Can Thiol/Disulphide Homeostasis Help in The Differential Diagnosis of Appendicitis in Children?

Tiyol / Disülfit Dengesinin Değerlendirilmesi Çocuklarda Apandisitin Ayırıcı Tanısında Yardımcı Olabilir mi?

Sabri DEMİR[1,2], Yasemin DERE GÜNAL[1], İsmail ÖZMEN[1,3], Nermin DİNDAR BADEM[4], Salim NEŞELİOĞLU[5], Özcan EREL[5]

[1] Kirikkale University, Faculty of Medicine, Department of Pediatric Surgery, Kirikkale, Turkey.
[2] Ankara City Hospital, Children Hospital, Department of Pediatric Surgery, Bilkent, Ankara, Turkey.
[3] Kilis State Hospital, Department of Pediatric Surgery, Kilis, Turkey.
[4] Kirikkale University, Faculty of Medicine, Department of Biochemistry, Kirikkale, Turkey.
[5] Yildirim Beyazit University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey.

ABSTRACT

Objective: We aimed to investigate the potential of assessing thiol/disulfide homeostasis as novel oxidative stress markers to improve the challenging diagnosis of acute appendicitis in children.

Material and Methods: A total of 60 pediatric patients (0-18 years) were enrolled in the study, 30 of which were in the control group and 30 in the acute appendicitis group. Native thiol, total thiol, dynamic disulfide, dynamic thiol, ischemia modified albümin, albumin, White blood cell, hemoglobin, hematocrit, and platelet counts of both groups were measured. The results of both groups were compared using the SPSS (Statistical Package for Social Sciences) version 17 (Chicago, USA) program. For all variables, p <0.05 was considered significant.

Results: Total thiol (p<0.001), native thiol (p<0.001), and albümin (p<0.001) levels were significantly decreased while dynamic disulfide, dynamic thiol (p=0.003), and ischemia modified albümin (p<0.001) levels those indicating oxidant side were increased in acute appendicitis group compared to control. White blood cell counts in acute appendicitis group were higher (p<0.001), and platelet counts were lower (p=0.03) than the control group.

Conclusion: In the differential diagnosis of acute appendicitis, especially in case that are difficult to diagnose, besides a physical examination, imaging, and current laboratory tests, quantification of thiol/disulfide homeostasis may be helpful in diagnosing. In addition, evaluating albumin and IMA levels may increase the specificity of the test. This test can be more helpful in cases diagnosis is difficult such as children small in their ages and mental retardation.

Key Words: Acute appendicitis, Appendectomy, Children, Oxidative stress, Thiol/disulphide homeostasis

ÖZ

Amaç: Çalışmamızın amacı, tanı konulmasında zorlanılan çocuklarda, akut apandisitin ayırıcı tanısında, oksidatif stresin yeni bir belirteci olan tiyol/disülfit dengesinin, kullanılabilirliğini araştırmaktır.

Gereç ve Yöntemler: Çalışmaya, yaşları 0-18 yaşları aralığında bulunan ve kontrol grubunda 30, akut apandisit grubunda 30 olmak üzere toplam 60 gönüllü çocuk dahil edildi. Her iki grubun da nativ tiyol, total tiyol, dinamik disülfit, dinamik tiyol, iskemi modifiye albümin, albümin, lökosit, hemoglobin, hematokrit ve trombosit sayıları bakıldı. Veriler, SPSS (Statistical Package for Social Sciences) versiyon 17 (Chicago, USA) programı kullanılarak karşılaştırıldı. Tüm değişkenler için p <0.05 anlamlı kabul edildi.

