Clinical and Experimental Health Sciences

Dynamic Thiol/Disulphide Homeostasis a Promising New Marker in the Diagnosis of Acute Appendicitis in Children: A Case Control Study of Acute Appendicitis and Abdominal Pain

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ABSTRACT

Objectives: One of the most common emergency surgery in pediatric surgery is due to acute appendicitis (AA). The diagnosis of AA is usually made using with the clinical score using clinical signs, symptoms, and laboratory tests. But symptoms and signs are not always typical, and this situation put clinician in a compelling situation. The range of misdiagnosis of AA is between 28-57 % between 2-12 years old children. Thiol/ Disulphide homeostasis is an important indicator of oxidative stress and inflammation. This study is aimed to evaluate and compare the feasibility of thiol/disulphide levels in pediatric patients with AA and abdominal pain (AP).

Methods: In this case-control study three different group established with 25 healthy participants (NCG), 25 patients with abdominal pain (PCG), and 25 with AA (AAG). Demographics, white blood cell count, neutrophil-lymphocyte counts, hemoglobin, platelet, mean platelet volume, C-reactive protein, total thiol (TT), native thiol, (NT) and disulphide (DS) levels measure through blood samples.

Results: According to our result, the level of NT were significantly lower in AAG when compared with NCG and PCG ($p<0.001$). DS levels were significantly higher in AAG than in NCG ($p<0.001$). CRP levels were significantly higher in both PCG and AAG than those of NCG $p<0.001$).

Conclusion: Thiol/disulphide homeostasis is a valuable method to examine acute appendicitis in the pediatric patients. Fluctuations of thiol/disulphide homeostasis could be used as a marker in daily clinical practice for diagnosis of appendicitis.

Keywords: Thiol/disulphide homeostasis, appendicitis, pediatric population, pediatric appendicitis

1. INTRODUCTION

Acute appendicitis (AA) is the most common emergency surgery in pediatric surgery department (1). Although the diagnosis of AA is often made with a clinical approach, symptoms, and signs which are not always typical, and diagnosis can be difficult in these patients (2). While the misdiagnosis rate is between 28-57% in the age range of 2-12, this rate rises to 100% under the age of two (3). Delayed diagnosis and treatment increase the risk of perforation in AA, which is related to increased mortality and morbidity. Various clinical scoring systems have been developed for diagnosis of AA, but these scoring systems are not routinely used in clinical practice (4). In some cases, different scoring systems are not accurate enough for diagnosis in terms of sensitivity and specificity.

Delays in diagnosis may cause perforation, which is an important complication of AA. Mortality significantly increases with perforation of AA due to intra-abdominal abscess (1). Especially in young children, complications, and uncommon events may emerge rapidly (5). Diagnosis should be made quickly and accurately to avoid the undesirable consequences and complications of AA (6).

Along with imaging techniques such as ultrasonography and computed tomography, easily accessible and applicable tests such as leukocyte count, neutrophil ratio, C reactive protein (CRP) and bilirubin levels also evaluated for diagnosis of AA (7). On the other hand, there are studies in which different markers such as calprotectin and leucine-rich alpha glycoprotein are used other than standard tests.

Pathophysiology of AA has been defined in detail; however, the factors affect the AA remains unclear (8). The progression of AA has been related to oxidative stress markers by de

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Oliveira et al. (9). The potential role of oxidative damage also been investigated by other studies as well (8).

Plasma thiols have a crucial role in radical scavenging activities in the body and therefore serve as an antioxidant through various mechanisms. Thiol-Disulphide Homeostasis (TDH) plays an important role in antioxidant status, detoxification, signal transmission, apoptosis, and enzymatic activities (10). Abnormal thiol-disulphide homeostasis is known to play a role in the pathogenesis of various diseases such as diabetes (11), cancer (12), cardiovascular diseases (13), rheumatoid arthritis (14), chronic kidney diseases (15), AIDS (16), Parkinson, Alzheimer's, Multiple Sclerosis and Amyotrophic Lateral Sclerosis (17). This homeostasis is thought to play a role in the etiopathogenesis of AA. The aim of this study was to investigate dynamic thiol-disulphide homeostasis, as a promising biomarker for children with AA and in children with AP.

