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Diagnostic value of serum signal peptide-CUB-EGF-like domain-containing protein 1 levels in patients with acute appendicitis

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SUMMARY

OBJECTIVE: Acute appendicitis is one of the most common surgical causes of an acute abdomen among patients admitted to the emergency room due to abdominal pain. The clinical diagnosis of acute appendicitis is usually difficult and is made by evaluating the clinical, laboratory, and radiological findings together. The aim of this study was to investigate the diagnostic potential of signal peptide-CUB-EGF-like domain-containing protein 1 as a biomarker for acute appendicitis.

METHODS: A total of 67 adult patients without any comorbidities who presented to the emergency department with abdominal pain and were clinically diagnosed with acute appendicitis were included in the case group. The patients included in the study were classified into the negative appendectomy group and the acute appendicitis group according to their histopathological final diagnosis. In addition, 48 healthy volunteers without comorbidities were included in the control group. Signal peptide-CUB-EGF-like domain-containing protein 1 levels of patients and the control group were measured.

RESULTS: According to postoperative histopathological examinations of the patients, 7 (10.4%) patients were diagnosed with negative appendectomy, and 60 (89.6%) patients were diagnosed with acute appendicitis. Signal peptide-CUB-EGF-like domain-containing protein 1 levels were higher in the patients with acute appendicitis than in negative appendectomy patients (p=0.012). Signal peptide-CUB-EGF-like domain-containing protein 1 levels were also higher in the case group compared to the control group (p=0.001).

CONCLUSION: The admission signal peptide-CUB-EGF-like domain-containing protein 1 level was significantly higher in adults with acute appendicitis. The SCUBE1 level is a novel but promising biomarker that aids in the diagnosis of acute appendicitis.

KEYWORDS: Acute disease. Appendicitis. Biomarkers. Leukocyte count. Membrane proteins. SCUBE1 protein, human.

INTRODUCTION

Acute appendicitis (AA) is one of the most common surgical causes of an acute abdomen among patients admitted to the emergency department (ED) due to abdominal pain[1]. Clinicians diagnose AA by evaluating the results obtained from laboratory and radiological examinations, primarily anamnesis and physical examination findings. In addition, diagnostic evaluation can be supported by adding clinical scoring systems, including physical examination findings and various laboratory markers. Many simple and usable scoring systems (the Alvarado score, the modified Alvarado score, the Appendicitis Inflammatory Response score, the RIPASA score, etc.) have been developed for the prediction of AA risk[2]. But their use alone is controversial[3,4]. The role of ultrasonography (USG) and computer tomography (CT) imaging methods remains important in the diagnosis of AA. Despite all diagnostic methods, negative pathology results ranging from 3 to 25% in patients with AA diagnosis and surgical treatment method can be found[5,6]. New methods are needed to make the correct diagnosis in AA and to reduce the rate of negative surgical treatment methods. For this purpose, studies have been carried out showing the relationship of various biochemical markers with AA[1,7].

SCUBE1 is a glycoprotein found on platelet and endothelial cell surfaces. This is a novel molecule with matrix-bound or soluble forms released from the platelet surface as a result of platelet aggregation, which has been shown to play an adhesive role in platelet-platelet or platelet matrix interaction. Studies were carried out on various levels of cardiovascular diseases, inflammatory events, and ischemic processes[8,9]. Platelet activation has a role in the pathophysiology of thrombosis and

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inflammatory diseases. Various platelet markers have been investigated in association with both thrombosis and inflammation[10]. There are few studies in the literature that investigate SCUBE1 levels in adult patients diagnosed with AA[11]. The aim of this study was to investigate the diagnostic value of serum SCUBE1 levels in adult patients who admit to the ED with abdominal pain and are diagnosed with AA.

METHODS

Study design and patient selection

This is a single-centered, prospective study that examines patients who were admitted to the ED with abdominal pain and have been diagnosed with AA. Approval was received from the local ethics committee (Decision No: 2017/109) before starting the study.

The study included patients aged 18 and over who had been admitted to the ED with abdominal pain, had no accompanying disease, were clinically diagnosed with AA, and underwent surgery. Pregnant women, trauma patients, patients who underwent medical treatment methods in the treatment of AA, and patients who voluntarily did not give the necessary consent to participate in the study were excluded from the study. Participants were informed about the study, and written informed consent was obtained from all participants to participate in the study. Symptoms, physical examination findings, laboratory parameters, imaging findings, Alvarado scores, and clinical and demographic characteristics of the patients included in the study were recorded in the study form. In our study, the postoperative histopathological diagnoses of patients were considered the gold standard.