Bulgular: Akut apandisit grubunda, kontrol grubu ile karşılaştırıldığında; total tiyol (p<0.001), nativ tiyol (p<0.001) ve albümin (p<0.001) düzeyleri anlamlı düzeyde düşük bulunmasına karşın, oksidatif tarafa kaymayı gösteren dinamik sülfit,

DEMİR S : 0000-0003-4720-912X
DERE GUNAL Y : 0000-0003-4488-236X
OZMEN I : 0000-0002-4101-5677
DINDAR BADEM N : 0000-0002-5095-7818
NESELIOGLU S :0000-0002-0974-5717
EREL O : 0000-0002-2996-3236

Conflict of Interest / *Çıkar Çatışması*: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics Committee Approval / *Etik Kurul Onayı*: Ethical approval for the study was obtained from the ethics committee for clinical research ethics in Kirikkale University (Date: 22.03.2016, Number: 08/08)

Contribution of the Authors / Yazarların katkısı*: *DEMİR S: Conceiving and designing the study, data analysis, and interpretation, manuscript preparation. ***DERE GUNAL Y:*** Collecting blood samples. ***OZMEN I***: Collecting and transfer of the blood samples. ***DINDAR BADEM N:*** Conducting and interpretation of the hematological tests. ***NESELIOGLU S:*** Conducting and interpretation of the biochemical tests. ***EREL O:*** conducting and interpretation of the biochemical tests, analysis and interpretation of data.

How to cite / *Atıf yazım şekli*: Demir S, Dere Gunal Y, Ozmen I, Dindar Badem N, Neselioglu S, Erel O. Can Thiol/Disulphide Homeostasis Help in The Differential Diagnosis of Appendicitis in Children?. Turkish J Pediatr Dis 2020; 14: 236-243.

Correspondence Address:/ Yazışma Adresi:
Sabri DEMIR
Ankara City Hospital, Children Hospital,
Department of Pediatric Surgery, Bilkent, Ankara, Turkey
E-posta: drsabridemir@gmail.com

Received/ Geliş tarihi : 01.10.2019
Accepted/ Kabul tarihi : 21.11.2019
Online published : 27.03.2020
Elektronik yayın tarihi
DOI: 10.12956/tchd.627936

dinamik tiyol (p=0.003) ve iskemi modifiye albümin (p<0.001) düzeyleri yüksek bulundu. Akut apandisit grubunda lökosit sayısı kontrol grubuna göre daha yüksek (p <0.001) bulunurken trombosit sayısı kontrol grubundan daha bulundu (p = 0.03).

Sonuç: Akut apandisitin ayırıcı tanısında, özellikle teşhis konmakta zorlanılan olgularda, fizik muayene, görüntüleme ve güncel laboratuvar testlerinin yanı sıra, tiyol/disülfid dengesinin değerlendirilmesi tanı koymada yardımcı olabilir. Ek olarak albümin ve iskemi modifiye albümin düzeylerinin değerlendirilmesi testin özgüllüğünü artırabilir. Bu test, yaşları küçük olan çocuklar ve mental retarde hastalar gibi tanının zor konabildiği hastaların ayırıcı tanısında daha yararlı olabilir.

Anahtar Kelimeler: Akut apandisit, Apendektomi, Çocuk, Oksdatif stress, Tiyol/disülfit dengesi

INTRODUCTION

Acute appendicitis (AA) is one of the most common causes of the acute abdomen encountered by pediatric surgeons. Accurate diagnosis still remains a serious problem because its symptoms can be confused with those associated with other diseases. Diagnosis is primarily based on anamnesis, clinical symptoms, and physical examination. Laboratory tests and radiological imaging such as roentgenogram, ultrasonography (USG), computed tomography (CT), and magnetic resonance imaging (MRI) are used for differential diagnosis. Although increases in white blood cell count (WBC) and C-reactive protein (CRP) level support diagnosis, they are not specific tests (1, 2). Alvarado test can be used to confirm the diagnosis (3). Unfortunately, currently, we do not have a specific test to diagnose AA accurately.

In AA, owing to either a faecolith or inflammation, luminal obstruction arises and consequently, the permeability of the appendiceal mucosal barrier increases, thereby resulting in increased involvement of neutrophils and other inflammatory cells in the tissue caused by the developing inflammatory response. These cells also increase the release of soluble mediators such as pro-inflammatory cytokines (IL-1, IL-6, IL-8), tumor necrosis factor-α (TNF- α), interferon-gamma (INF-γ) and anti-inflammatory cytokines (IL-4 and IL-10) (4). Therefore, it has been suggested that measurement of some of these cytokine levels in the diagnosis of acute infections and sepsis can be used to diagnose the infection and determine its severity (5). Some authors claimed that inflammatory markers such as IL-6, IL-8, and IL-10 in serum could be used for diagnosing AA (6, 7). However, none of these biochemical markers are specific for AA.

