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THE MOST USEFUL MARKER FOR THE DIAGNOSIS OF ACUTE APPENDICITIS: SERUM AMYLOID-A

AKUT APANDİSİT TANISI KOYMADA EN DEĞERLİ BELİRTEÇ: SERUM AMİLOİD-A

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Abstract

Objective: Leukocytosis and high CRP levels which are used in the diagnosis of acute appendicitis may be insufficient to support the diagnosis of recurrent infections in children. The objective of our study was to evaluate the Serum Amyloid A protein (SAA) levels in children with confirmed acute appendicitis and to compare the sensitivity and specificity of this marker of inflammation with WBC and CRP.
Methods: Sixty nine children (27 girls, 42 boys) who were admitted to the emergency service with abdominal pain were included in the study. Patients were divided into three groups as follows; Group-I: Patients with acute abdomen was operated with pre-diagnosis of acute appendicitis; Group-II: Patients who were hospitalized with the diagnosis of acute abdominal pain, but not operated; Group C: Patients who applied to emergency service for reasons other than acute abdominal pain, and cared for amyloid levels. The blood serum SAA, ESR, CRP and WBC at first admission, 6th hours, and 24th hours were measured and compared.
Results: In first admission, at the 6th and 24th hours SAA and CRP levels were significantly higher in *Group-I* when compared with *Group-II* and *Group-C* (p<0.001, p<0.05, respectively). There was no statistically significant difference between the two groups in terms of the WBC (p>0.07).
Conclusion: SAA levels have better discriminatory value than WBC or CRP in the assessment of acute appendicitis in children. Thus, this test appears to be of higher value than the current standards of care in the diagnosis of this condition.

Keywords: *Abdominal pain, acute appendicitis, SAA, CRP, WBC*

Öz

Amaç: Çocuk yaş grubunda akut karın ağrısının en sık nedenlerinden birisi olan akut apandisitin tanısında kullanılan lökositoz ve CRP yüksekliği, tekrarlayan enfeksiyonlar sebebi ile tanıyı desteklemekte yetersiz kalabilmektedir. Bu çalışmada akut karın ile başvuran hastalarda serum-amiloid A (SAA) seviyesinin akut apandisit teşhisinde ve cerrahi endikasyonu belirlemede güvenilirlik düzeyini ortaya koymak amaçlandı.
Yöntem: Altmış dokuz hasta (27 kız, 42 erkek) çalışmaya dahil edildi. Hastalar 23' er hastanın olduğu üç gruba ayrıldı. *Grup-I* akut apandisit ön tanısı ile operasyona alınan akut karınlı hastalardan, *Grup-II* akut karın ön tanısı ile servise yatırılıp takip edilen ancak cerrahi akut karın düşünülmediği için opere edilmeyen hastalardan, *Grup-C* ise akut karın ağrısı dışı nedenlerle acil servise başvuran sadece amiloid seviyesi bakılan hastalardan oluştu. Her üç gruptaki tüm hastalardan ilk başvuru anında kan alınarak SAA, ESR, CRP ve BK değerleri ölçüldü. Grup-I' de ameliyat sonrası 6. ve 24. saatte; Grup-II' de yatışı takiben 6. ve 24. saatte SAA, CRP ve BK değerlerinin tekrarı için kan alındı. Üç grubun kan ESR, SAA, CRP ve BK seviyeleri karşılaştırıldı.
Bulgular: Grup-I' deki hastaların başvuru anında, post-operatif 6. ve 24. saatte alınan SAA ve CRP seviyeleri Grup-II ve Grup-C ile karşılaştırıldığında istatistiksel olarak anlamlı yüksek saptanırken BK seviyelerinde anlamlı bir fark saptanmadı.
Sonuç: SAA çocuklarda akut apandisit tanısını koymada CRP ve BK' ye göre daha değerlidir ve enflamasyonun şiddetiyle doğru orantılı olarak artar.