As a control group, 48 healthy volunteers without comorbidities or an active inflammatory disease were selected. A voluntary consent form was obtained from the individuals in the control group. Plasma samples were taken from patients and the control group to measure SCUBE1 levels at the time of admission. After centrifuging for 10 min at 4,000 rpm, the plasma was separated and stored at -80°C. 24 h before the start of the SCUBE1 examination, the separated plasmas were removed from the -80°C environment and placed at +4 degrees. Dissolved plasmas were brought to room temperature, and SCUBE1 levels were measured.

Determination of signal peptide-CUB-EGF-like domain-containing protein 1 levels

SCUBE1 levels in plasma samples were measured by sandwich immunoassay (Enzyme-Linked Immunosorbent Assay (ELISA)) in accordance with the manufacturer's instructions. Commercially purchased Human SCUBE1 (floor no: E-EL-H5405) ELISA kits were used in the analysis. The results were expressed as ng/mL.

The endpoint of the study

In our study, it was determined to investigate the usability of initial SCUBE1 levels from patients in the diagnosis. For this purpose, the use of SCUBE1 levels in the distinction between a negative appendectomy and an AA diagnosis according to histopathological classification was determined as the primary endpoint. In addition, according to histopathological results, the distinction of SCUBE1 levels between the AA diagnosis and the control group was determined as the secondary endpoint.

Statistical method

Statistical analysis was carried out using the Jamovi v.1.6 statistical program (Jamovi Project Computer Software, Version 1.6, Sydney, Australia). Continuous variables were defined as mean and standard deviation for the data with normal distribution, and for the abnormal distribution with median and interquartile range (IQR). Categorical data were shown as frequency (n) and percentage (%). The Shapiro-Wilk test was used to check whether the data were normally distributed. Normal distribution data were expressed with a mean±SD, and abnormal distribution data were expressed with a median (IQR 25–75). The Student's t-test was used to compare normally distributed data, and Mann-Whitney U test was used to compare data showing abnormal distribution.

RESULTS

According to postoperative histopathological examinations of the patients, 7 (10.4%) patients were diagnosed with negative appendectomy, and 60 (89.6%) patients were diagnosed with AA. In the study, 65% (n=39) of the AA group (n=60) was male; 54.2% (n=26) of the control group (n=48) was male; and 28.5% (n=2) of the negative appendectomy group (n=7) was male. The median age of the AA group was 33 (25.0–43.5) years, while the median age of the control group was 30 (22.5–41.5). The gender and age distribution of patients and control groups is shown in Table 1.

Laboratory data, imaging findings, and Alvarado scores of the patients included in the study were calculated using the values at the time of initial admission and presented in Table 2. When laboratory parameters were examined, leukocyte (p=0.016), neutrophil (p=0.003), and neutrophil-lymphocyte

ratio (NLR) (p=0.004) levels were statistically significantly higher in the group with AA than the negative appendectomy group. Again, Alvarado scores were higher in the AA group and statistically significant (p=0.025). When the imaging findings of the patients included in the study were examined, 18 patients were diagnosed with USG and 49 patients were diagnosed with CT. The appendix diameter measured by CT was larger in the group diagnosed with AA (p=0.049). SCUBE1 levels, which were examined at the time of the first admission of the patients and the control group included in the study, are presented in Table 3. When the SCUBE1 levels of the patients were examined, it was shown that they were higher in the AA group (p=0.012) when compared with the negative appendectomy group. In addition, SCUBE1 levels in patients with AA were higher than those in the control group, and this was found to be statistically significant (p=0.001).

DISCUSSION

AA is one of the most common surgical causes of an acute abdomen. The clinical diagnosis of AA is often difficult and is made by co-evaluation of clinical, laboratory, and radiological findings. To help with diagnosis, a number of scoring systems have been developed that incorporate physical examination findings and various laboratory markers. However, many of

Table 1. Gender and age distribution of the patient and control groups.

	Gender		Age distribution (years)
	Male	Female	Median (IQR 25–75)
Negative appendectomy (n=7)	2 (28.5%)	5 (71.5%)	40 (IQR 28.5–42.0)
Acute appendicitis (n=60)	39 (65.0%)	21 (35.0%)	33 (IQR 25.0–43.5)
Control (n=48)	26 (54.2%)	22 (45.8%)	30 (IQR 22.5–41.5)

IQR: interquartile range.

Table 2. Laboratory, imaging, and alvarado scores of patients according to histopathological classification.