Free radicals, which are highly reactive molecules that contain one more unpaired electron, are formed during normal cell metabolism. The organism maintains a very delicate balance between the production of free radicals and antioxidant-based defence mechanism. This balance is essential for the survival and health of living organisms (8). Whenever it shifts to the oxidative side, consequently oxidative stress (OS) develops. OS-induced free oxygen radicals cause damage to cellular structures, nucleic acids, lipids, and proteins. It is known that in inflammatory events, OS increases as a consequence of neutrophil and macrophage activation and overproduction of free oxygen radicals (9,10). In inflammation, free oxygen radicals produced by polymorphonuclear leukocytes cause lipid peroxidation in cell membranes. Oxidative damage in the membranes results in increased microvascular permeability, edema, inflammatory cell infiltration, neutrophil activation, and, eventually, cell death (9).

Thiols are organic compounds containing a sulfhydryl group (-SH) attached to a carbon atom, also known as mercaptans (11). The thiol compounds present in plasma scavenge free radicals by binding to them and thus act as antioxidants.(12) Thiols (R-SH) react with free radicals in the organism to form disulphide bonds (RSSR). These disulphide bonds are reduced back to thiol groups with the help of antioxidant compounds. Thus, the organism's dynamic thiol/disulphide homeostasis is maintained in a stable state (11).

The status of the dynamic thiol/disulphide balance plays a critical role in the regulation of antioxidant protection, detoxification, signal transduction, apoptosis, enzymatic activity, transcription factors, and cellular signaling mechanisms (13). It has also been shown that the deterioration of dynamic thiol/disulphide homeostasis plays a role in the pathogenesis of many diseases such as diabetes, cardiovascular diseases, cancer, rheumatoid arthritis, chronic kidney disease, AIDS, Parkinson's disease, Alzheimer's disease, Friedrich's ataxia, multiple sclerosis, amyotrophic lateral sclerosis, and liver diseases. Thus, the determination of the dynamic thiol/disulphide homeostasis may provide valuable information about various normal or abnormal biochemical processes (11, 14).