Anahtar Kelimeler: *Karın ağrısı, akut apandisit, SAA, CRP, BK*

Introduction

It is reported in pediatric patients that acute appendicitis is responsible for 22-39 % of acute abdomen cases. It can be difficult to diagnose acute appendicitis especially in pre-school children. A definitive diagnosis can be made in 43-72% of patients with initial evaluation. Negative appendectomy rate among children varies between 4-50 % in different studies. The history and physical examination of patients play an important role in diagnosis. Laboratory tests and radiological imaging methods are used for diagnosis but they are not sensitive and specific.[1, 2] The low reliability of diagnostic methods has led to the search for new diagnostic methods. The aim of improving the reliability of diagnostic methods is to reduce the negative appendectomy and perforation rates.[1- 3] The aim of this study was to determine the reliability of high levels of SAA (serum amyloid-A) in making the definitive diagnosis of appendicitis and in decision-making for surgical treatment in pediatric patients admitted to the emergency department with abdominal pain.

Methods

This study included the patients aged 0-17 years-old who admitted to our emergency department with abdominal pain. Patients who were diagnosed with FMF (familial mediterrean fever) and who underwent surgery for reasons other than acute appendicitis were excluded from the study. Patients were divided into three equal groups. Group I consisted of patients with acute abdomen who underwent appendectomy with the preliminary diagnosis of appendicitis. Group II consisted of patients who were hospitalized with the preliminary diagnosis of acute abdomen but who were conservatively followed without surgery. Group C (control) consisted of patients who were admitted to the emergency department for reasons other than abdominal pain and who were examined to determine SAA levels.

Laboratory examinations and radiological imaging methods were performed due to suspicion of acute abdomen in the patients in Group I and II. Patients who were diagnosed with acute appendicitis based on clinical observation and adjunctive diagnostic methods underwent surgery. Other patients who were admitted to the emergency department with acute abdomen symptoms were hospitalized for observation purposes because there was no indication for surgery. The radiological examinations and pathology results of the patients in Group I and II were examined after the treatment to reach a definitive diagnosis.

Blood samples were taken from all patients in first admission, and then SAA, CRP, and WBC levels were measured. SAA, CRP, and WBC levels were measured again at 6^{th} and 24^{th} postoperative hours in Group I and at 6^{th} and 24^{th} hours after hospital admission in Group II. SAA, CRP, and WBC levels measured at baseline in all groups were statistically compared. SAA, CRP, and WBC levels measured at 6^{th} and 24^{th} hours in Group I and II were statistically compared as well. Moreover, SAA, CRP, and WBC levels measured at baseline and at 6^{th} and 24^{th} hours were statistically compared in Group I and II.

Biochemical Analysis

Serum CRP levels were measured using the COBAS INTEGRA 800 analyzer with immunoturbidimetric method (CRPLX from Roche Diagnostics) at the Biochemistry Laboratory of Kocaeli University Medical Faculty Hospital. Blood samples were taken to the straight tubes and were centrifuged at 3000 rpm for 10 minutes. The serum samples were separated and then were stored at -80°C in microsantrifuge tubes.

SAA levels were measured by adding 0.5 cc of patient's serum into a commercial kit (Invitrogen Corporation, Camarillo, CA, USA; Catalog No. KHA0011KHA0012KHA0011C) using the ELISA method. The measurement unit of the kit used in the study was ng/ml. The values below 2.0 x 10^6 ng/ml were considered as normal. The values between 2.0 - 4.0x10^6 ng/ml represented moderate-severe inflammation. The values above 5.0 x10^6 ng/ml represented severe systemic inflammation.[4]

WBC count was performed using an automated blood cell counter (Sysmex XT 2000 i, Roche diagnostics, Sysmex Corporation, Kobe, Japan). White blood cell count ≥ 10.000 cells/mm^3 was considered as leukocytosis.

Ultrasonography was performed with the Toshiba Nevio device. Firstly, the entire abdomen was scanned with a 3.5 MHz convex probe. Then, the right lower quadrant was scanned for acute appendicitis with an 8 MHz superficial probe. The inflamed appendix was defined as a non-compressible, immobilized intestinal segment over 6 mm. It appeared as a tubular structure extending from the cecum to the blunt end in the longitudinal section and as a target form in the axial section.

Statistical Analysis

Statistical analysis was performed using the SPSS for Windows 10.0 package program. The mean and standard deviation values for WBC, ESR, CRP, and SAA levels were determined in all groups.

