

Full Length Research Paper

Oxidative stress in diagnosis of acute appendicitis patients

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The most common acute surgical infection seen in emergency department is acute appendicitis (AA). Although many advances in diagnostic system, the diagnosis of AA is not always straight forward. Early diagnosis of AA is important for reducing morbidity rates. The objective of the current study was to investigate the value of oxidative stress in the accuracy of preoperative AA diagnosis. A total of thirty consecutive patients with histopathologically confirmed AA were retrospectively included in the study. Thirty volunteers were included as control group. Serum antioxidative status was evaluated by measuring total antioxidant status (TAS) levels in patients with AA and in healthy individuals. Serum oxidative status was evaluated by measuring total oxidant status (TOS). Then also oxidative stress index (OSI) was calculated. The male/female ratio of patients was 22/8. Mean age: 31.83±1.06. TOS and OSI levels increased in patient group compared to control group (respectively, 22.71±24.82 $\mu\text{mol H}_2\text{O}_2$ equivalent/L vs 8.34±2.26 $\mu\text{mol H}_2\text{O}_2$ equivalent/L, $p = 0.003$; 1.05±1.10 arbitrary unit vs 0.40±0.09 arbitrary unit, $p = 0.002$). But there were no differences in TAS levels of both groups, $p>0.05$. Oxidative stress parameters beside other diagnostic tools should be considered as a diagnostic marker in AA.

Key words: Acute appendicitis, diagnosis, oxidative stress, total oxidant status, total antioxidant status.

INTRODUCTION

Number of published studies about the oxidative status of acute appendicitis (AA) is very limited. AA is one of the most common causes of the acute abdomen (Binnebösel et al., 2009) AA remains a difficult diagnosis. To support the diagnosis of appendicitis, leukocyte counting and C-reactive protein (CRP) have been investigated in different studies (Mohammed et al., 2004; Stefanutti et al., 2007; Yang et al., 2006). It has also been reported that leukocytosis and elevated CRP levels confirm the diagnosis of AA (Sengupta et al., 2009). Reactive oxygen species (ROS) are oxygen-containing molecules that is produced during normal metabolism. The organism has enzymatic and non-enzymatic antioxidant systems neutralizing the harmful effects of the endogenous ROS products (Aycicek and Erel, 2007). Oxidative stress which can be defined as an increase in oxidants and/or a decrease

in antioxidant capacity is well known in inflammatory conditions. Because the existing inflammation characterized by activated neutrophils and macrophages is possibly associated to the high production of ROS (Bolukbas et al., 2005; Ozdogan et al., 2006; Serefhanoglu et al., 2009).

The aim of this study was to investigate the assistance of oxidative stress markers in diagnosis of AA.

MATERIALS AND METHODS

Study population and protocol

The study was performed in 2010. It was approved by institution's ethic committee. All patients with suspicion of AA admitted to the emergency department of our hospital were evaluated. Patients were included in the study if diagnosis of appendicitis was confirmed histopathologically postoperatively.

Routine preoperative diagnostic tools for AA included white blood count (WBC), CRP and ultrasonography (US) evaluation. Data were collected retrospectively for confirmed cases of AA. The cases with histopathological signs incompatible to AA were excluded. The

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control group included 30 healthy individuals who volunteered to participate in this study. They did not have any condition that would affect study parameters. Leukocyte counting was performed in the Coulter MAXM hemocounter. CRP levels were measured nephelometrically (Beckman Image). On the other hand, on admission to the emergency department, venous blood was drawn into blood tubes from AA patients, postoperatively confirmed later, and serum was separated from the cells by centrifugation at 1500 g for 10 min and the serum samples were stored at -80°C until the analyses. Serum antioxidative status was evaluated by measuring TAS levels in patients with AA and in control group. Serum oxidative status was evaluated by measuring TOS. The percent ratio of TOS to TAS level was accepted as OSI.

Similar studies were also performed in the control group. All these data were compared between AA patients and healthy control individuals.

Determination of serum total oxidant status (TOS) levels

TOS levels were measured using commercially available kits (Rel assay, Turkey). In the new method, oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) (Erel, 2005).