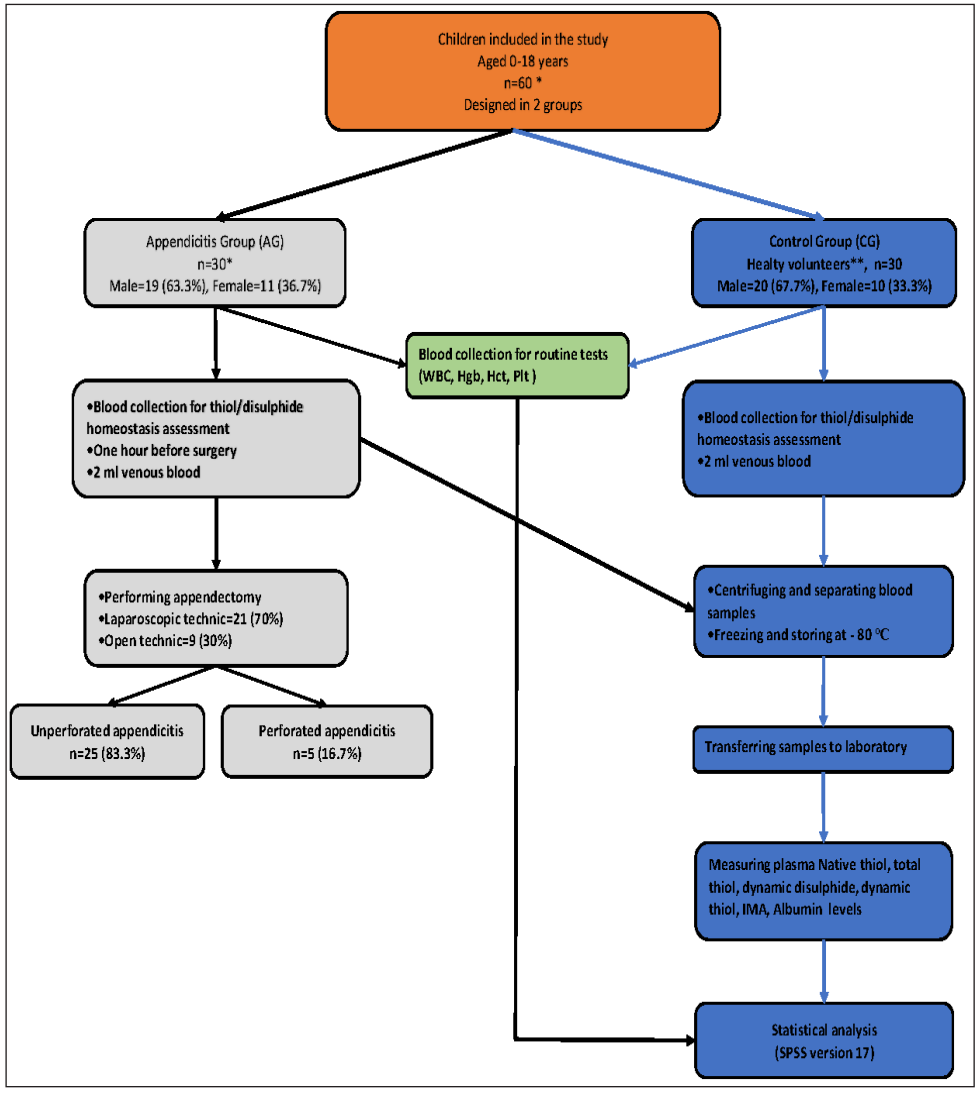
OS assessment is done either by analyzing the products resulting from oxidative damage or by determining the antioxidant defence capacity of the organism (4). Oxidative damage-induced lipid peroxidation results in the formation of malonyl dialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS). Thus, the determination of OS levels has been attempted by measuring the levels of biochemical markers such as superoxide dismutase (SOD), catalase (CAT), MDA, and TBARS (15-17). Recently, a new method has been developed by Erel et al. (11), which involves assessment of OS level in the body by thiol/disulphide homeostasis evaluation. Some studies have shown that this new method is a reliable OS indicator (18-23). This new method has revealed increased OS in adult AA patients, and it has been suggested that detection of this increase can aid the diagnosis of appendicitis (22). Because it is a new method and may help in the diagnosis of AA patients, we investigated the relationship between AA and OS in children.

PATIENTS and METHODS

Our study was designed in two groups: the patient group and control group. The patient group included 30 children, aged 0–18 years, who had an appendectomy in the pediatric surgery clinic between March and October 2016; this group was named as appendicitis group (AG). Thirty volunteers and healthy children were included in the control group (CG); these children were of the same age group and sex as those in the patient group and had registered at the pediatric surgery clinic owing to various reasons but did not suffer from any disease. The study was prospectively designed. Ethical approval for the study was obtained from the ethics committee for clinical research ethics in Kirikkale University (Date: 22.03.2016, Number: 08/08). Informed consent forms were taken from the parents of patients and volunteers belonging to AG and CG, respectively. Patients whose specimens were evaluated as normal by histopathological evaluation (negative appendectomy) were excluded from the study. The flowchart of the study is shown in Figure 1.

Collection and transfer of samples

Two milliliters of venous blood was taken from the AG patients 1 h before the surgery and once from CG volunteers. The blood samples were immediately centrifuged; then, the serum was separated, frozen and stored at -80 °C. When a sufficient number of blood samples were collected, they were transferred to the laboratory after being frozen in styrofoam boxes containing dry ice. Native thiol, total thiol, dynamic disulphide, dynamic thiol, ischemia modified albumin (IMA) and albumin levels of both groups were measured. Thiol and disulphide levels were analyzed using a newly developed method by Erel and Neselioglu. (11) Laboratory staff performing



* Although 32 patients underwent surgery, 2 patients were excluded from the study because their histopathological results were negative.
** Healthy children whose admitted to pediatric surgery clinic for various reasons but cannot be diagnosed with any disease.

