ORIGINAL ARTICLE

Can serum soluble urokinase plasminogen activator receptor be an effective marker in the diagnosis of appendicitis and differentiation of complicated cases?

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ABSTRACT

BACKGROUND: Soluble urokinase plasminogen activator receptor (suPAR) is a new biomarker of inflammation level. The aim of the study was to evaluate whether suPAR levels could be useful to detect acute appendicitis and to differentiate uncomplicated appendicitis (UA) from complicated appendicitis (CA).

METHODS: We prospectively studied 105 patients consisting of 40 UA cases, 40 CA cases, and 25 control patients. Blood samples were collected to measure suPAR level, C-reactive protein level, leukocyte counts, neutrophil counts, and neutrophil percentages preoperatively.

RESULTS: Median values of suPAR level, C-reactive protein level, leukocyte counts, neutrophil counts, and neutrophil percentages in UA and CA were significantly higher than control patients. suPAR levels of the UA and CA groups showed a statistically significant difference (p=0.016).

CONCLUSION: The current study demonstrated that serum suPAR concentrations can be helpful in differentiating CA from UA and in diagnosing acute appendicitis.

Keywords: Complicated appendicitis; soluble urokinase plasminogen activator receptor; uncomplicated appendicitis.

INTRODUCTION

Traditionally, the standard diagnostic method for acute appendicitis is physical examination, and the most common treatment is appendectomy. Additionally, there are randomized controlled studies on medical treatment for appendicitis. The treatment of patients with complicated appendicitis is controversial, and there is no clear consensus on the optimal treatment.

Acute appendicitis is the most common surgical emergency in children and adolescents.[1] Many inflammatory markers such as white blood cell count (WBC), C-reactive protein (CRP), procalcitonin, and D-dimer were used for the diagnosis of appendicitis.[2,3] These biomarkers have been shown to be useful in diagnosing appendicitis. In literature, no biomarkers have been identified for the preoperative differentiation of uncomplicated appendicitis (UA) from complicated appendicitis (CA).

The soluble urokinase plasminogen activator receptor (suPAR), a soluble form of the urokinase-type plasminogen activator receptor, is a biomarker that is produced by monocytes, macrophages, neutrophils, endothelial cells, active T cells, and tumor cells in serum, plasma, and cerebrospinal fluid. Urokinase bound to the cytoplasm and membrane of the proteolytic

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pathway is secreted by the plasminogen activator receptor.[4,5] suPAR levels increase in inflammation levels in cases such as pneumonia, urinary tract infections, Human Immunodeficiency virus (HIV) infection, urosepsis, sepsis, and malignancy.[6–8]

The aim of this study was to investigate the efficiency of serum suPAR levels in the diagnosis of appendicitis and differentiation of the complicated cases.

MATERIALS AND METHODS

Patient Selection and Study Design

This was a single-center, prospective study conducted between March 2012 and February 2015. Written informed consent was obtained from each patient's family, and the study was approved by the University of Health Sciences, Şişli Hamidiye Etfal Training and Research Hospital's Ethics Committee (04.12.2012-129). This study was supported by Istanbul Bilim University's Scientific Research Project Coordination Unit, which is under the category of Research Projects pursuant to sub-clause a of article 5 in the Directive of the Board of Evaluation for Scientific Projects (Project No: 2012-01-01). The study groups consisted of 40 patients with UA (acute, suppurative) and 40 patients with CA (gangrenous, perforated). The control group consisted of 25 patients who had undergone surgery for inguinal hernia, lipoma, pilonoidal sinus without infections, circumcision, or hypospadias. UA was defined as inflamed or suppurative appendicitis, and CA was defined as gangrenous or ruptured appendicitis.[9] Demographic variables and clinical findings were recorded and statistically compared. Blood samples were collected to measure suPAR level, CRP level, and WBC preoperatively.

