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Research Article/ Araştırma Makalesi

Diagnostic Value of Serum Thiobarbituric Acid Reactive Substances Levels in Pediatric Acute Appendicitis

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Purpose: This study aims to show the diagnostic value of Serum Thiobarbituric Acid Reactive Substances in pediatric appendicitis.

Method: Eighty-five pediatric patients hospitalized in the pediatric surgery ward with acute appendicitis and a control group of 50 pediatric patients with unspecific abdominal pain were included in this prospective case-control study. Forty-five patients whose pathology specimens confirmed acute appendicitis made up the final appendicitis group.

Results: Patients with appendicitis had higher Serum Thiobarbituric Acid Reactive Substances ($p<0.001$) levels than the control group. In receiver operating characteristic analysis, areas under the curve were 0.654 for Serum Thiobarbituric Acid Reactive Substances.

Conclusion: Serum Thiobarbituric Acid Reactive Substances test of patients with appendicitis provides limited accuracy in the diagnosis of appendicitis.

Keywords: Appendicitis, Thiobarbituric Acid, TBARS, MDA, Oxidative stress, Pediatrics

1.INTRODUCTION

Acute appendicitis (AA) is an emergency surgical condition characterized by an inflammatory response. Diagnosis of AA remains a surgical challenge due to significant differences in clinical presentation. Misdiagnosis rates range from 5% to 30%, and a 5% to 15% misdiagnosis rate is considered acceptable to reduce the risk of perforation.[1–3]

The pathophysiology of AA is characterized by the luminal obstruction, which leads to increased permeability of the appendiceal mucosal barrier and triggers an inflammatory response.[4] Previous studies have explored various markers as diagnostic tools in acute inflammatory states.[5–7] There is substantial evidence indicating the involvement of reactive oxygen species (ROS), leading to oxidative stress, in the physiopathology of this inflammatory process. Evaluating oxidative stress in humans typically involves examining products caused by oxidative damage or identifying the antioxidant defense capacity of the body. However, there is a need for unanimity on the parameters to measure oxidative stress and antioxidant status in different pathologies.[8–10]

ROS are released from macrophages, neutrophils, and various tissue cells. Antioxidant enzymes (like superoxide dismutase and catalase) regulate ROS to maintain cellular oxidative balance by directly suppressing free radicals. Superoxide dismutase and catalase activities were previously investigated among patients with AA and healthy individuals.[11] Because ROS have extremely short half-lives, they are difficult to measure directly. Instead, has been investigated through the presence of lipid peroxidation products such as malondialdehyde (MDA) using the serum thiobarbituric acid reactive substances (TBARS) assay.

The diagnosis of AA is primarily based on the clin-

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ical history and physical examination, complemented by laboratory investigations such as white blood cell (WBC) count and differential blood count. For many years, these, along with C-reactive protein (CRP) levels, have been the main laboratory diagnostic methods for AA.[12] In addition, various techniques, including ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), have been employed for early diagnosis.[13,14] However, given the controversy surrounding suspected cases of AA, more research is needed to reduce the rates of negative or unnecessary appendectomies and related complications. Despite advances in diagnostic and treatment technologies, AA remains a clinical challenge due to its high prevalence and varied clinical presentation. Moreover, the diagnostic value of extensively studied markers has yielded contradictory results. However, the routine use of excellent diagnostic methods such as ultrasound and CT is limited by their cost, ionizing radiation, and operator requirements, which restricts their availability in all healthcare institutions.

This study aims to contribute to the search for new markers that can provide additional information and improve the accuracy of AA diagnosis.

2. MATERIALS and METHODS

2.1.Patient Selection

The study was approved by the Clinical Researches Ethics Committee (2022/152). Eighty-five patients admitted to our hospital's Emergency Department (ED) with abdominal pain and were hospitalized in our pediatric surgery wards with the presumptive diagnosis of AA after anamnesis, physical examination, laboratory tests, and ultrasonography were included in the study. Fifteen patients were excluded from the study due to missing laboratory tests. Twelve patients were discharged with nonoperative management (NOM). A total of 58 patients underwent laparotomy with a diagnosis of AA during this study. Four cases that presented complicated acute appendicitis (AAWC) diagnoses during surgery were excluded. Based on the histopathological examination, 45 patients were included in acute appendicitis with no complications (AANC) subgroup. The histopathological examination results of nine patients were interpreted as normal appendectomy materials.