2. METHODS

In this prospective case control study involving healthy volunteers, AA and, AP. Patients with the same demographics, children between 5-14 years old who had AP and were diagnosed with AA included 25 children with appendicitis, 25 children with AP, and 25 healthy volunteers who admitted a university hospital in Istanbul, Turkey between August 2018 – February 2020. This study has been approved by local Ethics committee with the number of (13/20). An informed consent form signed by one of the parents/guardians of the children. Participants with acute appendicitis called AA group (AAG), participants with AP called positive control (PCG), and healthy participants called negative control group (NCG).

Participants' demographics were recorded. White Blood cell count, neutrophil ratio, leukocyte ratio, hemoglobin levels, platelet count, mean platelet volume, CRP levels, TT, NT, and DS levels were recorded in all groups. Patients diagnosed with any acute appendicitis and complaining abdominal pain have been included in contrary patients with a history of vitamins or any antioxidant substance, and not signed consent form was excluded from the study.

Patients with AA were diagnosed based on clinical symptoms, physical examination, WBC count, NLR, CRP, abdominal ultrasonography (used to measure the appendiceal diameter and wall thickness). Patients with an appendiceal diameter greater than 6 mm and wall thickness greater than 2 mm were considered to have appendicitis (18). All patients with AA were confirmed by examination of pathological specimens after the operation.

Venous blood samples were taken to measure thiol/disulphide homeostasis parameters of all participants who were included in the study. Blood samples were withdrawn after 8 hours of fasting to measure thiol/disulphide blood levels. Serum samples centrifugated at 2500 x rpm for 10 minutes and stored at – 80 °C. Then the serum samples tested for thiol/disulphide levels. Complete blood count, biochemistry, and CRP levels of all participants were measured at the time they were enrolled in the study. The reduced DS bonds were reduced to form free functional thiol groups according to Erel et al. (19). Both thiols either reduced or native thiol groups were determined. The amount of dynamic disulphide bonds was found by determining half of the difference between total thiol and native thiol groups. After calculating the amount of native, total thiol, disulphide, disulphide/total thiol ratio, native thiol/total thiol rates, and disulphide/native thiol ratios were determined.

DS levels ratios were compared between AAG, PCG, and NCG. Also, the clinical relationship between NT and NT/TT levels, DS, DS/NT and DS/TT levels and clinical manifestations also investigated.

Descriptive statistics are presented as frequencies and percentages. The normality of continues variables was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical variables shown as numbers with percentages and normally distributed continues variables mean ± standard deviation and means (with interquartile range) used. Intergroup comparison between continues variables has been estimated with variance analyses, the ANOVA and student t-test. For the comparison of categorical variables we preferred chi-sguare or Fisher exact tests. For statistical analyses Jamovi, and the SPSS software (ver. 22.0; IBM SPSS Inc., Chicago, IL) was used. A p value < 0.05 was considered as statistical significance.

3. RESULTS

In this study 75 participants were included. The demographics, biochemical results, and the thiol/disulphide homeostasis parameters of the NCG, PCG, and AAG are summarized in Table 1. The ages of the participant were 9.56 ± 2.38, 9.72 ± 3.45, and 9.64 ± 3.16 for NCG, PCG, and AAG respectively (Table 1). No statistically significant difference observed among the groups in terms of age and sex ($p>0.05$). On the other hand, thiol ratios were found statistically significant with white blood cell count, neutrophil ratio, leucocyte ratio, hemoglobin levels, platelet number, mean platelet volume ($p<0.005$) (Table 1).