Serum suPAR Analysis

Blood (2 mL) from the patients was centrifuged for 10 min at 1000 g (=3000 rpm), and the serum samples were stored at −80°C; these samples were collectively evaluated. The serum suPAR values were quantified using the suPARnostic assay (ViroGates, Copenhagen, Denmark), a suPARnostic TM ELISA kit, according to the manufacturer's instructions. Briefly, serum specimens (25 µL) were added to the wells of the ELISA plate, followed by addition of a peroxidase conjugate solution (225 µL). A second test was carried out using 100 µL of serum and the conjugate in duplicate wells. The reaction mixtures were incubated for 1 h at room temperature (18°C–26°C) in the dark. After washing, TMB substrate (100 µL) was added to each well and incubated for another 20 min at room temperature. The reaction was stopped by adding a stopping solution (100 µL) to each well. The absorbance was read at 450 nm within 30 min of stopping the reaction.[5]

Statistical Analysis

Statistical calculations were performed with the NCSS 2007 program for Windows. Besides standard descriptive statistical calculations [mean, standard deviation, median, interquartile range (IQR)], Kruskal–Wallis test was used in the comparison of the groups. Post-hoc Dunn's multiple comparison test was used in the comparison of the subgroups, and a Chi square test was used to evaluate the qualitative data. The results were evaluated within a 95% confidence interval (CI). The statistical significance level was $p<0.05$.

To calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio (LR) for the suPAR level, CRP level, WBC count, neutrophil count (NC), and neutrophil percentage (NP) at varying cutoff values, a conventional receiver operating characteristic (ROC) curve was generated. The area under the curve (AUC) was calculated for suPAR, CRP, WBC, NC, and NP as a biomarker. ROC analysis was used to calculate AUC for the suPAR, CRP, WBC, NC, and NP cutoff values and to identify the progression to appendicitis.

RESULTS

The study group comprised 34 females and 71 males. The median ages were 10 (IQR, 4.5–12) years in the control group, 10 (IQR, 8.25–14) years in the UA group, and 10.5 (IQR, 7.25–13) years in the CA group (p=0.226). There were no significant differences between the demographic properties of the groups. The median length of hospital stay was 2 (IQR, 1–2) in the UA group and 6 (IQR, 5–8) in the CA group (p=0.0001). There was a significant difference between the duration of hospitalization in the two groups (Table 1).

As the three groups marker were compared, suPAR level, CRP level, WBC count, NC, and NP were found to be significantly increased in the UA and CA groups than in the control

Table 1. Median and IQR values of age (years) and gender

	Control group	Uncomplicated appendicitis group	Complicated appendicitis group	p
Age Median (IQR)	10 (4.5–12)	10 (8.25–14)	10.5 (7.25–13)	0.226
Gender				
Female	9 (36.0%)	10 (25.0%)	15 (37.5%)	0.345
Male	16 (64.0%)	30 (75.0%)	25 (62.5%)	

IQR: Interquartile range.

Table 2. Median and IQR values of the serum suPAR (ng/mL) and CRP (mg/dL) levels, WBC (mm^3) and NC (mm^3), and NP (%) in the three groups of patients

	Control group	Uncomplicated appendicitis group	Complicated appendicitis group	p
suPAR	2.84 (2.63–3.41)	3.76 (2.97–4.37)	4.17 (3.55–5.5)	0.0001
CRP	3.19 (1.14–3.19)	16.5 (2.6–87.43)	21.75 (11.35–67.35)	0.0001
WBC	7.8 (7.3–9.7)	16.05 (11.08–19.3)	16.85 (14.2–19.6)	0.0001
NC	3.2 (2.2–3.5)	13.55 (8.29–16.93)	14.23 (11.69–16.65)	0.0001
NP	35.5 (28.45–44.3)	84.25 (73.23–88.65)	86.86 (79.28–89.83)	0.0001

suPAR: Soluble urokinase plasminogen activator receptor; CRP: C-reactive protein; WBC: White blood cell count; NC: Neutrophil count; NP: Neutrophil percentage.