Determination of serum total antioxidant status (TAS) levels

TAS levels were measured using commercially available kits (Rel assay, Turkey). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L (Erel, 2004).

Calculation of oxidative stress index (OSI)

The ratio of TOS to TAS was accepted as the OSI. For calculation, the resulting unit of TAS was converted to $\mu\text{mol/L}$ and the OSI value was calculated according to the following formula:

$\text{OSI (arbitrary unit)} = \text{TOS } (\mu\text{mol H}_2\text{O}_2 \text{ equivalent/L}) / \text{TAS } (\mu\text{mol Trolox equivalent/L})$ (Harma and Erel, 2003; Kosecik et al., 2005; Yumru et al., 2009).

Statistics

For statistical evaluation, we used the software package SPSS 15.0 and probability value of less than 0.05 was accepted as statistically significant. As the data were normally distributed and independent, statistical analysis was performed using Student's t-test when comparing groups. The results are given as the mean \pm standard deviation (SD).

RESULTS

Thirty consecutive patients with histopathologically

confirmed diagnosis of AA were included in the study. The male to female ratio of groups 1 and 2 were 22/8 and 21/9 respectively. Mean age was 31.83 ± 1.06 years. Laboratory examinations revealed a WBC of $12.5 \times 10^3 \pm 3.90$ a CRP concentration of 12.74 ± 7.92 mg/dl, in group 1 and a WBC of $6.59 \times 10^3 \pm 1.52$ a CRP concentration of 5.53 ± 1.5 in group 2. All parameters were significantly higher in AA group compared to the control group. US was found positive in 63.3% of the patients with AA. TOS and OSI levels increased in patient group compared to control group (respectively, 22.71 ± 24.82 $\mu\text{mol H}_2\text{O}_2$ equivalent/L vs 8.34 ± 2.26 $\mu\text{mol H}_2\text{O}_2$ equivalent/L, $p = 0.003$; 1.05 ± 1.10 arbitrary unit vs 0.40 ± 0.09 arbitrary unit, $p = 0.002$) (Figures 1 and 3). But there was no differences in TAS levels in both groups, $p > 0.05$ (Figure 2). These values for all parameters are presented as mean \pm SD in Table 1.

DISCUSSION

In this study, levels of TOS and OSI as predictors of oxidative status in AA patients were significantly higher than those of healthy individuals. But there were no statistically differences in TAS levels for both groups. These results show that the oxidative/antioxidative balance shifted towards oxidative status, namely: increased oxidative stress was present in patients with AA compared with healthy control individuals. The diagnosis of AA may become a considerable problem. However, to obtain minimal morbidity, prompt and accurate diagnosis is essential. Delayed initiation of adequate therapy or unnecessary operations are results of diagnostic mistakes. Development and refinement of a number of new strategies and diagnostic procedures, including structured patient interview pathways, scoring systems, ultrasound, computed tomography (CT), and diagnostic laparoscopy, occurred during the past years (Beasley, 2000; Zielke, 2002). In suspected AA the number of unnecessary operations and the frequency of complications may be reduced by determining the optimum algorithm for diagnostic procedure. This may result in reducing the costs for treatment of patients with acute abdominal conditions. A combination of clinical and laboratory findings are bases of the indication for operation (Andersson et al., 1999; Bergeron et al., 1999; Korner et al., 2000). According to reports, the rate of correct diagnosis using physical examination and leukocyte counting has been declared as 80%. Despite utilization of US, CT, and scintigraphy, the rate of correct diagnosis has remained lower than 90%. CT can be useful in diagnosis but, in our department it is not a routine diagnostic tool when appendicitis is suspected. This management is in line with the literature (Lin et al., 2008). Due to CT scans performed for the diagnosis of AA in the emergency department, the patient may be exposed a significant amount of radiation.

