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Correlation between the serum and tissue levels of oxidative stress markers and the extent of inflammation in acute appendicitis

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OBJECTIVES: To determine the serum and tissue levels of markers of impaired oxidative metabolism and correlate these levels with the histopathology and Alvarado score of acute appendicitis patients.

METHOD: Sixty-five acute appendicitis patients (mean age, 31.4±12.06 years; male/female, 30/35) and 30 healthy control subjects were studied. The Alvarado score was recorded. Serum samples were obtained before surgery and 12 hours postoperatively to examine the total antioxidant status, total oxidant status, paraoxonase, stimulated paraoxonase, arylesterase, catalase, myeloperoxidase, ceruloplasmin, oxidative stress markers (advanced oxidized protein products and total thiol level) and ischemia-modified albumin. Surgical specimens were also evaluated.

RESULTS: The diagnoses were acute appendicitis (n = 37), perforated appendicitis (n = 8), phlegmonous appendicitis (n = 12), perforated+phlegmonous appendicitis (n = 4), or no appendicitis (n = 4). The Alvarado score of the acute appendicitis group was significantly lower than that of the perforated+phlegmonous appendicitis group ($p = 0.004$). The serum total antioxidant status, total thiol level, advanced oxidized protein products, total oxidant status, catalase, arylesterase, and ischemia-modified albumin levels were significantly different between the acute appendicitis and control groups. There was no correlation between the pathological extent of acute appendicitis and the tissue levels of the markers; additionally, there was no correlation between the tissue and serum levels of any of the parameters.

CONCLUSIONS: The imbalance of oxidant/antioxidant systems plays a role in the pathogenesis acute appendicitis. The Alvarado score can successfully predict the presence and extent of acute appendicitis.

KEYWORDS: Appendicitis; Oxidative Stress; Inflammation.

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INTRODUCTION

Acute appendicitis is the most common cause of acute abdominal pain requiring appendectomy, which is the most frequently applied intervention in the daily clinical practice of general surgery (1).

The diagnosis of acute appendicitis is primarily based on clinical symptoms, physical examination and history. However, because symptoms of appendicitis overlap with many urologic, abdominal and gynecologic conditions, achieving a definitive diagnosis is a clinical challenge. Indeed, after clinical diagnosis, a negative appendicectomy rate of 12% and missed perforated appendicitis rate of 3.4% have been reported in the literature (2,3). To support the diagnosis, preoperative scoring systems such as the Alvarado score, which enables risk stratification for acute appendicitis in patients presenting with acute abdominal symptoms, were developed (4); however, these systems are rarely used in practice. Furthermore, although radiological and blood tests can be used to aid in the diagnosis and decrease the negative appendectomy and perforation rates (2,3), there is currently no specific diagnostic test for appendicitis.

To develop specific diagnostic tests, the molecular and biochemical etiology and course of acute appendicitis must be determined. While the etiology of acute appendicitis has

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been described in detail, the factors that affect the progression and predict the degree of appendicitis have yet to be investigated (1). Some biochemical markers, such as those used to identify oxidative stress and inflammation, have been proposed as indicators of the presence and extent of acute appendicitis (5,6). However, data on the tissue and plasma levels of these markers as well as the histopathological characteristics are limited. Developing specific diagnostic tools for appendicitis would both prevent unnecessary surgery and help to define the advanced stages of inflammation requiring urgent intervention, such as perforated appendicitis, which is associated with increased morbidity and mortality.

In this study, we hypothesized that oxidative stress has a role in the pathogenesis of acute appendicitis and we aimed to determine the serum and tissue levels of markers of impaired oxidative metabolism as well as their correlation with the histopathological diagnosis and Alvarado score of patients with acute appendicitis. We also evaluated the variables preoperatively and postoperatively to determine the effect of inflammation on these variables.

MATERIALS AND METHODS

Study design and patients

All patients who underwent appendectomy at the Department of General Surgery of Ataturk Training and Research Hospital between May 2013 and October 2013 were included in this prospective study, which was approved by the Institutional Ethics Committee of Yildirim Beyazit University Medical School (approval date: April 29, 2013; number: 57) and conducted in accordance with the latest version of the Helsinki Declaration. Patients signed written informed consent forms before participating in the study.