Figure 1: The patient flow diagram of the study.

the plasma thiol/disulphide homeostasis measurement analysis were blinded to the clinical information and outcomes of the patients; results were not available to the treating physicians, staff, or investigators during the study period. Data regarding WBC, hemoglobin (Hgb), haematocrit (Hct) and platelet (Plt) count from both groups were obtained from the database of the hospital (Figure 1).

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences (SPSS), version 17, (Chicago Inc. 2008, USA) program. First, for analysing the descriptive properties of the variables, the mean, median, interquartile range (IQR) and frequency values were found. For evaluating whether the numerical variables are normally distributed, visual (histogram and probability plots) and analytical methods (Kolmogorov–Smirnov test) were used. While dynamic disulphide (SS), dynamic thiol (SS/SH %), IMA, albumin, WBC, Hgb and Hct values were normally distributed, native thiol (SH), total thiol (SS+SH), and Plt counts were not normally distributed. The descriptive analysis of abnormally distributed numerical variables was performed using the Mann–Whitney U test and by employing median and IQR values, while the analysis of normally distributed variables was performed using Student t-test by employing the mean values of the variables. Comparisons among categorical variables were made using the Chi-Square test. For all variables, $p<0.05$ was considered significant.

RESULTS

During the study period, 32 patients were operated upon owing to AA preliminary diagnosis. Of them, two were excluded because of negative histopathological findings. The remaining 30 patients were compared with 30 healthy volunteers with similar demographic characteristics. In the AG (n=30), 5 patients (16.7%) had perforated appendicitis, and 25 (83.3%) had non-perforated appendicitis. Appendectomy was performed on 21 patients (70%) as the laparoscopic technic and performed to nine patients (30%) as the open technic.

The demographic results of groups

In the AG, 11 (36.7%) of the patients were females, and 19 (63.3%) were males. In the CG, 10 of the volunteers (33.3%) were females, and 20 (67.7%) were males. The mean age in the CG was 11.51 years (Min-Max=7.0–17.0, Std Dev=2.86) and in the AG was 12.34 years (6.0–17.0; 3.35). There were no statistically significant differences between the two groups in age and sex (p=0.30 and p=0.59, respectively) (Table I).

Laboratory results

The results of the AG and the CG are given in Table II, and the results of the statistical analysis of both groups are shown in Table III. The levels of native thiol, total thiol, and albumin, as well as Plt count in the AG, were significantly lower than those in the CG, whereas dynamic thiol level, IMA, and WBC were significantly higher. An increase in dynamic disulphide (SS) level was also observed, but it was not statistically significant (p=0.47) (Table III). In the CG, the mean dynamic disulphide level was observed to be 17.15, whereas, in the AG, it was noted to be 18.05 (Table II). As revealed in earlier studies, our study also revealed that WBC count was higher in the AG than in the CG (p=0.001). There was no significant difference in Hgb and Hct values between both the groups (Table III). However, Plt count of patients in the AG was significantly lower than that in the CG (p=0.03). Some researchers reported that Plt count decreased in the appendicitis patients, consistent with the findings of our study (24).

Native thiol levels in the AG were lower than those in the CG. IMA levels were significantly higher in the AG (mean; min-max=1.25; 0.98–1.70) than those in the CG (mean; min-max=0.97; 0.42–1.2) (p<0.001). Albumin levels in the AG (mean, min-max=3.42; 2.90–3.68) were significantly lower than those in the CG (mean, min-max=3:56; 3.35–3.84) (p<0.001).

When the results for perforated and non-perforated patients in the AG were compared, 5 of the patients (16.7%) were found to have perforated appendicitis, and 25 (83.3%) were found to have non-perforated appendicitis. Although the number of patients between the two groups differed, the results of the two groups were compared. The findings for both groups are shown in Table IV. There were no statistically significant differences between the groups, except for the Plt counts. Plt counts which were found to be significantly lower in the non-perforated group than those in the perforated group (p=0.03).

DISCUSSION

In our study, total thiol, native thiol, and albumin levels,

Table I: Demographic properties of the groups.

	Control (n=30)	Appendicitis (n=30)	p
Gender			
Male (n,%)	20 (66.7)	19 (63.3)	0.590*
Female (n,%)	10 (33.3)	11 (36.7)	
Age			
Mean (Year) (Min-Max), (Std. Dev.)	11.51 (7-17) (2.86)	12.34 (6-17) (3.35)	0.300**

** Pearson Chi-Square test was used, ** Student t-test was used.*

Table II: The laboratory results and descriptive statistics of the groups (n=30).