Table 3. Dunn's multiple comparison test for serum suPAR and CRP levels, NC, and NP in the three groups

Groups	suPAR	CRP	WBC	NC	NP
Control/UA	0.001	0.0001	0.0001	0.0001	0.0001
Control/CA	0.0001	0.0001	0.0001	0.0001	0.0001
UA/CA	0.016	0.279	0.199	0.240	0.153

suPAR: Soluble urokinase plasminogen activator receptor; CRP: C-reactive protein; WBC: White blood cell count; NC: Neutrophil count; NP: Neutrophil percentage; UA: uncomplicated appendicitis; CA: Complicated appendicitis.

group (p=0.0001). The suPAR levels in the CA group were significantly higher than those in the UA group (p=0.016). This parameter was the only statistically significant difference between these two groups (Tables 2, 3).

The ROC curves of sensitivity (true positive rate) versus specificity (false positive rate) for the different cutoff values of the suPAR, CRP, NC, NP, and WBC in relation to different outcomes in the control and appendicitis groups are illustrated in Figure 1. The AUC was 0.811 (95% CI 0.723–0.881) for suPAR, 0.864 (95% CI 0.784–0.923) for CRP, 0.912 (95% CI 0.841–0.958) for WBC, 0.975 (95% CI. 0.925–0.995) for NC, and 0.980 (95% CI 0.931–0.997) for NP. There was a correlation between suPAR and WBC, suPAR and NC, and suPAR and NC between the appendicitis and control groups. To investigate the value of suPAR as a diagnostic marker for appendicitis, a cutoff value of 3.5 ng/mL and an LR equal to 4.37 were used (LR >2: statistically significant value). The results of other markers value are showed in Table 4.

The ROC curves of sensitivity (true positive rate) versus specificity (false positive rate) for the different cutoff values of suPAR, CRP, NC, NP, and WBC in relation to outcomes in the UA and CA groups is illustrated in Figure 2. The AUC was 0.656 (95% CI 0.542–0.759) for suPAR, 0.570 (95% CI 0.455–0.681) for CRP, 0.583 (95% CI0.468–0.693) for WBC, 0.576 (95% CI 0.461–0.686) for NC, and 0.593 (95% CI 0.477–0.701) for NP. There was no significant correlation between the ability of the markers to differentiate UA from CA.

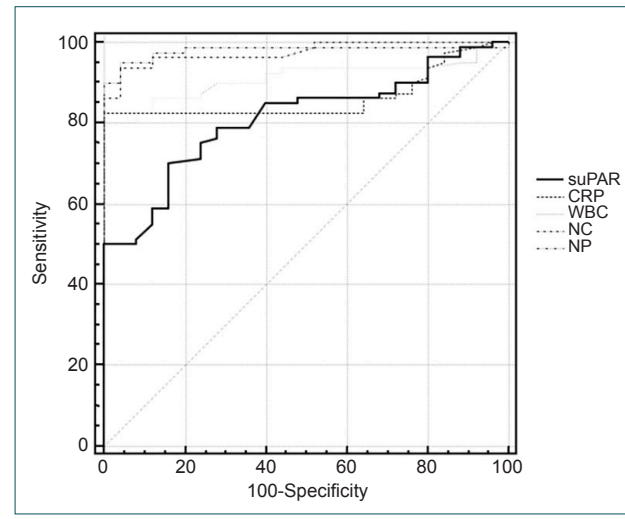

Figure 1. ROC curves of the discrimination ability of serum suPAR, CRP, WBC, NC, and NP to differentiate the control and the appendicitis cases.

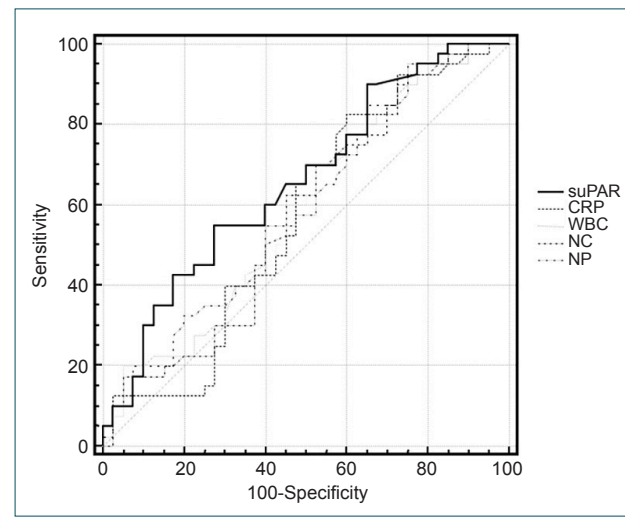

Figure 2. ROC curves of the discrimination ability of serum suPAR, CRP, WBC, NC, and NP to differentiate UA and CA.