The results were evaluated by the Mann-Whitney U test and t-test. They were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

The Wilcoxon Two-Sample Paired Signed Rank Test was used to compare the values of the blood samples taken simultaneously in each group. The error probability value less than 5% ($p<0.05$) was considered statistically significant.

Results

A total of 69 patients (27 girls, 42 boys) were enrolled in the study. 23 patients were included in each group. There was no significant difference between the groups in terms of age and gender distribution (Table 1).

Table 1. General distribution of patients included in groups

Group	Gender		Age	Most common symptom	Treatment
	M	F			
1	17	6	9.83 (1-17) years	Abdominal pain, fever, vomiting	Surgical
2	13	10	7.80 (3-17) years	Widespread abdominal pain, nausea	5 patient surgical 18 patient medical
C*	12	11	5.87 (0-17) years	Symptoms other than abdominal pain	Symptomatic
Total	42	27	7.83 (for the three groups)		28 patient surgical 41 patient medical

*C: Control

In Group I, 16 patients had acute appendicitis, 3 patients had phlegmonous appendicitis, and 7 patients had perforated appendicitis.

In Group II, 5 patients who were followed up with acute abdominal pain underwent emergency surgery because acute abdomen symptoms did not decrease during follow-up. Laparotomy revealed ileus, partial volvulus, ruptured ovarian cyst, and two different invaginations (one was the starting point of the Meckel Diverticulum, and the other was the starting point of the polyp).

The mean SAA level at baseline was measured as 935.219±35.319 mg/l in Group I, as 259.691±28.413 mg/l in Group II, and as 308.615±585.73 mg/l in Group C. The mean SAA level at baseline was higher in Group I at a statistically significant level when compared with Group II and Group C (p<0.05). There was no statistically significant difference between Group II and Group C in terms of mean SAA level at baseline.

The mean WBC level at baseline was measured as 16.223±1.169x10^3/ul in Group I, as 13.333±1.260 x10^3/ul in Group II, and as 13.304±1.310x10^3/ul in Group C. There was no statistically significant difference between the three groups in terms of mean WBC level at baseline (p=0.17).

The mean CRP level at baseline was measured as 3.96 ±1.05 mg/dl in Group I, as 3.08±1.09 mg/dl in Group II, and as 0.90±0.94 in Group C. There was no statistically significant difference between Group I and Group II in terms of mean CRP level at baseline. However, the mean CRP level at baseline was lower in Group C at a statistically significant level when compared with Group I and Group II (p=0.01) (Table 2).

Table 2. Comparison of all parameters of patients with acute appendicitis and control groups

	AA (n=23)	Control (n=23)	*p*
Age	9±3	8±4	0.243
Gender, F	6	7	0.892
Serum Amyloid-A	950±35	950±35	<0.001
CRP	4±0.9	1±0.2	0.01
Sedimentation	16.000±200	13.000±300	0.17
WBC	17±1	10±1	0.15

AA: Acute appendicitis, CRP: C-reactive protein, WBC: white blood count, F: Female

When SAA, CRP, and WBC levels measured at 6th and 24th hours in Group I and II were examined the following results were obtained:

In Group I, WBC, SAA, and CRP levels measured at 6th hour were 15.79130, 959.273, and 7.36, respectively.

In Group II, WBC, SAA, and CRP levels measured at 6th hour were 13.164, 593.195, and 2.36, respectively.

There was no statistically significant difference between Group I and II in terms of WBC levels measured at 6th hour.

There was a statistically significant difference between Group I and II in terms of SAA and CRP levels measured at 6th hour.

In Group I, WBC, SAA, and CRP levels measured at 24th hour were 14.757, 905.558, and 9.24, respectively.

In Group II, WBC, SAA, and CRP levels measured at 6th hour were 13.043, 485.917, and 1.94, respectively.

There was a statistically significant difference between Group I and II in terms of SAA and CRP levels measured at 24th hour.

In Group I, when comparing the WBC, SAA, and CRP levels obtained at three different times, there was a statistically significant difference in only CRP level. There was no statistically significant difference in SAA and WBC levels.