	Mean	Median	Std. Deviation	Minimum	Maximum	IQR**			p*
						25	50	75	
Native Thiol (SH) (µmol/L)									
Control	470.26	454.25	40.15	392.3	593.7	444.1	454.25	491.35	0.010
Appendicitis	395.13	414.85	55.59	273.4	475.5	331.17	414.85	432.8	0.040
Total Thiol(SH+SS) (µmol/L)									
Control	504.56	497.54	42.1	430.4	634.5	472.9	497.54	529.29	0.200
Appendicitis	431.25	452.84	58.18	318.4	525.6	370.26	452.84	470.01	0.010
Dynamic disulphid(SS) (µmol/L)									
Control	17.15	18.5	5.02	7.24	27.42	11.7	18.5	20.95	0.080
Appendicitis	18.05	19.12	4.64	7.36	25.62	15.84	19.12	21.69	0.200
Dynamic thiol (% SS/SH)									
Control	3.66	3.83	1.11	1.46	6.27	2.6	3.83	4.44	0.200
Appendicitis	4.64	4.7	1.34	2.02	8.22	3.71	4.7	5.48	0.200
IMA (ABSU)* **									
Control	0.97	92.88	0.14	0.42	1.2	0.91	1.01	1.08	0.170
Appendicitis	1.25	1.23	0.14	0.98	1.7	1.15	1.23	1.31	0.100
Albumin (g/dL)									
Control	3.56	92.88	0.11	3.35	3.84	3.51	3.57	3.63	0.200
Appendicitis	3.42	3.45	0.16	2.9	3.68	3.35	3.45	3.52	0.130
WBC (µL)									
Control	7801	92.88	1807.4	4010	11610	6917.5	7710	8620	0.130
Appendicitis	14297	13860	4573.6	5750	24520	11825	13860	16643	0.200
Hemoglobin (g/dL)									
Control	13.36	92.88	1.1	11.5	15.3	12.45	13.4	14.22	0.200
Appendicitis	13.06	13.05	1.16	11	15.4	12.35	13.05	13.8	0.200
Platelet counts									
Control	316267	92.88	70961.8	127000	494000	278000	313000	341000	0.030
Appendicitis	284233	273000	72274.2	168000	452000	239000	273000	335000	0.160

** Kolmogorov_Smirnov test used, **IQR (Interquartile Range), *** ABSU (Absorbance Unit)*

which represent the anti-oxidative side in thiol/disulphide homeostasis, were significantly lower in the AG than in the CG. Contrastingly, dynamic disulphide, dynamic thiol, and IMA level, which represent the oxidative side, were found to be higher in the AG than in the CG. The level of dynamic disulphide (SS) was higher in the AG, but the difference was not statistically significant (p=0.47) (Table II,III). These results indicated that the thiol/disulphide balance was shifted towards the oxidative side in favor of OS in the AG, thereby indicating that OS increases in AA.

Table III: Comparison of the results of the control and the acute appendicitis groups.

Normally distrubuted variables*			
Variable	**Control (n=30) Mean (Min-Max)**	**Appendicitis (n=30) Mean (Min-Max)**	**p***
Dynamic disulphid (SS) (µmol/L)	17.15 (7.24-27.42)	18.05 (7.36-25.62)	0.470
Dynamic thiol (% SS/SH)	3.66 (1.46-6.27)	4.64 (2.02-8.22)	0.003
IMA (ABSU)* **	0.97 (0.42-1.2)	1.25 (0.98-1.70)	<0.001
Albumin (g/dL)	3.56 (3.35-3.84)	3.42 (2.90-3.68)	<0.001
WBC (µL)	7801.0 (4010.0-11610.0)	14 296.6 (5750-24520)	<0.001
Hemoglobin (g/dL)	13.36 (11.50-15.30)	13.06 (11.00-15.40)	0.310
**Abnormally distributed variables ** **			
Variable	**Control (n=30) Median (IQR 25-75)**	**Appendicitis (n=30) Median (IQR 25-75)**	**p****
Native thiol (SH) (µmol/L)	454.25 (444.10-491.35)	414.85 (331.17-432.80)	<0.001
Total thiol (SH)+(SS) (µmol/L)	497.54 (472.90-529.29)	452.84 (370.26-470.01)	<0.001
Platelet counts	313000 (278000-341000)	273000 (239000-335000)	0.030

** Student t test was used,** Mann-Whitney U test was used,*** ABSU (Absorbance Unit)*

Table IV: The laboratory results and comparison of the non-perforated (n=25) and perforated (n=5) groups.

