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ORIGINAL ARTICLE

The Diagnostic Value of Plasma Copeptin Level in Patients with Acute Appendicitis

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SUMMARY

Background: **This study aims to determine the effectiveness of plasma copeptin level, leukocyte, and neutrophil-to-lymphocyte ratio (NLR) in the diagnosis of patients with acute appendicitis presenting to the emergency department.**
Methods: **Patients over the age of 18 presenting to the emergency department due to abdominal pain and diagnosed with acute appendicitis as a result of clinical, laboratory, and radiological evaluation were included in the study. The control group consisted of healthy volunteers. Blood samples were taken from all groups at the time of admission. Plasma copeptin level was studied using the ELISA kit. Statistical Package for the Social Sciences v.22 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) was used for statistical analysis.**
Results: **Fifty patients and 50 healthy volunteers were included in the study. Plasma copeptin, leukocytes, and NLR levels were found to be significantly higher in the appendicitis group compared to the control group. In receiver operating characteristic curve (ROC) analysis, copeptin had 96% sensitivity and 92% specificity at the optimal cutoff value of 3.2 ng/mL to predict patients with appendicitis [area under the curve (AUC): 0.994, 95% confidence interval (CI): 0.984 - 1, p < 0.0001)].**
Conclusions: **In this study, plasma copeptin levels, leukocytes, and NLR were found to be significantly higher in AA patients compared to healthy volunteers. Increased plasma copeptin level in AA patients may play a potential role in the diagnosis of appendicitis.**
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KEY WORDS

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INTRODUCTION

Acute appendicitis (AA) is the most common reason for surgical intervention in patients presenting to the emergency department with abdominal pain [1], and the lifetime risk of acute appendicitis in the general population is approximately 7% [2]. The diagnosis of appendicitis is made by anamnesis, physical examination, laboratory, and radiological imaging [3]. While the clinical diagnosis may be simple in patients presenting with classical signs and symptoms, there may be a delay in diagnosis and treatment in patients presenting with atypical presentation [4]. The diagnosis of appendicitis is missed

in 3.8 - 15% of children and 5.9 - 23.5% of adults in emergency department admissions [5]. Radiological imaging methods such as ultrasonography and computed tomography (CT) are widely used in the diagnosis of acute appendicitis [6]. However, these methods require special equipment and an experienced radiologist and there is radiation exposure in the use of CT, which increases the cost [6,7]. The Alvarado score is a scoring system based on clinical symptoms, findings, and laboratory parameters developed for the diagnosis of acute appendicitis [8]. Migration of pain to the right lower quadrant is 1 point, loss of appetite is 1 point, nausea-vomiting is 1 point, right lower quadrant tenderness is 2 points, rebound is 1 point, fever > 37.5°C is 1 point, and leukocytosis is 2 points. The incidence of acute appendicitis is 3.7% in those with a score of 3 or less, and these cases are discharged with a recommendation for follow-up. In patients with a score of 7 or higher, the incidence of AA is 77.7%, and surgical consultation is recommended if clinically compatible. In cases with a moderate probability with a score between 4 and 6, radiological imaging is recommended [7]. The use of scores in the diagnosis of AA increases the accuracy of the diagnosis and decreases the rate of negative appendectomy [9]. However, despite clinical, diagnostic imaging, and clinical prediction scores, diagnosis continues to be difficult. Although many biochemical parameters have been studied, there is no single biochemical test to be used for diagnosis. In addition to leukocytosis, the neutrophil count and neutrophil-to-lymphocyte ratio (NLR) increase while the lymphocyte count decreases in acute appendicitis [6,10,11]. Copeptin is a 39-amino-acid glycopeptide produced from preprovasopressin [12]. Released from the neurohypophysis together with arginine-vasopressin [13]. Copeptin level was found to be highly correlated with disease severity and mortality in critically ill patients such as myocardial infarction, heart failure, stroke, traumatic brain injury, sepsis, and chronic obstructive pulmonary disease [12-14]. In our literature review, we could not find any study investigating copeptin levels in patients with acute appendicitis. In this study, we planned to investigate the plasma copeptin level in patients who came to the emergency department with the complaint of abdominal pain and were diagnosed with acute appendicitis.

MATERIALS AND METHODS

The study was approved by the Ethics Committee of Firat University (No.: 2021/06-14). Written informed consent was obtained from all participants and the study was conducted in accordance with the Declaration of Helsinki. The study group included 50 patients older than 18 years of age, who were admitted to the emergency department during the 6-month period with the complaint of abdominal pain and were diagnosed with acute appendicitis as a result of clinical, laboratory, and radiological evaluation. The control group consisted of 50 people without a known disease who agreed to participate in the study. Demographic data of the groups were recorded. The Alvarado score was determined by the emergency physician at the time of admission of the patients. Those with known cardiovascular disease, malignancy, renal failure, liver disease, stroke, and acute infection were excluded from the study.