The control group consisted of 50 pediatric patients who applied to the ED with unspecific abdominal pain within the study period, for whom the diagnosis of acute appendicitis was excluded by anamnesis, physical examination, laboratory tests, and ultrasonography.

The informed consent form was taken from the individuals and their families in the study and control groups.

2.2.Collection and Storage of Blood Samples

Venous blood samples taken from the patients routinely during emergency service admissions were taken into anticoagulant-free biochemistry tubes under CLSI GP41-A6 guidelines. Blood samples for serum were centrifuged at 4000 rpm for 10 minutes after coagulation was completed. After the centrifuge, routine tests requested from the patients were studied immediately and the excess serum samples were kept at -80 ºC until the study day.

2.3.Biochemistry and Hemogram Measurement

Serum biochemistry parameters were studied in the Abbott Architect c16000 autoanalyzer, which makes spectrophotometric measurements by using commercial kits. Hemogram was studied from complete blood by using the flow cytometry method in the Sysmex XN-1000 autoanalyzer.

2.4.Serum TBARS Determination Study Protocol

100 µL of plasma was mixed with 500 µL of 10% TCA solution and vortexed. The mixture was incubated at 95°C for 10 minutes and then centrifuged at 4000 rpm for 10 minutes. 400 µL of the supernatant was taken and mixed with 200 µL of 0.67% TBA solution. The mixture was vortexed and incubated again at 95°C for 10 minutes. After incubation, it was centrifuged at 4000 rpm for 10 minutes. The resulting color was read at 532 nm and analyzed spectrophotometrically. The results were determined using a prepared 40-2.5 nmol/mL standard curve of 1.1.3.3-tetramethoxypropane and expressed in pmol/mL.

2.5.Statistical Analysis

The normal distribution of continuous data was tested with the Kolmogorov-Smirnov test, Histogram, and Q-Q plots. Parametric data were reported as mean and standard deviation (SD), nonparametric data were reported as median and IQR and categorical variables were reported as number and frequency (%).

Student t-test was used to analyze continuous variables, as in comparing TBARS levels between pathologically confirmed acute appendicitis and healthy control groups. Pearson's Chi-square test was used to compare categorical variables. Receiver operating characteristic (ROC) curve analysis was conducted for continuous variables and the areas under the curve (AUC) were calculated. Cut-off points were determined using the Youden index and diagnostic value criteria were calculated with 95% confidence intervals. Significance was accepted as $p < 0.05$ in statistical analysis. All analyses were made with R based Jamovi statistical program (version 1.1.5.0; https://jamovi.org) and Statistical Package for Social Sciences (SPSS version 26).

3.RESULTS

Eighty-five AA cases were analyzed from September 2022 to March 2023, younger than 18 years. The negative appendectomy (NA) rate was 15.5% (9 of 58). The AANC group comprised 31 male patients (68.9%) and 14 female (31.1%). The mean age of the AANC group was 11.4 (4.29) years, while the mean age of the control group was 10.9 (3.44) years. Age was not significantly different between the AANC and the control groups ($p=0.411$) (Table 1). WBC, ANC and TBARS levels were significantly higher in AANC patients versus healthy control subjects ($p<0.001$). The diagnostic values of statistically significant parameters were evaluated using ROC analysis. In the AANC group, AUC was above 0.900 for WBC and ANC, above 0.600 for while TBARS. ROC curves for laboratory data are given in Figure 1. TBARS cut-off value were calculated from the respective ROC curves. For TBARS, the cut-off value of >0.5 pmol/ml had a sensitivity of 64.4% and a specificity of 76% (AUC=0.654 ± 0.06; $p<0.001$). While a 70.4% (60.9-78.4) negative predictive value was found for TBARS, this rate was 94.2% (84.5-97.9) for WBC, and 92.5% (82.8-96.9) for ANC. Table 2 shows areas under the curve (AUC), cut-off values, sensitivities, specificities, positive predictive values (+PV), negative predictive values (-PV), positive likelihood ratio (+LR), negative likelihood ratio (-LR), and p values in the prediction of AA. Laboratory data of AANC, NA, NOM, AAWC, and Control groups are given in Figure 2.