In Group II, when comparing the WBC, SAA, and CRP levels obtained at three different times, there was a statistically significant difference between SAA levels measured at baseline and at 6th and 24th hours. When the SAA levels in Group II were examined, it was noticed that the SAA levels of five patients who underwent surgery increased during follow-up.

CRP and WBC levels at baseline were found to be higher in Group I and II. However, SAA elevation was more prominent in Group I than in Group II (Figure 1).

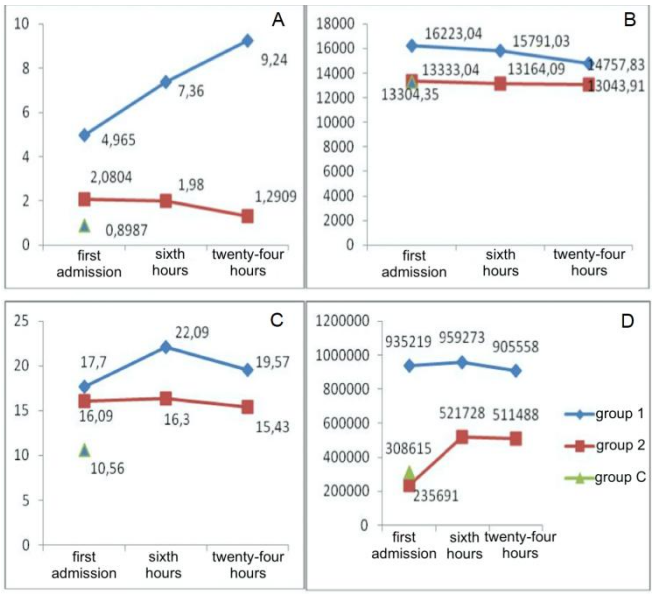

Figure 1. CRP (A), WBC (B), ESR (C), and SAA (D) values of all groups

The ROC analysis, which was performed to compare the parameters at first admission after making a definitive diagnosis, revealed that an SAA value above 500 mg/ml was 100% specific and 9 % sensitive for the diagnosis of acute abdomen requiring surgery. It was concluded that SAA is a better parameter than CRP and WBC in the diagnosis of acute abdomen requiring surgery (Figure 2).

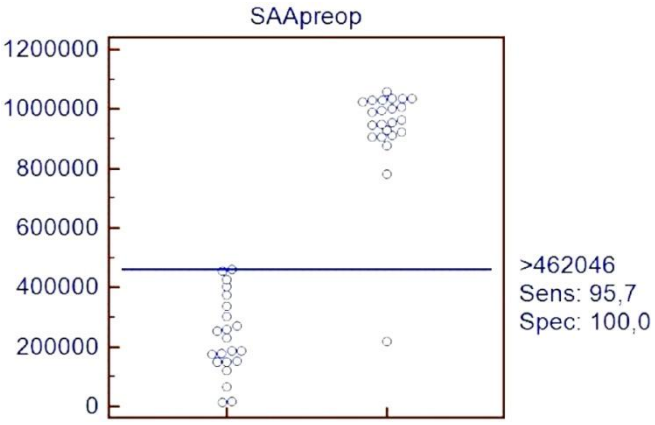

Figure 2. ROC analysis of SAA values

Discussion

Acute appendicitis is the most common cause of acute abdomen in children and ranks first among diseases that require urgent surgery. Eighteen percent of children who refer to emergency departments with abdominal pain are

diagnosed with appendicitis. The risk of perforation is 20 – 50% in children who are diagnosed as having appendicitis, and the rate of unnecessary appendectomy is 40%. [1,2,6]

Since the development of inflammation plays a role in the pathophysiology of acute appendicitis, a diffuse inflammatory response is prominent . For this reason, laboratory tests in patients with acute appendicitis should detect systemic inflammation and should be widespread and easy to use. In addition, the test should be low-cost and should be repeated if necessary. Laboratory tests such as WBC and CRP have been used for a long time to support clinical data at the final decision stage. However, these tests have low specificity and low positive predictive value for diagnosing appendicitis because they increase other inflammatory conditions mimicking appendicitis. CRP, a non-specific inflammatory marker, has a sensitivity rate of 43 – 92% and a specificity rate of 33 – 95% for diagnosing appendicitis in children with acute abdominal pain. The reasons why the results of the studies show a wide range are the collection of data at different times, the use of different methods, and the use of different positive criteria. Although CRP is the best known and most widely used parameter for infection, CRP alone is inadequate in the early diagnosis of infection in some cases.