Blood samples were taken at the time of admission to the emergency department for laboratory analysis. For the copeptin level, 3 mL blood was collected from the patient and control groups into BD® Vacutainer glass Aprotinine K3EDTA tubes (BD Diagnostics, Franklin Lakes, NJ, USA), centrifuged at 3,000 x g for 6 minutes, and stored in a -80°C deep freezer until the study day. Plasma copeptin levels were measured using a commercially available kit, ELISA (Human Copeptin ELISA Kit, Catalog No.: 201-12-5463 Sunred Biological Technology Co. Ltd., Shanghai), with a lower sensitivity limit of 0.067 ng/mL. The sample was measured twice in one experiment. The variance coefficient within and between assays for this kit was < 10% and < 12% as stated by the manufacturer of the test. The detection range for copeptin was 0.07 - 20 ng/mL. The complete blood count was performed on the same day. Tripotassium EDTA tubes (Vacuette) were used to collect blood samples. Thirty minutes after the blood sampling, the leukocyte (range: 3.8 - 8.6 10e3/µL), neutrophil (range: 2.1 - 6.1 10e3/µL), and lymphocyte (range: 1.3 - 3.5 10e3/µL) measurements were completed with an automatic blood counter (Siemens Advia 2120, Diagnostic Solutions, Milan, Italy).

Statistical Package for the Social Sciences v.22 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) package program was used for statistical analysis. The results were given as the mean ± standard deviation (SD) when they were in a normal distribution, and as the median (interquartile range (IQR)) when they did not fit the normal distribution. Categorical variables were given as numbers (percentage). The chi-squared test was used for non-metric data, Student's *t*-test was used for comparison of parameters between groups, Mann Whitney U test was used for comparison of non-parametric groups, and Pearson's correlation test was used for examining the relationships between parameters in groups. Receiver operating characteristic (ROC) curve analysis was performed to assess the ability of copeptin level to predict AA. p-values < 0.05 were accepted as the lowest level of significance.

RESULTS

A total of 50 patients with appendicitis and 50 healthy volunteers were included in the study. Thirty-one of those in the appendicitis group were male and the mean age was 29.5 ± 10.3. In the control group, 26 patients were male and their mean age was 31.3 ± 6.7. There was no difference between the groups in terms of age and gender (p = 0.06, p = 0.31, respectively).

Table 1. Laboratory values of appendicitis and control groups.

Laboratory values	Appendicitis (n = 50)	Control (n = 50)	p-value
Copeptin ng/mL Median (IQR)	5.4 (4.3 - 7.8)	2.1 (1.7 - 2.6)	0.0001
Leukocyte 10e3/µL Median (IQR)	11.95 (9.72 - 13.95)	6.49 (5.68 - 7.25)	0.0001
Neutrophil 10e3/µL Median (IQR)	8.79 (6.55 - 10.88)	3.58 (3 - 4.33)	0.0001
Lymphocyte 10e3/µL Median (IQR)	1.77 (1.4 - 2.2)	2.055 (1.64 - 2.35)	0.087
NLR Median (IQR)	4.6 (3.4 - 7.6)	1.8 (1.5 - 2.3)	0.0001

IQR: interquartile range, NLR: neutrophil-to-lymphocyte ratio.

Table 2. Optimal cutoff, sensitivity, specificity, and AUC values of copeptin, leukocytes, and NLR.

	Cutoff	Sensitivity %	Specificity %	AUC (CI 95%)	p-value
Copeptin ng/mL	3.2	96	92	0.994 (0.984 - 1)	0.0001
Leukocyte 10e3/µL	8.6	86	90	0.954 (0.908 - 1)	0.0001
NLR	2.8	88	88	0.931 (0.880 - 0.982)	0.0001

AUC: area under the curve, CI: confidence intervals, NLR: neutrophil-to-lymphocyte ratio.

