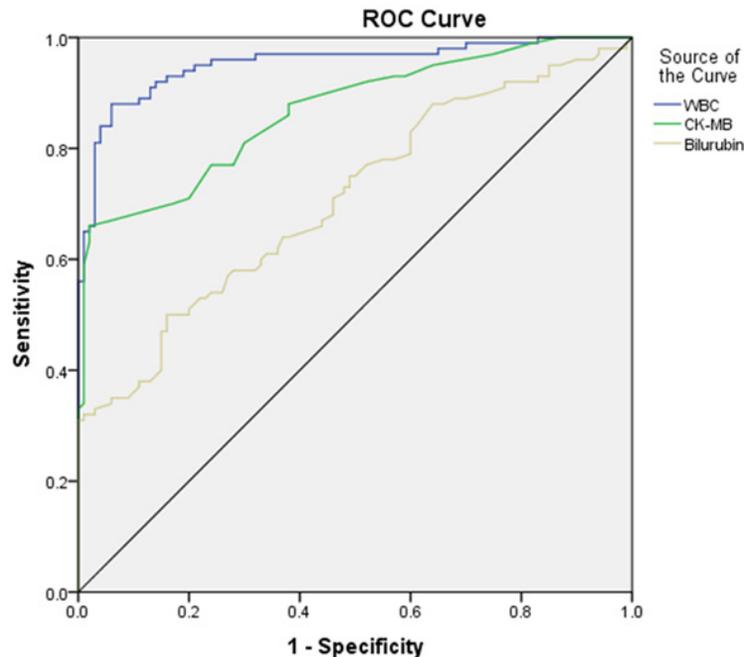
Figure 1. ROC curve. Diagonal segments are produced by ties.

WBC, CK-MB, and Bilirubin were significant predictors of AA in logistic regression analysis (P ≤ 0.0001, 0.002, 0.024, respectively, Nagelkerke r² = 0.862, **Table 2**).

Evaluating cut-off values in ROC analysis, we identified diagnostic cut-off values for WBC, CK-MB, and Bilirubin (**Figure 1**; **Table 3**). The diagnostic cut-off value for WBC was 9.500 u/l; sensitivity was 93% and specificity was 83%. The cut-off value for CK-MB was 17.5 u/l; sensitivity was 81% and specificity was 70%. The cut-off value for bilirubin was 0.51 mg/dl; sensitivity was 79% and specificity was 40%.

Discussion

AA occurs more frequently in males compared to females (1.4:1.0) [13]. Although non-perforated AA can be treated simply using appendectomy, gangrenous or perforated appendici-tis can cause complications that may be life threatening in some cases [14, 15].

WBC and neutrophil counts are widely used by clinicians in daily practice. These markers typically increase in proportion with the severity of inflammation [16, 17]. Birchley *et al.* [18] found that higher WBC values are associated with complicated appendicitis relative to uncomplicated appendicitis. Although WBC typically increases in AA patients, it is not a specific marker as increases in WBC occur in many other inflammatory conditions [19]. Although the sensitivity of WBC is high in the diagnosis of AA, specificity has been reported to be weak [20]. In a meta-analysis reviewing 14 studies and including a total of 3382 patients, WBC of 10.000/mm³ had a sensitivity of 83% and specificity of 67% in the diagnosis of AA [21]. The sensitivity of WBC has been reported to be 67-97.8% and the specificity of WBC has been reported to be 31.9-90.8% across a range of individual studies [18, 22]. WBC count was significantly elevated in the AA group in the present study. WBC had a sensitivity of 93% and specificity of 73% using a cut-off value of 9.500 u/l.

MPV is reported routinely in the complete blood count. Complete blood count is usually neglected by doctors in AA diagnosis with the exception of WBC and neutrophil counts. MPV is a widely used marker of platelet production and function. However, changes in MPV value are also important in cardiovascular, cerebrovascular, acute pancreatitis, rheumatoid diseases,

Table 3. ROC curve analysis

Variables	AUC	SE	P	95% CI		Cut-off value	SEN (%)	SPE (%)	PPV (%)	NPV (%)
				Upper limit	Lower limit					
WBC	0.952	0.015	< 0.0001	0.922	0.982	9.500 u/l	93	83	84.5	92.2
CK-MB	0.867	0.025	< 0.0001	0.818	0.917	17.5 u/l	81	70	72.9	78.6
Bilirubin	0.667	0.038	< 0.0001	0.593	0.741	0.51 mg/dl	79	40	56.8	65.6

AUC: Area under curve, SE: Standard error, CI: Confidence interval. SEN: Sensitivity, SPE: Specificity, PPV: Positive predictive value, NPV: Negative predictive value.