The Alvarado score, which is a 10-point clinical scoring system for the diagnosis of acute appendicitis based on the symptoms, signs and diagnostic tests of patients presenting with suspected acute appendicitis, was used to stratify patients before surgery (4) (Table 1). The Alvarado score is a valid and useful diagnostic tool for acute appendicitis. It rules out appendicitis at a cut-point of 5 with high sensitivity and specificity for all patient groups (7).

Open surgical appendectomy was applied for the majority of patients. For open appendectomy, a Rockey-Davis or McBurney incision was used for the laparotomy. The anterolateral abdominal muscles were split and the peritoneum was incised. The mesoappendix was ligated with 2/0 polyglactin and then divided. The base of the appendix was ligated with 2/0 polyglactin. A hemostat was applied to the distal side of the specimen, and the appendix was transected. The incision was closed in an anatomic fashion.

Table 1 - Alvarado score for appendicitis (4).

	Score
Symptoms	
Migratory right iliac fossa pain	1
Nausea/vomiting	1
Anorexia	1
Signs	
Tenderness in the right iliac fossa	2
Rebound tenderness in the right iliac fossa	1
Elevated temperature	1
Laboratory findings	
Leukocytosis	1
Neutrophil shift to the left	1
Total	10

The laparoscopic approach was applied in only four patients. In these patients, pneumoperitoneum was induced with a Veress needle. A 3-trocar technique using 5- and 10-mm cannulas was used to perform laparoscopic appendectomy. The mesoappendix was dissected using an electrocautery and the stump was controlled using pre-tied suture loops. The appendix was retrieved inside a disposable bag to avoid contamination.

The excised appendices were categorized into the following five groups: acute appendicitis, perforated appendicitis, gangrenous (phlegmonous) appendicitis, perforated+phlegmonous appendicitis and normal tissue (no appendicitis) (8). Microscopically, the changes in the appendix tissue range from minimal focal inflammation to total necrosis of the appendix wall. In early lesions, neutrophils appear at the base of the crypt adjacent to a small defect in the epithelium, which is called acute appendicitis. After this inflammatory process reaches the submucosa, it spreads quickly to the remaining appendix. In the advanced stages, when the mucosa is lost and the wall is necrotic, the appendicitis is defined as gangrenous (phlegmonous). When the tissue integrity is impaired, it is called perforation.

Study procedures

Serum samples were obtained before and 12 hours after the surgery to study the following parameters: total antioxidant status (TAS), total oxidant status (TOS), paraoxonase (PON), stimulated paraoxonase (SPON), arylesterase, catalase, myeloperoxidase, ceruloplasmin, oxidative stress markers [advanced oxidized protein products (AOPP) and total thiol level (TTL)] and ischemia-modified albumin (IMA).

The serum samples of 30 healthy control subjects (age range, 19-64 years) were collected and analyzed after obtaining subjects' written consent for laboratory tests.

The specimen that was resected during surgery was evaluated to determine the tissue protein level (mg/mL) according to the Lowry method (9). Furthermore, the following parameters were determined in tissue samples: TAS, TOS, arylesterase, catalase, AOPP and myeloperoxidase. The surgical specimen was studied 12 hours after ligation to avoid possible immediate elevation of free radicals in the tissue.

To measure the TOS and TAS, fully automated calorimetric measurement methods were used (10,11). Commercial Rel assay kits were used for the measurement of PON and arylesterase activity (12,13). PON activity was measured in the absence and presence of NaCl (SPON).

Statistical analysis

The study data were summarized with descriptive statistics (mean and standard deviation for continuous variables; frequency and percentage for categorical variables). To determine the normality of the data distribution, the Kolmogorov-Smirnov test was applied. Repeated measures analysis of variance was used to compare the normally distributed continuous data before and after surgery. For comparison of categorical variables, Fisher's exact test was used. The correlations between the parameters were tested by

Table 2 - Demographic and clinical characteristics of patients with according to their pathological diagnosis.