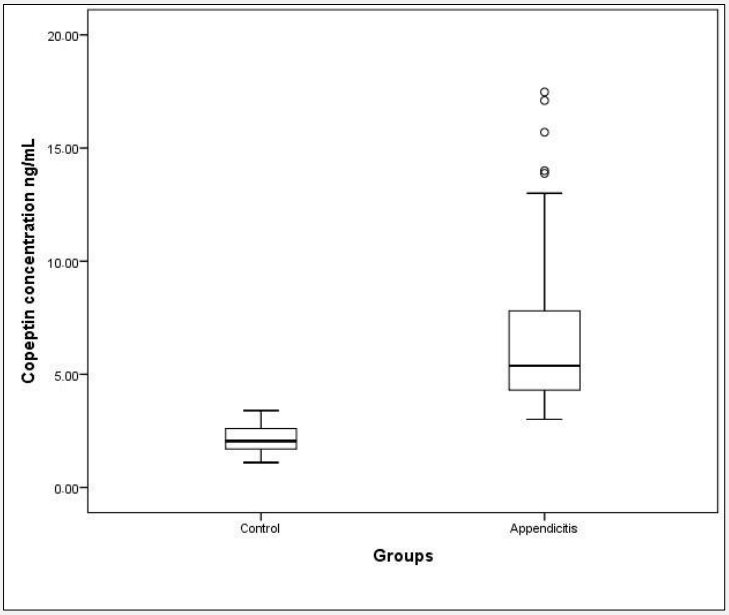

Figure 1. Copeptin level box-and-whisker plot of appendicitis and control groups.

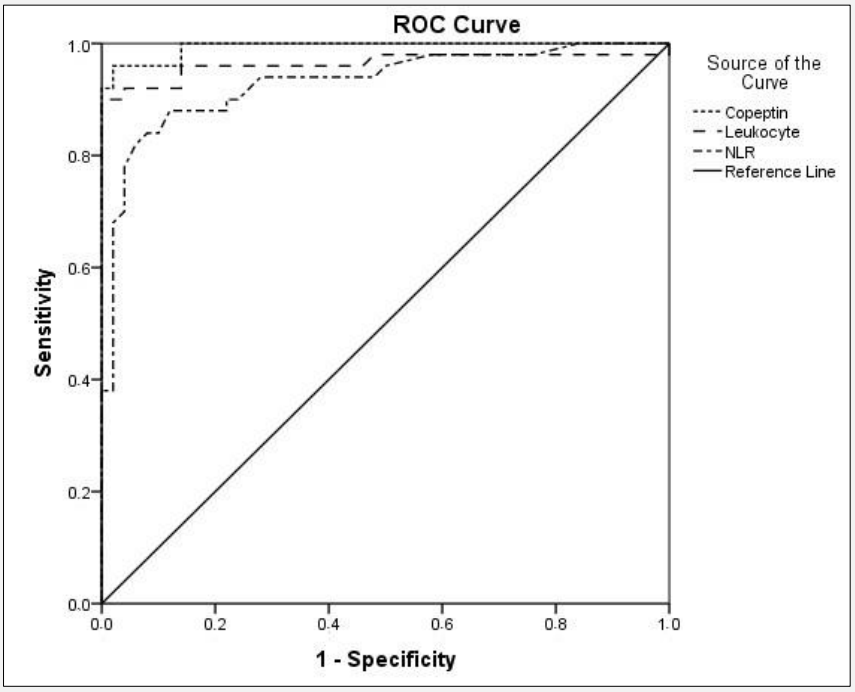

Figure 2: Evaluation of copeptin, leukocyte, and NLR levels on the ROC curve.

When the laboratory values of the groups were compared, leukocyte, neutrophil, and NLR were significantly higher in the appendicitis group than in the control group. Similarly, plasma copeptin level was found to be significantly higher in the appendicitis group than in the control group. Figure 1 shows the box-and-whisker plot indicating the plasma copeptin level of appendicitis and control groups. Laboratory values of the groups are given in Table 1.

In receiver operating characteristic curve (ROC) analysis, to predict patients with appendicitis, copeptin had 96% sensitivity and 92% specificity at the optimal cut-off value of 3.2 ng/mL (area under the curve (AUC): 0.994, 95% confidence interval (CI): 0.984 - 1, $p < 0.0001$)). The sensitivity, specificity, and AUC values of copeptin, leukocytes, and NLR at the optimal cutoff value are shown in Table 2, while the ROC curve of copeptin, leukocytes, and NLR are presented in Figure 2. The mean Alvarado score of the appendicitis group was 6 ± 1.3 (min: 4 - max: 8). There was no significant correlation between plasma copeptin level and Alvarado score ($r = 1.31$, $p = 0.363$). There was no significant difference between plasma copeptin levels of patients with an Alvarado score of 4 - 6 with a moderate probability for AA and patients with an Alvarado score of 7 and above with a high probability for AA ($p = 0.38$).

DISCUSSION

In this study, we found that copeptin, leukocytes, and NLR were significantly higher in AA patients than in healthy volunteers. Copeptin level was strongly associated with the diagnosis of AA.