Table 1.

Comparison of demographics and laboratory data between AANC and the control groups

Variables, Mean (SD)	AANC n=45	Control n=50	p value
Age	11.4 (4.29)	10.9 (3.44)	0.411
WBC (cells /mm3)	17530 (5360)	8156 (1541)	<0.001
ANC (cells /mm3)	13541 (5728)	4219 (1232)	<0.001
TBARS (pmol/ml)	0.591 (0.247)	0.459 (0.076)	<0.001
AANC: Acute Appendicitis with no Complications, WBC: White Blood Count, ANC: Absolute Neutrophil Count, TBARS: Thiobarbituric Acid Reactive Substances, SD: Standard Deviation			

Table 2.

Diagnostic accuracy metrics of WBC, ANC and TBARS in the diagnosis of acute appendicitis with no Complications

Metric	WBC	ANC	TBARS
AUC ± SE	0.986 ± 0.01	0.952 ± 0.02	0.654 ± 0.06
Cut off Value	10.750 cells/mm3	6.680 cells/ mm3	0.5 pmol/ ml
Sensitivity (95 % CI)	93.3 (81.7-98.6)	91.1 (78.8-97.5)	64.4 (48.8-78.1)
Specificity (95 % Cl)	98 (89.4-99.9)	98 (89.4-99.9)	76 (61.8-86.9)
+PV (95 % Cl)	97.6 (85.8-99.7)	97.6 (85.5-99.7)	70.7 (58.5-80.6)
-PV (95 % Cl)	94.2 (84.5-.97.9)	92.5 (82.8-96.9)	70.4 (60.9-78.4)
+LR (95 % Cl)	46.7 (6.7-325.4)	45.6 (6.5-317.8)	2.7 (1.6-4.6)
-LR (95 % Cl)	0.07 (0.0-0.2)	0.09 (0.0-0.2)	0.5 (0.3-0.7)
Accuracy (95 % Cl)	95.8 (89.6-98.8)	94.7 (88.1-97.3)	70.5 (60.3-79.4)
p-value a	0.001	0.001	0.003
WBC: White Blood Count, ANC: Absolute Neutrophil Count, TBARS: Thiobarbituric Acid Reactive Substances, AUC: Area Under the Curve, CI: Confidence Interval, LR: Likelihood Ratio, PV: Predictive Value, a: The value in groups were calculated by using ROC curve.			

Figure 1.

Receiver operating characteristic (ROC) curve analyses of important parameters for the diagnosis of appendicitis (WBC, ANC and TBARS)