	No appendicitis (negative)	Acute appendicitis	Perforated appendicitis	Phlegmonous appendicitis	Perforated+phlegmonous appendicitis	*p*
n	4	37	8	12	4	
Age (years)	30.5±11.1	32.06±10.47	34.88±15.74	25.92±6.49	33.5±9.82	0.371
Gender (male/female)	1/3 (25/75)	15/22 (40.5/59.5)	5/3 (62.5/37.5)	8/4 (66.7/33.3)	1/3 (25/75)	0.352
Surgical technique (open/ laparoscopic)	4/0 (100/0)	34/3 (91.9/8.1)	8/0 (100/0)	12/0 (100/0)	3/1 (75/25)	0.419
US evaluation (no/yes)	4/0 (100/0)	30/7 (81.1/18.9)	8/0 (100/0)	9/3 (75/25)	2/2 (50/50)	0.234
CT (not performed/ performed)	4/0 (100/0)	35/2 (94.6/5.4)	3/5 (37.5/62.5)	11/1 (91.7/8.3)	1/3 (25/75)	<0.001
Alvarado score	5.5±2.52	5.82±1.58	6.38±1.69	7.25±1.55	9±1.16	0.002
Hospitalization duration[a]	1.25±0.5	1.28±0.87	3.38±1.99	1.75±1.14	5±1.41	<0.001
Leukocytes (K/μL)	9650±2145.54	12021.63±4751.62	12737.5±3601.17	11583.34±4683.41	14475±8109.82	0.674

[a]The hospitalization durations of patients without appendicitis and pathologically diagnosed acute appendicitis were significantly shorter than those of the perforated and perforated+phlegmonous appendicitis groups ($p = 0.025$ and $p<0.001$ for no appendicitis; $p<0.001$ for appendicitis, respectively). The duration of hospitalization was significantly longer in perforated+phlegmonous appendicitis group than in the phlegmonous appendicitis group ($p<0.001$), while it was longer in the perforated appendicitis group than in the phlegmonous appendicitis group ($p = 0.020$).

Pearson's correlation analysis and expressed as correlation coefficients (r).

Computer software (Statistical Package for the Social Sciences, Version 17.0, SPSS Inc., Chicago, Illinois, USA) was used for all analysis. Statistical significance was set at $p<0.05$.

RESULTS

Sixty-five patients (mean age, 31.4±12.06 years; age range, 17-73 years; male/female, 30/35) with acute appendicitis were included in the study. The pathological diagnoses were acute appendicitis in 37, perforated appendicitis in 8, phlegmonous appendicitis in 12, perforated+phlegmonous appendicitis in 4 and normal tissue (no appendicitis) in 4 cases. The demographic and clinical findings of the pathological diagnosis groups were similar except for the hospitalization duration, Alvarado score and presence of CT imaging (Table 2). The Alvarado scores of the no appendicitis and acute appendicitis groups were significantly lower than those of the perforated+phlegmonous appendicitis group ($p = 0.028$ and $p = 0.004$, respectively). The duration of hospitalization was the longest in the perforated+phlegmonous appendicitis group, which was followed by the perforated appendicitis, phlegmonous appendicitis, acute appendicitis and no appendicitis groups ($p<0.001$). CT was performed for more patients in the perforated+phlegmonous (3/4 patients) and perforated appendicitis groups (3/5 patients) than the other pathology groups.

Serum parameters

The preoperative and postoperative levels of TAS, TTL and AOPP were significantly higher, while TOS, catalase and IMA were lower in the control group than in the patients with appendicitis ($p<0.05$ for all) (Table 3). The postoperative arylesterase level of the appendectomized subjects was significantly lower than of control subjects ($p = 0.043$). However, there was no significant difference between the preoperative and postoperative levels of any of the serum parameters ($p>0.05$ for all).

The TOS and catalase levels were significantly higher in patients with perforated appendicitis than in those with acute appendicitis ($p<0.05$ for both) (Table 4). Other serum parameters did not show a significant difference between pathology groups.