CRP is an important acute phase reactant that is used clinically to distinguish between viral and bacterial infections that generally show the same findings, to assess the response to antibiotic treatment of severe bacterial infections, and to identify diseases that develop as complications.

Serial measurements of CRP together with clinical findings rather than a single value give more information about the course of the disease. [5,6] In 5 patients (Group II) in which we did not consider surgery at first admission but followed up due to abdominal pain, SAA levels increased significantly at 6th and 24th hours after hospital admission, but there was no increase in CRP levels at the same rate. 5 patients underwent surgery due to the lack of improvement during follow-up. 2 patients had invagination, 1 patient had a ruptured ovarian cyst, and 2 patients had brid ileus. This indicates that SAA rises more distinctly not only in acute appendicitis but in all pathologies requiring surgery compared with other APRs.

The increase in WBC level as an early inflammatory response is a known condition in the diagnosis of acute appendicitis. However, WBC has a sensitivity rate of 77% and a specificity rate of 62% for diagnosing acute appendicitis. Stefanutti et al. showed that the increase in CRP and WBC levels in patients with acute abdomen was not a reliable parameter in making a decision about surgery.[4,6] The use of currently available inflammatory tests as single or dual combination has limited value in the diagnosis of acute appendicitis.[1,2,7] In the study, it was also found that CRP and WBC levels were high and correlated in patients who responded to medical treatment and who were discharged without need of surgery.

It is thought that the etiology of appendicitis in children is mostly of viral origin. Among all APRs used for appendicitis, the most sensitive marker for viral infections is SAA. This information also indicates that SAA is the most important marker in the diagnosis of acute appendicitis, especially in children.

Today, SAA is used as a specific acute phase protein in clinical practice. SAA and CRP reflect endotoxin levels. Immune activation in the liver is triggered by inflammation, tissue damage, and infection. SAA release begins within a few hours. SAA levels peak within 2 - 3 days (100 to 1000 - fold increase) and then return to basal levels 5 - 7 days later. SAA rises arlier and more sharply than CRP. Unlike CRP, it tends to increase in both viral and bacterial infections. No statistically significant difference was found between SAA and CRP levels measured at first admission in Group I (p<0.05). However, SAA levels were approximately 2.6 times higher in Group I than Group II and Group C.

It is reported that the SAA value in healthy individuals is 9.7 mg/l.[3] The measurement unit of the kit used in the study was ng/ml. The values below 2.0 x 10^6 ng/ml were considered as normal. In the study, the fact that 10 patients in Group C had higher serum levels of SAA according to blood tests done for reasons other than abdominal pain was because the patients had infectious diseases of the other organ systems.

In Group II, SAA levels were elevated during follow-up in 5 of the 23 patients who were followed up due to acute abdomen. The clinical findings of these patients with high SAA levels did not improve. Therefore, surgical procedure was decided. They were seen to have surgical pathologies other than acute appendicitis. ccording to the obtained data, we found that SAA had a sensitivity rate of 82.1% and a specificity rate of 56.4% for diagnosing appendicitis.

In the ROC analysis, it was concluded that SAA can be used as a better parameter than CRP and WBC in the diagnosis of acute abdomen requiring surgery in patients with SAA > 500 mg/ml.

With the results of the this study, it was concluded that SAA can be used as a better parameter than CRP and WBC in the diagnosis of acute abdomen requiring surgery in patients with SAA>500 mg/ml.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Compliance with Ethical Statement

This study was approved by The Ethics Committee of Kocaeli University, Turkey (Project number; 2010/53). Written informed consents were obtained from all patients.

Author Contributions

GEY: Hypotesis; MAA: Design; MAA, HMK, ÇV: Data collection; ELE, GEY: Analysis; GEY, MAA: Literature search; MAA: Manuscript writing.

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