Radiation risk can be eliminated by US but it has

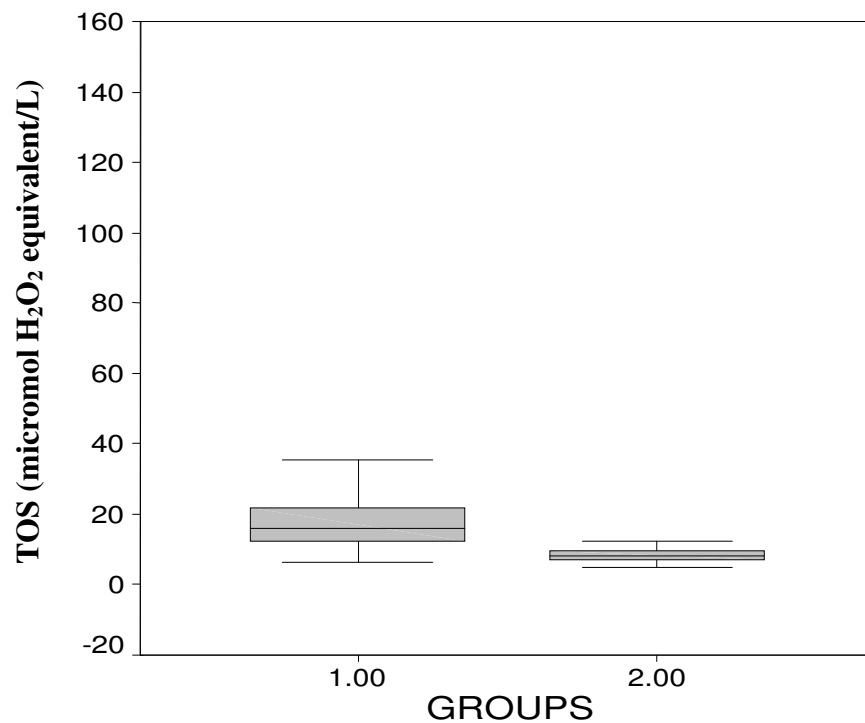


Figure 1. Differences in TOS levels between acute appendicitis patients (Group 1) and control group (Group 2).

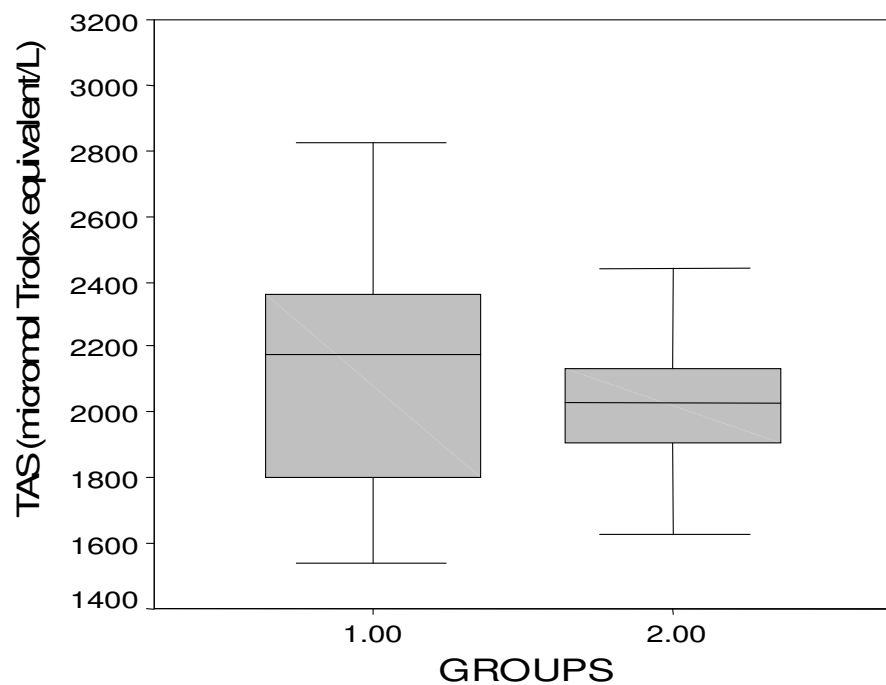


Figure 2. TAS levels in acute appendicitis patients (Group 1) and control group (Group 2).

sensitivity inferior to that of CT (Old et al., 2005). In this study, abdomen US as an adjunct to physical

examination was performed in all patients. Only 63.3% of the patients with AA could be diagnosed with US, in the