Tissue parameters

In pathologically acute appendicitis patients (n = 37), the mean levels of the parameters in the tissue were as follows: protein, 2.88±1.25 mg/mL; TAS, 0.33±0.23 μmol Trolox Eq/mg; TOS, 1.78±2.3 nmol H_2O_2 Eq/mg; arylesterase, 692.27±251.58 U/mg; catalase, 210.49±103.99 mU/mg; AOPP, 60.06±53.27 nmol chloramine-T Eq/mg; and

Table 3 - Levels of serum parameters in control subjects and appendectomized patients.

	Control (n = 30)	Appendectomized subjects		*p*		
		Preoperative (Pre)	Postoperative (Post) *12 hours after the surgery*	Pre *vs.* Post	Pre *vs.* Control	Post vs. Control
TAS (mmol Trolox Eq/L)	2.28±0.24	2.08±0.25	2.05±0.27	0.590	***0.042***	***0.014***
TOS (μmol H_2O_2 Eq/L)	3.15±1.4	5.89±4.8	5.23±3.78	0.593	***0.007***	***0.001***
Paraoxonase (U/L)	208.98±100.13	199.09±114.18	188.2±116.8	0.111	0.838	0.467
Stimulated paraoxonase (U/L)	589.25±309.88	561.85±346.97	528.88±354.26	0.160	0.854	0.526
Arylesterase (KU/L)	234.53±50.15	197.53±55.81	194.42±50.03	0.096	0.176	***0.043***
Ceruloplasmin (U/L)	17.57±6.74	14.76±6.42	14.62±6.93	0.535	0.056	0.088
Catalase (U/L)	104.03±82.4	252.38±170.65	229.33±158.44	0.583	***<0.001***	***<0.001***
TTL (μmol/L)	260.8±54.3	177.61±38.82	181.6±38.72	0.951	***<0.001***	***<0.001***
Myeloperoxidase (U/L)	111.64±40.21	136.73±49.98	137.59±48.4	0.826	0.080	0.057
AOPP (μmol chloramine-T Eq/L)	136.39±163.7	53.29±24.24	47.86±24.24	0.039	**0.013**	**0.007**
IMA (absorbance unit, ABSU)	0.31±0.09	0.64±0.09	0.66±0.08	0.197	***<0.001***	***<0.001***

Table 4 - Levels of serum parameters with respect to pathological diagnosis.

	No appendicitis (negative)	Acute appendicitis	Perforated appendicitis	Phlegmonous appendicitis	Perforated+phlegmonous appendicitis	*p*
TAS (mmol Trolox Eq/L)	2.16±0.13	2.06±0.26	2.07±0.3	2.05±0.26	1.99±0.28	0.738
TOS (μmol H_2O_2 Eq/L)	4.22±1.37	4.82±3.03	8.74±6.21	5.67±4.9	7.16±7.11	***0.011***
Paraoxonase (U/L)	227.24±99.47	197.34±133.32	169.26±87.54	175.93±74.87	227.73±95.89	0.608
Stimulated paraoxonase (U/L)	650.47±297.46	556.19±405.37	470.52±268.69	496.6±229.5	636.15±281.06	0.645
Arylesterase (KU/L)	214.69±48.26	196.5±51.76	193.78±49.76	182.81±61.03	216.3±45.98	0.451
Ceruloplasmin (U/L)	13.15±3.87	15.11±6.87	14.19±4.96	13.93±7.23	15.6±8.68	0.868
Catalase (U/L)	193.02±103.15	211.09±145.06	361.04±191	262.41±157.84	258.97±247.33	***0.014***
TTL (μmol/L)	194.34±39.01	182.26±36.63	155.59±23.89	182.75±44.26	178.94±52.07	0.095
Myeloperoxidase (U/L)	152.62±34.76	139.77±44.35	136.34±69.76	137.73±47.55	97.45±50.33	0.18
AOPP (μmol chloramine-T Eq/L)	47.33±12.51	46.66±20.78	66.45±31.25	51.48±26.25	55.56±32.93	0.052
IMA (absorbance unit, ABSU)	0.65±0.09	0.64±0.09	0.67±0.09	0.67±0.09	0.67±0.1	0.337

myeloperoxidase, 30.91±28.24 mU/mg. The tissue parameters did not show any difference between the pathological diagnosis groups ($p>0.05$ for all) (Table 5).