	Mean	Median	Std. Deviation	Minimum	Maximum	IQR*			p**
						25	50	75	
Native thiol (SH) (µmol/L)									
Non-perfoprated	399.02	416.30	54.65	273.4	475.50	358.05	416.30	432.80	0.380
Perforated	375.72	364.90	62.62	312.2	454.70	317.10	364.90	439.75	
Total Thiol (SH+SS) (µmol/L)									
Non-perfoprated	435.50	452.96	55.85	318.36	525.58	389.16	452.96	471.90	0.510
Perforated	410.04	379.62	71.84	335.4	505.94	350.24	379.62	485.05	
Dynamic disulphid (SS) (µmol/L)									
Non-perfoprated	18.24	18.98	4.07	8.96	25.04	16.19	18.98	21.28	1.000
Perforated	17.16	19.68	7.48	7.36	25.62	9.49	19.68	23.57	
Dynamic thiol (% SS/SH)									
Non-perfoprated	4.66	4.76	1.29	2.31	8.22	3.73	4.76	5.36	0.950
Perforated	4.54	4.63	1.79	2.02	6.68	2.87	4.63	6.16	
IMA (ABSU) ***									
Non-perfoprated	1.24	1.22	0.16	.98	1.70	1.15	1.22	1.30	0.170
Perforated	1.31	1.30	0.10	1.21	1.45	1.22	1.30	1.41	
Albumin (g/dL)									
Non-perfoprated	3.44	3.49	0.17	2.90	3.68	3.37	3.49	3.55	0.110
Perforated	3.35	3.41	0.11	3.17	3.43	3.25	3.41	3.43	
WBC (µL)									
Non-perfoprated	13962	13680	4462	5750	22970	11700	13680	16965	0.550
Perforated	15970	15850	5292	11090	24520	11595	15850	20405	
Hemoglobin (g/dL)									
Non-perfoprated	13.16	13.10	1.15	11.30	15.40	12.40	13.10	13.85	0.510
Perforated	12.60	13.00	1.24	11.00	13.80	11.30	13.00	13.70	
Platelet counts									
Non-perfoprated	271680	266000	67704	168000	452000	225000	266000	303500	0.030
Perforated	347000	360000	66768	270000	440000	283000	360000	404500	

***** *Inter Quartile Ranges,* ****** *Mann-Whitney U test was applied,* *******ABSU (Absorbance Unit)

Albumin level was found to be decreased despite an increase in the IMA level in the AG as compared with the CG. Seventy percent of the total thiol pool in serum is of albumin origin. In ischemia, the structure of albumin modifies to IMA, and IMA levels increase as albumin levels decrease. IMA has been proposed to be an indicator of ischemia (25). In circumstances where OS increases, the extent of albumin structure modification also increases. Recently, IMA has been widely accepted as a marker of OS in many pathological conditions including AA (26-28). In their study, Dumlu et al. (29) also observed increased IMA levels in the AG than in the CG. In a study conducted in children, Nazik et al. (30) observed that IMA levels were elevated in AA. Kılıç et al. (31) suggested that IMA levels in adult AA patients are significantly elevated and can be used as an indicator of appendicitis severit. Consistent with the findings of previous studies, our study revealed higher IMA levels in the AG than in the CG.