The mean results of WBC levels, neutrophil ratios, lymphocyte ratios, hemoglobin (Hg) levels, platelet (Plt) count, the mean platelet volume (MPV), CRP levels, among the groups were statistically different ($p<0.05$) (table 2 and figure 1).

TT levels associated with the antioxidant profile. The mean results of TT levels measured were NCG, PCG, AAG were 0.472 ± 0.0217, 0.470 ± 0.0237, 0.371 ± 0.01333 mmol/L respectively (Table 1) and a statistically significant difference observed between, NCG and AAG ($p<0.001$), PCG and AAG ($p<0.001$) (Table 2 and figure 3). On the other hand, measured NT levels were NCG, PCG, AAG was 0.340 ± 0.0304, 0.332 ± 0.0223, 0.197 ± 0.0288 mmol/L respectively and a statistically difference obtained ($p<0.05$) (Table 1, 2 and figure 3). The mean results of DS levels were NCG, PCG, and AAG 0.0664 ± 0.0150, 0.0676 ± 0.0159, 0.0876 ± 0.0159 mmol/L, respectively which given in table 1 and 2. Disulphide levels

were significantly different among the groups (p<0.001) (Table 2 and Figure 3). Our measurement showed that the NT/TT (%) ratio for NCG, PCG, and AAG were 72.1 ± 5.74 %, 71.2 ± 5.81 %, 53.1 ± 7.96 % respectively which given in table 1 and 2. Among the groups a statistically significant difference was present (Table 2) (p<0.001).

In this study, DS/NT(%) ratios for NCG, PCG and AAG were respectively 19.7 ± 5.37 %, 20.6 ± 5.49 %, 46.4 ± 15.7 % and a statistically significant difference were found (p<0.001) (Table 1, 2 and Figure 3). DS/TT (%) ratios for NCG, PCG and AAG were 13.9 ± 2.87 %, 14.4 ± 2.91 %, 23.5 ± 3.98 % respectively which given in table 1 and 2. In the comparison of DS/TT levels, a statistically significant difference has been obtained given in table 2 (p<0.001).

Table 1. *The demographic and laboratory findings of groups.*

	NCG	PCG	AAG	P
N	25	25	25	
Sex (n, M/F)	13/12	13/12	13/12	
Age	9.56 ± 2.38	9.72 ± 3.45	9.64 ± 3.16	
WBC (x10³/µL)	7.61 ±1.57	11.8 ± 3.46	24.1 ± 7.34	<0.001
Neut %	62.3 ± 8.26	70.5 ± 17.3	85.4 ± 16.6	<0.001
Lymph%	23.0 ± 5.16	33.6 ± 8.51	41.9 ± 7.81	<0.001
Hb (g/dL)	14.9 ± 1.77	13.3 ± 1.16	12.0 ± 1.51	<0.001
PLT (x10³/µL)	277 ± 51.6	261 ± 46.4	189 ± 11.2	<0.001
MPV (fL)	8.62 ± 0.697	8.21 ± 1.15	6.26 ± 1.14	<0.001
CRP (mg/dL)	0.237 ± 0.0943	0.628 ± 0.485	7.56 ± 2.92	<0.001
Total Thiol (mmol/L)	0.472 ± 0.0217	0.470 ± 0.0237	0.371 ± 0.01333	<0.001
Native Thiol (mmol/L)	0.340 ± 0.0304	0.332 ± 0.0223	0.197 ± 0.0288	<0.001
Disulphide (mmol/L)	0.0664 ± 0.0150	0.0676 ± 0.0159	0.0876 ± 0.0159	<0.001
Disulphide /Native Thiol (%)	19.7 ± 5.37	20.6 ± 5.49	46.4 ± 15.7	<0.001
Disulphide /Total thiol (%)	13.9 ± 2.87	14.4 ± 2.91	23.5 ± 3.98	<0.001
Native Thiol/Total Thiol (%)	72.1 ± 5.74	71.2 ± 5.81	53.1 ± 7.96	<0.001