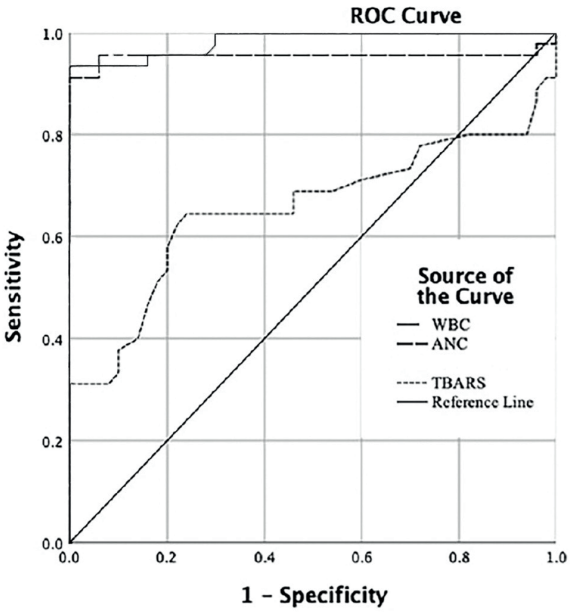
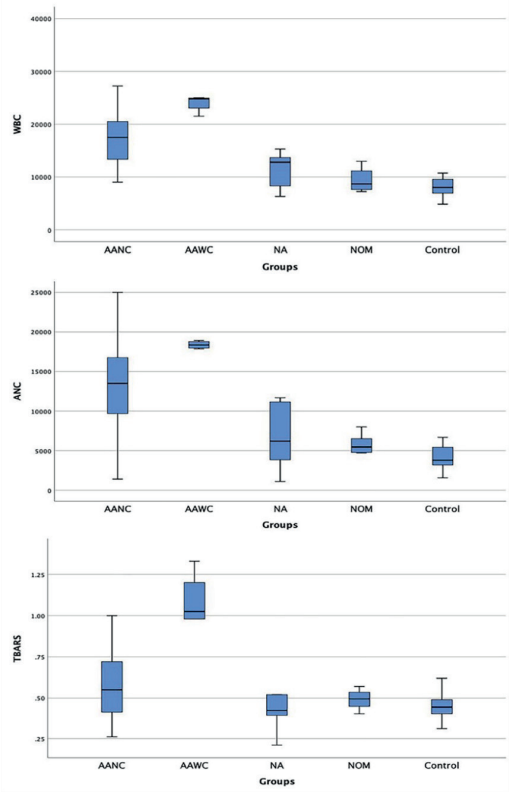


Figure 2.

Box plots presenting the median of WBC, ANC and TBARS levels



4.DISCUSSION

Acute appendicitis is a condition characterized by inflammation of the appendix. This condition leads to tissue damage in the wall of the appendix.[14] Increased oxidative stress during the inflammatory process may contribute to cell damage and tissue degradation. Activation of inflammatory cells and release of cytokines can increase oxidative stress. In addition, due to tissue hypoxia caused by tissue damage, oxygen metabolism may be impaired, and oxidative stress may increase. Compared with the number of studies evaluating inflammatory markers in the diagnosis of acute appendicitis, only a limited number of studies have evaluated the diagnostic value of ischemic and oxidative stress-related markers. Some studies have shown increased oxidative stress markers in patients with acute appendicitis. Among these markers, parameters such as malondialdehyde (MDA), total oxidant capacity, nitric oxide (NO), and superoxide dismutase (SOD) be found. These findings suggest that oxidative stress may play a role in the pathophysiology of acute appendicitis.[8,15]

However, it has not been fully determined whether oxidative stress is the cause or consequence of acute appendicitis. Some research suggests that oxidative stress may contribute to the development of appendicitis, while others think that oxidative stress is a result of the tissue damage that occurs as a result of appendicitis.[8,11,15–17]

In this study, appendicitis patients were found to have higher TBARS levels compared to the control group. According to the ROC analysis results, AUC for TBARS was calculated as 0.654. This result suggests that the test provides limited accuracy for diagnosing appendicitis. The +PV (70.7%) and -PV (70.4%) are similar, indicating that the TBARS test provides limited value in diagnosing appendicitis. The +LR was 2.7 and the -LR was 0.5. These results show that the TBARS test alone is insufficient for diagnosing appendicitis and should be evaluated with other clinical and laboratory findings. A limited number of studies in the literature have investigated MDA levels in AA and have reported conflicting results. In the study of Machado et al., which compared AANC, AAWC, and Control groups, oxidative stress parameters exhibited different behaviors. The SOD, CAT, and TBARS levels did not show any significant difference among all assessed groups (SOD: p= 0.29, n= 41; CAT: p= 0.19, n= 40; and TBARS: p= 0.18, n= 63).11 In a study performed by Koltuksuz et al. involving pediatric patients with AA, MDA was found to be significantly elevated in cases of acute suppurative and acute perforated appendicitis when compared to cases of acute focal appendicitis and the control group.18 In the study of Hakkoymaz et al., no significant difference was found between MDA levels of AA patients and healthy controls (p= 0.107).[8]

5.CONCLUSION

The results show that TBARS levels can help diagnose appendicitis however provides limited accuracy. It is essential their usability in future research should be further examined.

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