Recently, the relationship between OS markers and various disorders has been investigated; one of these disorders is AA. Some researchers have claimed that increased OS plays a role in the etiology of AA, while other researchers have claimed that an increase in OS is the consequence of inflammatory processes that occur owing to AA (16). Thus, the relationship between AA and OS remains controversial. The relationship between AA and OS has been investigated in several experimental and clinical studies (9, 15, 29, 32, 33). Very few studies have been conducted on children in this respect (15). Dumlu et al. (29) showed that OS marker levels were increased in both plasma and appendiceal tissues and were higher in AA cases than in controls. As OS markers, they investigated total antioxidant status (TAS), total oxidant status (TOS), paraoxonase (PON), stimulated paraoxonase (SPON), arylesterase, catalase, myeloperoxidase, ceruloplasmin, advanced oxidized protein products (AOPP) and IMA. They suggested that OS plays a role in the pathogenesis of AA and demonstrated that increase OS is directly proportioned to the severity of the disease and the Alvarado score.

Özyazıcı et al. (22) have shown that the thiol/disulphide balance shifts towards the oxidative side in adult AA patients. Yılmaz et al. (17) have shown that total thiol levels in adult AA patients decreased compared with those in controls. The results of our study were similar to the aforementioned studies.

Koltuksuz et al. (15) who observed cases of childhood AA by investigating SOD and MDA levels revealed that those children had increased OS and suggested that increased SOR owing to inflammatory processes may play a role in the progression of AA. OS was found to be higher in perforated AA cases than in non-perforated AA cases. They suggested that an excessive

increase in OS indicates advanced AA. Kavaklı et al. (34) measured oxidative stress index (OSI) by measuring TAS and TOS levels in adult AA cases. As a result, they found that TOS and OSI were higher in AA cases than in controls. Similarly, Köksal et al. (35) measured TAS, TOS and paraoxonase levels in adult AA patients and demonstrated that they had higher OS as opposed to controls. All these studies show that OS is elevated in both adults and children with AA. However, in contrast to the view of other researchers, we believe that OS does not play a role in the pathogenesis of AA and instead, OS is a consequence of the inflammatory process that occurs during AA (16).

Platelets play an essential role in the regulation of hemostasis and inflammatory events. Recently, Plt counts and Plt-related parameters (such as PBW, MPW) have become the focus of interest for various diseases. Some studies have shown that Plt counts decrease in AA (36). In our study, Plt counts were significantly lower in the AG than in the CG ($p<0.03$).

Consistent with the findings of previous studies, in our study, a significant increase in WBC counts was found in patients in the AG compared with volunteers in the CG (36-38).

There was no significant difference in thiol/disulphide homeostasis between non-perforated and perforated AA patients. However, this result was thought to be owing to the low number of patients in the perforated AG. Previous studies have reported that OS is higher in perforated AA than in non-perforated AA (15, 29). However, our present study did not focus on investigating the differences between perforated and non-perforated AA. Thus, further study of this issue with the inclusion of more number of patients is warranted.

CONCLUSION

The differential diagnosis of AA continues to be a serious problem. The lack of a specific diagnostic laboratory test makes accurate diagnosis challenging, especially in younger children. Eventually, complicated cases owing to late diagnosis (plastron or perforated appendicitis) and negative appendectomies continue to be encountered. In the literature, negative appendectomy rate was reportedly around 12.2%, and perforated appendicitis rate was 3.4% in adult patients (26, 39). In a study conducted by Oyetunji et al. (40) which involved 250.873 pediatric patients on whom appendectomy had been performed, it was demonstrated that negative appendectomy rate was 6.7%. Therefore, scientists are still searching for specific laboratory tests to diagnose AA accurately. The development of a specific test for the diagnosis of AA will prevent both unnecessary negative appendectomy and early diagnosis of complicated cases, resulting in reduced morbidity and mortality. In the literature, the relationship between AA and OS has been investigated using different test methods (4, 9, 15, 16, 32). All methods have demonstrated that OS is increased in AA patients. OS assessment via evaluation of thiol/disulphide homeostasis promises to be a new and reliable method. Besides physical examination findings, imaging modalities and current laboratory tests, measuring thiol/disulphide homeostasis of patients may be helpful in diagnosing AA in both children and adults. In addition to thiol/disulphide homeostasis, evaluation of albumin and IMA levels may increase the test specificity. This test can be more helpful in cases where the diagnosis is difficult such as in young children and in patients with mental retardation. However, more clinical studies on the subject should be undertaken.

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