To investigate the value of suPAR as a differential marker of UA and CA, a cutoff value of 4.1 ng/mL was founded. LR was equal to 2 with statistically significant. The cutoff values of sensitivity and specificity of suPAR, CRP, WBC, NC, and

Table 4. Cutoff values, sensitivity, and specificity of the suPAR, CRP, NC, and NP for the prediction of outcomes in the control and appendicitis groups

Control/appendicitis	Cutoff values	Sensitivity	Specificity	PPV	NPV	Likelihood ratio
suPAR	3.5	70.00	84.00	93.3	46.7	4.37
CRP	3.19	82.50	36.00	80.5	39.1	1.29
NC	5.29	93.75	96.00	98.7	82.8	23.44
NP	55.8	95.00	96.00	98.7	85.7	23.75

suPAR: Soluble urokinase plasminogen activator receptor; CRP: C-reactive protein; NC: Neutrophil count; NP: Neutrophil percentage; PPV: positive predictive value; NPV: Negative predictive value.

Table 5. Cutoff values, sensitivity, and specificity of suPAR, CRP, NC, and NP for the prediction of outcomes in uncomplicated and complicated appendicitis groups

UA/CA groups	Cutoff values	Sensitivity	Specificity	PPV	NPV	Likelihood ratio
suPAR	4.1	55.00	72.50	66.7	61.7	2.00
CRP	7.6	82.50	40.00	57.9	69.6	1.37
WBC	15.4	70.00	47.50	57.1	61.3	1.33
NC	8.16	92.50	25.00	55.9	83.3	1.23
NP	71.9	95.00	25.00	55.9	83.3	1.27

suPAR: Soluble urokinase plasminogen activator receptor; CRP: C-reactive protein; NC: Neutrophil count; NP: Neutrophil percentage; PPV: positive predictive value; NPV: Negative predictive value; UA: uncomplicated appendicitis; CA: Complicated appendicitis.

NP for the prediction of outcomes in UA and CA groups are shown in Table 5.

DISCUSSION

Acute appendicitis is generally diagnosed by physical examination and clinical evaluation. Biochemical markers and radiologic evaluations are helpful for clinicians to diagnose this disease. Progression from UA to CA is fast in children. It can be difficult to differentiate preoperative CA from UA. Many markers for inflammation were evaluated during CA and UA periods. It has been reported that during the progression of appendicitis, WBC counts and CRP levels rise due to inflammation, yet these parameters have not proved adequate in determining the degree of inflammation for CA evaluation.[10] Beltrán et al.[11] have stated that BKS and CRP can be beneficial in the diagnosis of appendicitis and in distinguishing UA from CA. According to the findings of our study, differently from other parameters, serum suPAR level is an effective marker in both diagnosing appendicitis and distinguishing CA from UA. The results are statistically significant (Table 3). It has been found that increases in serum CRP level, WBC count, NC, and NP are also statistically significant in diagnosing acute appendicitis; yet none of these markers have proven to be adequate parameters for statistically distinguishing CA from UA (Table 3).

In our study, the cutoff value for a UA diagnosis has been measured to be 3.5 ng/mL, with 84% specificity and 93% PPV. In patients with acute abdominal findings, suPAR levels seem to be an effective parameter in establishing a diagnosis. The cutoff value for suPAR in distinguishing CA from UA has been measured to be 4.1 ng/mL, with 72.5% specificity, 66.7% PPV, and 55% sensitivity. Although sensitivity levels have not been found to be high, the serum suPAR level is an effective indicator in distinguishing CA from UA. As seen in Table 2, the IQR values of suPAR do not show wide fluctuations. These results demonstrated that serum suPAR levels are more effective in terms of their impact on diagnosis and differential diagnosis.