and other inflammatory disorders [23-26]. Given that MPV reflects ongoing inflammatory processes, MPV was as a marker for AA diagnosis [22, 27, 28]. However, increases in WBC and neutrophil counts are well known markers in AA diagnosis. A limited number of studies have begun to clarify the use of MPV in differential diagnosis. Some studies have proposed that evaluation of MPV in combination with WBC and neutrophil percentage is the most appropriate diagnostic approach. In a study evaluating diagnostic value of MPV, PMV was identified as an important marker when evaluated prior to surgery [16]. However, the use of MPV in AA diagnosis is controversial and recent reports have been inconsistent [22, 27-29]. Uyanık *et al*. [11] proposed MPV has no diagnostic value for AA. There was no difference in MPV values between groups in our study.

Damage to the appendix wall causes translocation of bacteria and endotoxins from the appendiceal lumen and into the portal system in AA. Inflammatory cytokines can lead to intrahepatic cholestasis. *E. coli* endotoxins can cause cholestasis in a dose-dependent manner [30]. Bilirubin is a useful positive predictive marker of AA. Several studies have demonstrated an association between hyperbilirubinemia and simple appendicitis. In addition, bilirubin is a valuable marker of appendiceal perforation. Bilirubin should be used in combination with other laboratory tests and clinical examination in the evaluation of suspected AA patients [31].

Estrada *et al*. [32] evaluated the association between hyperbilirubinemia and perforated appendicitis. They demonstrated that bilirubin values in patients with perforated appendicitis or gangrenous appendicitis are elevated relative to patients with simple appendicitis.

Sand *et al*. [33] compared biomarkers in cases of perforated and gangrenous appendicitis, demonstrating that the specificity of hyperbili-

rubinemia was 86% and the specificity of CRP was only 35%.

Hyperbilirubinemia is an important marker in both perforated appendicitis and in simple appendicitis. The probability of simple AA is higher in patients with hyperbilirubinemia than in patients with normal bilirubinemia. The specificity of hyperbilirubinemia was 70% for cases of perforated or gangrenous appendicitis, although other studies have reported lower specificity [34, 35]. In a different study, hyperbilirubinemia had a specificity of 88% and a positive predictive value of 91% [31]. Hyperbilirubinemia was more predictive of AA than WBC and CRP in one study [35].

However, none of these studies have demonstrated the predictive value of bilirubin elevation in simple acute appendicitis. Uncertain [7] and positive [37] results were presented in two prospective studies. Total bilirubin values were found to be significantly different in the study group compared to the control group in the present study. Total bilirubin values of AA patients (mean 0.8 mg/dl, range 0.5-1.1) were higher than among the control group (mean 0.5 mg/dl, range 0.4-0.8) in our series. The diagnostic cut-off value for bilirubin was 0.5 mg/dl, which had a sensitivity of 79% and specificity of 40%.

Graber *et al*. [38] showed in a study conducted on dogs that serum CK isozyme levels were significantly elevated following infarction. Ischemic or hemorrhagic intestinal infarcts promote bacteria growth and cause mucosal damage. Break down of intestinal smooth muscle cells leads to the release of CK isoenzymes. Serum CK activity, seromuscular enzyme, is increased in intestinal ischemia compared to other enzymes such as alkaline phosphatase and diamine oxidase. Leukocytosis, elevated serum lactate, and CK/CK-MB values were considered to be specific parameters for the diagnosis of this

disease in a study analyzing 62 non-occlusive mesenteric ischemia patients [39]. No previous study has evaluated serum CK in the diagnosis of AA. In a prospective study, 90 female patients were divided into 5 groups according to diagnosis: tubal pregnancies (n = 20), missed abortus (n = 20), control subjects (n = 20), pelvic inflammatory disease (n = 20), and acute appendicitis (n = 10). Serum CK concentrations were elevated in cases of tubal pregnancy. Increases in maternal serum CK were proposed as a possible marker of ectopic pregnancy [40]. CK-MB was significantly higher in the study group than among the control group in the present study ($P < 0.0001$). Using a cut-off value of 17.5 u/l, the sensitivity of serum CK was 81% and specificity was 70% for the diagnosis of AA. The retrospective design employed in this study and the small number of patients enrolled are significant limitations. Further prospective clinical studies including larger number of patients are necessary to evaluate the efficacy of CK-MB and bilirubin in the diagnosis of AA.

Conclusion

This study is the first study demonstrating that CK-MB is a valuable parameter in AA diagnosis. CK-MB may be particularly useful when evaluated in combination with WBC and bilirubin as well as physical examination and other laboratory findings in patients with suspected appendicitis symptoms. Further studies are necessary to identify specific and reliable markers or combinations of markers for the diagnosis of AA.

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Disclosure of conflict of interest

None.

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