	Negative appendectomy (n=7)	Acute appendicitis (n=60)	p-value
WBC (10^3/μL)	8.6±3.7	13.2±4.7	**0.016**
Neutrophil (10^3/μL)	5.3±3.0	10.5±4.3	**0.003**
Lymphocyte (10^3/μL)	2.4 (IQR 1.5–2.9)	2.0 (IQR 1.2–2.5)	0.296
PLT (10^3/μL)	247 (IQR 226–282)	224 (IQR 203–260)	0.122
MPV (fL)	9.5±0.8	9.8±1.3	0.469
CRP (mg/L)	1.6 (IQR 0.8–6.4)	0.9 (IQR 0.3–4.7)	0.559
Total protein (g/L)	7.7±0.7	7.5±0.5	0.706
Albumin (g/L)	4.9±0.3	4.4±0.3	0.866
NLR	1.44 (IQR 1.17–3.55)	5.83 (IQR 3.23–9.76)	**0.004**
Alvarado score	5 (IQR 4.5–6)	7 (IQR 5–8)	**0.025**
USG (mm) (n=18)	7±0	7.9±0.7	0.143
CT (mm) (n=49)	9.4±2.1	10.2±2.6	**0.049**

WBC: white blood cell; PLT: platelet; MPV: mean platelet volume; CRP: C-reactive protein; NLR: neutrophil lymphocyte rate; IQR: interquartile range; USG: ultrasonography; CT: computed tomography. Bold values indicate statistical significance at the p<0.05 level.

Table 3. Signal peptide-CUB-EGF-like domain-containing protein 1 levels of the patient and control groups.

	Negative appendectomy	Acute appendicitis	p-value
SCUBE1 (ng/mL)	19 (IQR 15.5–19.5)	23.5 (IQR 19–28.3)	0.012
	Control	**Acute appendicitis**	
SCUBE1 (ng/mL)	13.5 (IQR 11–16)	23.5 (IQR 19–28.3)	0.001

IQR: interquartile range.

these scoring systems have not been widely accepted. In addition, diagnostic imaging tools such as USG and CT are also used in the diagnosis of AA[3,7]. CT scanning has now become the gold standard for diagnosing AA. In cases where a CT scan cannot be performed, it has become difficult to reach the correct diagnosis[12]. Despite all diagnostic methods, a 3–25% negative appendectomy is encountered[6]. In our study, similar to the literature, we found a negative appendectomy rate of 10.4%. In order to reduce the negative effects of CT scanning and the negative appendectomy rate, clinicians have tended to investigate the role of clinical scoring systems, USG, and some biomarkers in the diagnosis of AA[1,7,11-15].

SCUBE1 is a cell surface glycoprotein found in platelets and endothelial cells[16]. Studies have been carried out on the possibility that SCUBE1 may have a role in various cardiovascular, metabolic, and ischemic diseases[8,9,17-19]. Güzel and her colleagues found SCUBE1 levels higher in hypertensive patients than in normal, healthy individuals[18]. Türkmen and his colleagues mentioned that SCUBE1 could be used to diagnose the early stage of acute mesenteric ischemia[9]. Erdoğan and his colleagues noted that SCUBE1 levels have the potential to be used to predict mortality in septic patients. They also argued that there would be endothelium damage as a result of severe inflammation, and consequently, SCUBE1 levels would increase[8]. We investigated the utility of SCUBE1 in the diagnosis of AA with the hypothesis that there would be endothelium damage after inflammation and therefore SCUBE1 levels could increase.

The results of our study support our hypothesis that SCUBE1 levels in patients diagnosed with AA were higher compared to both the negative appendectomy group and the control group. According to our study, high levels of SCUBE1 statistically support the AA diagnosis. When we look at the literature, there are a limited number of studies evaluating the availability of SCUBE1 in the diagnosis of AA. Sonmez and his colleagues evaluated SCUBE1 levels in the diagnosis of AA and argued that there was no diagnostic marker. However, they found SCUBE1 significantly higher in the CRP-positive group[11]. In a different study involving pediatric appendicitis patients, the difference between appendicitis and control group SCUBE1 values was found to be statistically significant[20].

Our study has some limitations. The most significant limitation is that it is a single-centered study, and the number of patients included in the study is small. Only patients who underwent an appendectomy were included in the study. Patients who initially suspected appendicitis but were not clinically diagnosed with it were not included in this study.

CONCLUSION

As a result, SCUBE1 levels can be used to help diagnose patients clinically diagnosed with AA. Some biochemical markers have produced promising results to help diagnose AA in adult patients. However, it is obvious that there is a need for a greater number of high-quality evidence-based studies.

AVAILABILITY OF DATA AND MATERIALS

The authors agree to the conditions of the publication including the availability of data and materials in our manuscript.

INFORMED CONSENT

Patients' consents were obtained from the patients before starting the study.

ETHICAL APPROVAL

This study was approved by the Recep Tayyip Erdogan University Clinical Research Ethics Committee (Decision No. 2017/109).

HUMAN RIGHTS

The principles outlined in the Declaration of Helsinki have been followed.

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AUTHORS' CONTRIBUTIONS

GA: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. **MA:** Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Project administration, Writing – review & editing. **MI:** Data curation, Writing – review & editing. **HAU:** Formal Analysis, Validation, Writing – review & editing. **RB:** Validation, Writing – review & editing. **MKÇ:** Supervision, Writing – review & editing.

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