Correlation between the serum and tissue parameters

There was no significant correlation between any of the tissue and serum parameters except the tissue protein level, which had a slight negative correlation with the serum paraoxonase (r = -0.280, $p = 0.024$) and stimulated paraoxonase levels (r = -0.272, $p = 0.028$) (Table 6).

DISCUSSION

The value of biochemical markers in diagnosing and predicting the severity of acute appendicitis has been reported in several studies in the literature. McGowan et al. (5) recently identified that the preoperative bilirubin, C-reactive protein and white cell count are indicative of perforation in acute appendicitis. Yildirim et al. (14) also reported that elevated levels of serum inflammatory markers could be used to confirm the diagnosis of acute appendicitis. In addition to inflammatory markers, oxidative stress markers have recently become an area of interest. Oxidative stress and imbalance in the prooxidant/oxidant defense system are proposed to be involved in the pathology and progression of appendicitis (15,16). In this respect, the serum levels of thiol and nitric oxide metabolites, oxidative stress markers and lipid peroxidation markers were studied. Yilmaz et al. (15) reported that serum nitric oxide levels and oxidative stress are elevated in acute appendicitis independent of the extent of the lesion. Kaya et al. (17) reported that along with the plasma levels of total protein, albumin, uric acid and bilirubin, the levels of plasma antioxidant components, including TAS, were elevated in all patients with acute appendicitis (n = 12). These authors suggested that the induction of the antioxidative response plays a role in the development of acute appendicitis. In another study by Koltuksuz et al. (18), superoxide dismutase and malondialdehyde levels were studied to determine the oxidative activities in the plasma of patients with various pathologic diagnoses of acute appendicitis. The authors found that the plasma level of these oxidative markers increased in acute appendicitis in response to increases in oxygen free radicals, which may play an important role in the extent of appendicitis. Furthermore, Satomi et al. (19) studied the tissue levels of markers and showed a correlation between tissue superoxide dismutase and the degree of inflammation, which indicates that active oxygen influences the degree of inflammation in particularly phlegmonous and gangrenous appendicitis. It should be noted that previous studies reporting the associations between the degree of appendicitis and variables related to oxidative stress were sometimes described from a diagnostic perspective and other times from an etiologic perspective. It is therefore not possible to distinguish the cause from the effect in these studies or in our present study.

Although a number of studies have focused on the serum level of oxidative stress markers, reports on the tissue levels of these markers are limited. As a result, we aimed to determine the levels of oxidative stress markers in both plasma and in tissue, identify a correlation between them and evaluate the correlation between the pathological diagnosis or Alvarado score and marker levels. We studied several markers indicative of oxidative stress and the activation of the antimicrobial defense system (20), such TAS, TOS, PON, SPON, arylesterase, catalase, myeloperoxidase, ceruloplasmin,

Table 5 - Levels of tissue parameters with respect to the pathological diagnosis.

	No appendicitis (negative)	Acute appendicitis	Perforated appendicitis	Phlegmonous appendicitis	Perforated+phlegmonous appendicitis	*p*
Protein level with the Lowry method (mg/mL)	1.87±1.9	2.88±1.25	2.63±1.87	3.19±1.72	3.22±0.76	0.568
TAS (μmol Trolox Eq/mg)	0.54±0.37	0.33±0.23	0.46±0.35	0.33±0.1	0.25±0.02	0.258
TOS (nmol H_2O_2 Eq/mg)	2.22±2.24	1.78±2.3	5.39±8.87	3.22±1.89	0.93±0.67	0.117
Arylesterase (U/mg)	590.8±117.13	692.27±251.58	541.07±291.24	637.47±258.54	737.4±76.91	0.516
Catalase (mU/mg)	200.93±170.43	210.49±103.99	146.85±71.91	174.6±69.5	193.93±35.25	0.495
AOPP (nmol chloramine-T Eq/mg)	122.37±105.85	60.06±53.27	97±128.6	54.76±23.37	56.82±30.04	0.261
Myeloperoxidase (mU/mg)	19.18±17.15	30.91±28.24	38.18±50.33	48.01±47	31.01±37.13	0.566

Table 6 - Coefficient (r) and significance of the correlation between the tissue and serum parameters.