Through a comparison with existing literature, it may be said that the results of our study have revealed less of an increase in suPAR levels in the case of appendicitis than in cases of sepsis, pneumonia, or pyelonephritis. In various studies, suPAR levels for community-acquired pneumonia and pyelonephritis have been measured as >8 ng/dL.[7,12] In another study, the median suPAR level in pneumonia patients has been found to be 10.5 ng/mL, and it has been reported that suPAR levels >12.9 ng/mL predict undesirable outcomes such as death, with 80% specificity and 76.1% PPV.[13] Different cutoff values in different illnesses may assist clinicians in establishing a differential diagnosis.

Similar results have been found in patients infected with HIV. While suPAR levels <3.28 ng/mL may indicate patients infected with HIV, suPAR levels >4.19 ng/mL are associated with mortality risk due to AIDS.[14] It appears that cutoff val-

ues for patients infected with HIV are close to the values of those infected with acute appendicitis.

There are studies demonstrating that suPAR levels are also important markers in terms of mortality and morbidity. suPAR levels in patients with sepsis have also been evaluated, and the cutoff value has been measured as 5.5 ng/dL, with 75% sensitivity and 72% specificity. In another study, the cutoff value has been found to be >6.61 mcg/L in the case of sepsis.[15,16] In yet another study, suPAR levels >10–12 ng/dL have been demonstrated as a prognostic marker for estimated mortality rates in pneumonia-related sepsis.[13] In a study by Okulu et al.,[17] suPAR level has been found to be an effective marker in infants with sepsis, and the cutoff value has been measured to be 11.3 ng/dL. In our study, the median suPAR level in the CA group amounted to 4.17 (IQR, 3.55–5.5) ng/mL. There were no patients in life-threatening or critical condition due to appendicitis in any group. No high suPAR levels were found. Among other markers, a wide range of results were observed, particularly in terms of CRP levels.

Consequently, it is important that an appendicitis diagnosis could be established clinically, especially with physical examination. None of the currently existing markers have yet proven to be definitively sensitive or selective. Biomarkers are helpful for formulating the diagnosis. High suPAR levels have been observed to be beneficial in diagnosing appendicitis, differentiating cases of CA, and demonstrating the seriousness of the illness. It is also beneficial in establishing a differential diagnosis for illnesses such as pneumonia, which give rise to symptoms such as stomach pain. For serum suPAR levels to attain clinical use, there is need for large-scale and well-attended studies evaluating patients with complaints of acute abdominal pain.

Conflict of interest: None declared.

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ORİJİNAL ÇALIŞMA - *ÖZET*

Çocuk hastalarda suPAR'ın (Serum soluble urokinase plasminogen activator receptor) akut panadisit tanısında yararı var mıdır?

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AMAÇ: suPAR (Soluble urokinase plasminogen activator receptor) enflamasyon düzeyinin tesbitinde kullanılan yeni bir biyobelirteçtir. Çalışmamızın amacı suPAR düzeylerinin çocuklarda akut apandidsit tanısındaki ve akut ve kronik apandisit ayırımındaki yerini tartışmaktır.

GEREÇ VE YÖNTEM: Çalışmamıza 40 komplike apandisit, 40 komplike olmaya apandisit ve 25 kontrol grubu oluştumak üzere 105 çocuk alındı. Tüm hastalardan ameliyat öncesinde suPAR, C-reaktif protein, lökosit, nötrofil ve nötrofil yüzdesi bakılmak üzere kan örnekleri alındı.

BULGULAR: Apandisitli gruplarda kontrol grubuna göre suPAR, C-reaktif protein, lökosit, nötrofil ve nötrofil yüzdesi anlamlı olarak yüksek bulundu. Komplike apandisitlerde suPAR değeri anlamlı olarak daha yüksek bulundu (p=0.016).

TARTIŞMA: Çalışmamız kan suPAR seviyelerinin akut ve komplike apandisit ayırımında faydalı olduğunu göstermiştir.

Anahtar sözcükler: komplike apandisit; komplike olmayan apandisit; soluble urokinase plasminogen activator receptor (suPAR).

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