Diagnosis of AA can be difficult, and delay in diagnosis and treatment is associated with increased complications and mortality. Therefore, early diagnosis and treatment are important. In addition to clinical and radiological imaging, the search for a simple and accessible biomarker for the diagnosis of appendicitis continues. The complete blood count is one of the most commonly used and easily accessible laboratory tests. Leukocytosis is usually an expected finding in AA patients. Increased leukocyte count is one of the earliest signs of appendiceal inflammation and is associated with increased inflammation [15,16]. In our study, leukocyte count was significantly higher in appendicitis patients compared to the healthy control group. Despite being an important parameter in the diagnosis of AA, the diagnostic value of leukocytosis is variable. Although leukocytosis is observed in most patients with appendicitis, it is not specific and increases with pain in the right lower quadrant, and a normal value does not exclude AA [17,18].

NLR is a simple and inexpensive inflammatory marker that can be easily calculated from differential blood count [10,19]. In the case of inflammation, as a component of the cellular response, the neutrophil count and NLR increase while the lymphocyte count decreases [6, 10]. Increasing NLR indicates the severity of the inflammatory response [6]. A relationship has been found between NLR with cardiovascular diseases, malignancies such as colorectal and gastric cancer, chronic inflammatory diseases such as acute cholecystitis, and inflammatory bowel disease, and it has been reported to be a good indicator of inflammation [6,19,20]. Goodman et al. [21] first reported that NLR could be used in the diagnosis of AA, it was a more sensitive parameter than leukocyte, and an NLR of 3.5 would be a sensitive indicator for the diagnosis of AA. In subsequent studies, NLR has been reported to be an important parameter both in the diagnosis of AA and in determining the complications and severity of appendicitis [6,11,15,20, 22]. However, in the literature, different cutoff values of NLR have been given in diagnosing AA and differentiating complicated appendicitis. In our study, we found the sensitivity and specificity of NLR 88% at a cutoff value of 2.8. Hajibandeh et al. [22] reported that the sensitivity of NLR was 88.9% and the specificity was 90.9% at a cutoff value of 4.7 in the diagnosis of AA in their study in which they examined 17 observational studies involving 8,914 patients. In another study conducted with 1,623 patients who underwent appendectomy with the diagnosis of AA, Pereira et al. [23] found the sensitivity of NLR as 70.1% and the specificity as 43.2% at a cutoff value of 2.4 in the diagnosis of AA. They explained the reason why the sensitivity was lower compared to other studies was that the study included only the patients who underwent appendectomy and the patients who received medical treatment were excluded. In their study evaluating 3,392 patients who underwent appendectomy, Sevinç et al. [6] reported the sensitivity of NLR as 81% and specificity as 53% at a cutoff value of 3 in the diagnosis of AA. The diagnostic value of NLR alone in identifying AA patients has been reported to be low [10] and the normal NLR value does not exclude the diagnosis [11].

Copeptin is a peptide that is co-released with vasopressin and its circulating role is not yet fully understood. Since it remains more stable in plasma and serum at room temperature and is easily measured, it is used in routine evaluation instead of vasopressin [13,14]. Copeptin indicates the individual stress level [24], and its level increases in critically ill patients [13,25]. While copeptin level is recommended as a diagnostic biomarker in some diseases such as diabetes insipidus [26] and sepsis [12], it may be a prognostic marker in many diseases such as lower respiratory tract infection, heart failure, stroke, myocardial infarction, septic shock, and traumatic brain injury [13,14]. It has also been shown that plasma copeptin level is associated with the severity and mortality of the disease in critically ill patients hospitalized in the intensive care unit [27]. Similarly, studies on patients with acute pancreatitis have shown that serum copeptin levels are significantly higher in pancreatitis patients compared to the control group, and high copeptin levels are associated with the severity and mortality [14,25]. Although the level of copeptin has been investigated in many diseases, to the best of our knowledge, there has been no study examining the capacity of copeptin in the diagnosis of acute appendicitis. In our study, we found that the copeptin level was significantly higher in AA patients compared to the healthy control group and was associated with 96% sensitivity and 92% specificity at a cutoff value of 3.2 (AUC: 0.993, 95% CI: 0.984 - 1, $p < 0.0001$). In the diagnosis of AA, its sensitivity was found to be higher than the leukocyte count and NLR.

Limitations: In this study, patients with acute appendicitis were compared with a healthy control group. Since other diseases causing abdominal pain were not included in the study, a comparison with those diseases could not be made. During the study period, complicated appendicitis was detected in only one case and none of the patients developed AA-related mortality. Therefore, the prognostic value of copeptin in AA patients could not be evaluated.

In conclusion, we found that the copeptin level increased significantly in AA patients compared to the healthy control group and predicted the diagnosis of appendicitis. Increased copeptin level may have a potential role in the diagnosis of appendicitis patients.

Declaration of Interest:
None.

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