Tissue parameters		Serum parameters										
		TAS	TOS	Paraoxonase	Stimulated paraoxonase	Arylesterase	Ceruloplasmin	Catalase	TTL	Myeloperoxidase	AOPP	IMA
Protein level	r	-0.105	0.078	-0.280	-0.272	-0.113	-0.019	0.129	0.070	0.063	0.030	-0.015
	p	0.405	0.536	***0.024***	***0.028***	0.370	0.878	0.307	0.580	0.619	0.812	0.904
TAS	r	0.022	-0.123	0.201	0.197	-0.062	-0.038	-0.182	-0.106	0.101	-0.133	-0.137
	p	0.864	0.328	0.108	0.116	0.621	0.762	0.148	0.399	0.422	0.293	0.278
TOS	r	-0.067	-0.087	0.070	0.067	0.038	-0.011	-0.144	-0.064	-0.038	-0.171	-0.121
	p	0.598	0.491	0.579	0.595	0.763	0.928	0.252	0.612	0.765	0.172	0.336
Arylesterase	r	0.012	0.009	0.059	0.060	0.036	0.040	0.024	-0.011	-0.026	0.065	0.072
	p	0.923	0.942	0.643	0.638	0.779	0.753	0.847	0.929	0.835	0.608	0.568
Catalase	r	0.025	-0.062	0.027	0.019	0.082	0.068	-0.020	-0.070	-0.130	0.077	-0.008
	p	0.842	0.625	0.832	0.878	0.514	0.592	0.872	0.578	0.303	0.541	0.951
AOPP	r	0.039	-0.115	0.187	0.179	0.043	0.034	-0.186	-0.134	-0.017	-0.165	-0.074
	p	0.761	0.364	0.135	0.154	0.734	0.786	0.138	0.288	0.891	0.189	0.556
Myeloperoxidase	r	0.011	0.046	-0.010	-0.006	-0.005	-0.147	0.105	0.070	-0.069	0.024	-0.130
	p	0.933	0.716	0.938	0.959	0.970	0.242	0.405	0.581	0.587	0.851	0.302

AOPP, TTL and IMA, in the serum samples and we examined tissue protein, TAS, TOS, arylesterase, catalase, AOPP and myeloperoxidase in the surgically resected tissue.

We found that the Alvarado score significantly increases with increasing severity of acute appendicitis. This finding supports the report by Ohle et al. (7), which suggested that an Alvarado score cut point of 5 could be used to rule out an acute appendicitis diagnosis. In our study, patients with acute appendicitis had an Alvarado score over 5 and the score increased significantly in perforated, phlegmonous and perforated+phlegmonous cases. This finding indicates that in addition to being useful in the diagnosis of acute appendicitis, the Alvarado score can be used to predict the severity of inflammation.

Although US and CT are commonly suggested for diagnosing acute appendicitis in the literature (1,21,22), these radiologic modalities are applied in our clinical practice only for patients with severe symptoms. Therefore, the rates of US and CT evaluation were higher in patients with perforated and/or phlegmonous appendicitis. Clinical evidence suggests that laparoscopic appendectomy has some advantages over surgery (1). However, in our clinic, due to limited availability of technical equipment, we still predominantly perform open surgery for patients with acute appendicitis.

The serum leukocyte count was higher in perforated and/or phlegmonous appendicitis patients in our study, but this difference was not statistically significant. This finding is not in agreement with the results of previous studies, which reported significantly higher white cell and neutrophil counts in acute appendicitis (22), particularly in perforated appendicitis (5). The differences between these findings might be due to the small sample size, particularly for perforated appendicitis cases in our study.