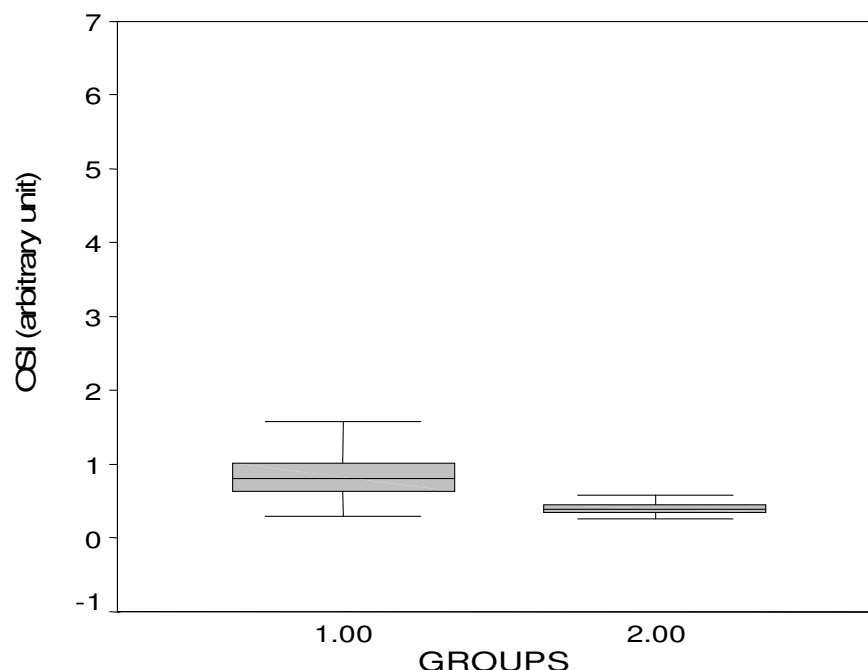


Figure 3. Differences in OSI levels between acute appendicitis patients (Group 1) and control group (Group 2).

Table 1. TOS, TAS and OSI levels between patient and control groups (mean \pm SD).

Parameters	Group	Mean	SD	P
TOS ($\mu\text{mol H}_2\text{O}_2$ equivalent/L)	Patient	22.71	24.82	0.003
	Control	8.34	2.26	
TAS ($\mu\text{mol Trolox}$ equivalent/L)	Patient	2104.40	356.33	>0.05
	Control	2046.33	267.12	
OSI (arbitrary unit)	Patient	1.05	1.10	0.002
	Control	0.40	0.09	

remaining patients, ultrasonographic evaluation was reported to be normal. It was suggested by a recent study that normal values of both WBC and CRP were very unlikely in pathologically confirmed appendicitis and inflammatory markers might be helpful in diagnosis of AA (Khan et al., 2004; Stefanutti et al., 2007; Yildirim et al., 2006). We believe that OSI level analysis would support these results. Oxidative stress has been implicated in more than 100 disorders (Harma and Erel, 2003). The factors affecting the progression and the mechanisms involved in the pathology of AA have still been investigated (Yilmaz et al., 2010). The association between AA and oxidative stress has been addressed in a few experimental and clinical studies (Ozdogan et al., 2006). But in those studies oxidative stress was not measured in total whereas, oxidants and antioxidants have additive effects. Although the concentration of

oxidant and antioxidant components can be measured individually, these measurements are time and cost consuming and require sophisticated systems. In addition, it may not accurately reflect the TAS and TOS (Horoz et al., 2005). For this reason, we measured oxidant status and antioxidant status totally and demonstrated that there is an immediate increase in oxidant status and OSI at the initiation of AA. In our study, we demonstrated positive relationship between TOS; OSI levels and AA. Therefore, we conclude that OSI might be a reliable diagnostic marker for AA. WBC and CRP levels are commonly used to support the clinical diagnosis of AA. The use of these diagnostic laboratory measurements with the addition of OSI in suspicion of AA can enhance the accuracy of diagnosis. None of these markers, including OSI levels are specific to AA, and all can be elevated in many clinical conditions. They may not

play a major role in differential diagnosis, but when there are clinical findings suggestive of AA they may aid to strongly support the diagnosis of it.

In conclusion, these results suggest that the measurement of oxidative stress parameters may be useful adjunct to laboratory and radiological findings in diagnosis of AA.

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