In our study, both the preoperative and postoperative serum levels of TAS, TTL, AOPP, TOS, catalase, arylesterase and IMA in patients with acute appendicitis were significantly different compared to the control group and there were no differences between the preoperative and postoperative values. On the basis of these findings, as suggested by previous studies, the imbalance between the oxidant and antioxidant defense systems plays a role in the pathogenesis of acute appendicitis. This imbalance is not restored to normal with surgery and a longer follow-up time may be needed to determine the time required to alleviate oxidative stress. Furthermore, the serum levels of TOS and catalase were significantly higher in patients with perforated appendicitis than in those with acute appendicitis, demonstrating that these markers may indicate the pathological extent of the disease.

We also measured the protein and oxidative marker levels in surgically resected tissue and found no correlation between the pathological extent of acute appendicitis and the tissue levels of these markers. Satomi et al. (19), on the other hand, reported increased tissue oxidative activity in phlegmonous and gangrenous appendicitis compared to uncomplicated acute appendicitis in a 56-patient study. The lack of significant findings in our study may be due to the small sample size, particularly for perforated and phlegmonous appendicitis cases. We also found no correlation between the tissue and serum levels of any of the parameters, although there was a weak negative correlation between serum PON and SPON levels and the tissue protein levels. Therefore, we suggest that serum oxidative stress markers can better predict oxidative imbalance in acute appendicitis compared with tissue markers.

The main limitation of our study is its limited sample size, which resulted in even smaller sample sizes for each pathological diagnosis group and low statistical power. Another limitation is that we did not compare the serum parameters among the different pathological diagnosis groups, which precludes the evaluation of the relationship between the serum levels of biochemical markers and the extent of inflammation. It is also worth noting that we did not analyze the diagnostic properties of variables with statistical tests, such as receiver operating characteristic analysis, sensitivity and specificity. Furthermore, we did not compare the diagnostic properties of the new variables with traditional markers of inflammation such as the white blood cell count, proportion of neutrophils, temperature, or C-reactive protein. Nevertheless, this pilot study is the first to examine the levels of biochemical markers in plasma and tissue together with the pathological diagnosis in patients with acute appendicitis. Therefore, our study will provide the basis for future studies on the role of oxidative stress in the pathogenesis and diagnosis of acute appendicitis.

In conclusion, the serum levels of oxidative stress markers change in patients with acute appendicitis, showing that the

imbalance between oxidant and antioxidant defense systems may play a role in the pathogenesis of acute appendicitis, which could be either etiologic or a consequence of inflammation. These serum markers may be used to confirm the diagnosis of appendicitis and to determine the extent of the disease. Furthermore, we found that the Alvarado score can successfully predict the presence and extent of acute appendicitis and should therefore be used more often in clinical diagnosis and treatment planning for patients with acute appendicitis.

AUTHOR CONTRIBUTIONS

Dumlu EG, Tokaç M, Bozkurt B, Yildirim MB, Ergin M, Yalçin A and Kiliç M designed the work, interpreted the data, drafted the manuscript, provided final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Humes DJ, Simpson J. Acute appendicitis. BMJ. 2006;333(7567):530-4, http://dx.doi.org/10.1136/bmj.38940.664363, http://dx.doi.org/10.1136/bmj.38940.664363.AE.
2. Park JS, Jeong JH, Lee JI, Lee JH, Park JK, Moon HJ. Accuracies of diagnostic methods for acute appendicitis. Am Surg. 2013;79(1):101-6.
3. Toorenvliet BR, Wiersma F, Bakker RF, Merkus JW, Breslau PJ, Hamming JF. Routine ultrasound and limited computed tomography for the diagnosis of acute appendicitis. World J Surg. 2010;34(10):2278-85.
4. Alvarado A. A practical score for the early diagnosis of acute appendicitis. Ann Emerg Med. 1986;15(5):557-64, http://dx.doi.org/10.1016/S0196-0644(86)80993-3.
5. McGowan DR, Sims HM, Zia K, Uheba M, Shaikh IA. The value of biochemical markers in predicting a perforation in acute appendicitis. ANZ J Surg. 2013;83(1-2):79-83.
6. Ozdogan M, Devay AO, Gurer A, Ersoy E, Devay SD, Kulacoglu H, et al. Plasma total anti-oxidant capacity correlates inversely with the extent of acute appendicitis: a case control study. World J Emerg Surg. 2006;1:6.
7. Ohle R, O'Reilly F, O'Brien KK, Fahey T, Dimitrov BD. The Alvarado score for predicting acute appendicitis: a systematic review. BMC Med. 2011;9:139, http://dx.doi.org/10.1186/1741-7015-9-139.
8. Rosai J. Rosai and Ackerman's surgical pathology. 10th ed. Elsevier, Mosby; St. Louis, MO, USA. 2011.
9. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
10. Behdad A, Hosseinpour M. Methylene blue usage in the evaluation of traumatic necrosis in a rabbit liver injury. Ulus Travma Acil Cerrahi Derg. 2009;15(4):337-41.
11. Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clin Biochem. 2004;37(2):112-9, http://dx.doi.org/10.1016/j.clinbiochem.2003.10.014
12. Erel O. A new colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11, http://dx.doi.org/10.1016/j.clinbiochem.2005.08.008.
13. Toy H, Camuzcuoğlu H, Celik H, Erel O, Aksoy N. Assesment of serum paraoxonase and arylesterase activities in early pregnancy failure. Swiss Med Wkly. 2009;139(5-6):76-81.
14. Yildirim O, Solak C, Koçert B, Unal B, Karabeyoğlu M, Bozkurt B, et al. The role of serum inflammatory markers in acute appendicitis and their success in preventing negative laparotomy. J Invest Surg. 2006;19(6):345-52, http://dx.doi.org/10.1080/08941930600985686.
15. Yilmaz FM, Yilmaz G, Erol MF, Köklü S, Yücel D. Nitric oxide, lipid peroxidation and total thiol levels in acute appendicitis. J Clin Lab Anal. 2010;24(2):63-6, http://dx.doi.org/10.1002/jcla.20301.
16. Zhavoronok TV, Stepovaia EA, Riazantseva NV, Petina GV, Sokolovich EG, Starikov IuV, et al. Impaired oxidative metabolism in acute inflammatory diseases. Klin Lab Diagn. 2006;12:10-4.
17. Kaya M, Boleken ME, Kanmaz T, Erel O, Yucesan S. Total antioxidant capacity in children with acute appendicitis. Eur J Pediatr Surg. 2006;16(1):34-8.
18. Koltuksuz U, Uz E, Ozen S, Aydinç M, Karaman A, Akyol O. Plasma superoxide dismutase activity and malondialdehyde level correlate with the extent of acute appendicitis. Pediatr Surg Int. 2000;16(8):559-61, http://dx.doi.org/10.1007/s003830000424.
19. Satomi A, Hashimoto T, Murakami S, Murai H, Kawase H, Takahashi S, et al. Tissue superoxide dismutase (SOD) activity and immunohistochemical staining in acute appendicitis: correlation with degree of inflammation. J Gastroenterol. 1996;31(5):639-45, http://dx.doi.org/10.1007/BF02347610.
20. Thomas EL. Myeloperoxidase-hydrogen peroxide-chloride antimicrobial system: effect of exogenous amines on antibacterial action against Escherichia coli. Infect Immun. 1979;25(1):110-6.
21. Linam LE, Munden M. Sonography as the first line of evaluation in children with suspected acute appendicitis. J Ultrasound Med. 2012;31(8):1153-7.
22. Kıyak G, Korukluoğlu B, Özgün Y, Devay AO, Kuşdemir A. Evaluation of Ohmann and Eskelinen scores, leukocyte count and ultrasonography findings for diagnosis of appendicitis. Ulus Travma Acil Cerrahi Derg. 2009;15(1):77-81.