NCG: Negative Control Group, PCG: Positive Control Group, AAG: Acute Appendicitis Groups, WBC: White Blood Count, Neut: Serum Neutrophil Ratio, Lymph: Serum Lymphocyte Ratio, Hb: Serum Hemoglobin Levels, MPV: Mean Platelet volume, PLT: Serum Platelet, CRP: C-Reactive Protein

Table 2. *One-way ANOVA (Welch's) of laboratory values and demographics.*

	F	df1	df2	p
Age	0.0187	2	46.7	NS
Sex	0.0000	2	48.0	NS
WBC	69.8762	2	38.4	<0.001
Neut %	19.5650	2	42.3	<0.001
Lymph%	52.2361	2	45.4	<0.001
Hb	18.1931	2	46.5	<0.001
PLT	58.6826	2	35.1	<0.001
MPV	39.1048	2	45.1	<0.001
CRP	84.2200	2	33.2	<0.001
Total Thiol	280.6810	2	44.6	<0.001
Native Thiol	202.2764	2	47.0	<0.001
Disulphide	14.1882	2	48.0	<0.001
Disulphide /Native Thiol	32.6414	2	44.2	<0.001
Disulphide /Total thiol	53.4055	2	47.2	<0.001
Native Thiol/Total Thiol	53.4055	2	47.2	<0.001

NCG: Negative Control Group, PCG: Positive Control Group, AAG: Acute Appendicitis Groups, WBC: White Blood Count, Neut: Serum Neutrophil Ratio, Lymph: Serum Lymphocyte Ratio, Hb: Serum Hemoglobin Levels, MPV: Mean Platelet volume, PLT: Serum Platelet, CRP: C-Reactive Protein

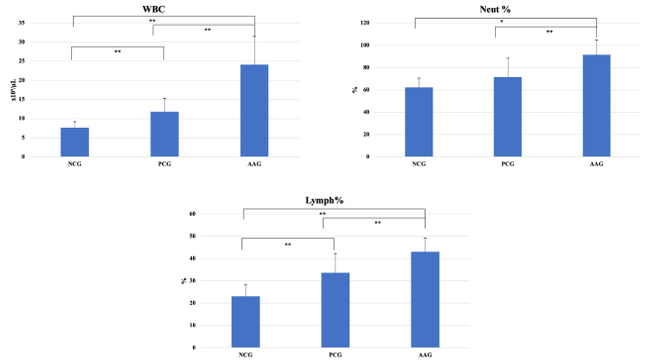

Figure 1. *Comparison white blood cell count, neutrophil ratio, and lymphocyte ratios. *p<0.05, **p<0.001. NCG: Negative Control Group, PCG: Positive Control Group, AAG: Acute Appendicitis Groups, WBC: White Blood Count, Neut: Serum Neutrophil Ratio, Lymph: Serum Lymphocyte Ratio.*

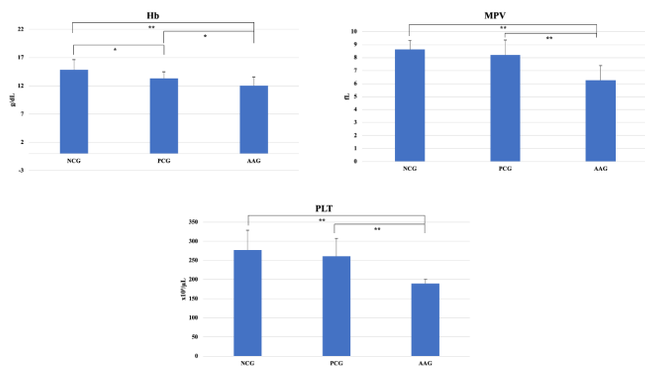

Figure 2. *Comparison serum hemoglobin, mean platelet volume, and platelet levels *p<0.05, **p<0.001. NCG: Negative Control Group, PCG: Positive Control Group, AAG: Acute Appendicitis Groups, Hb: Serum Hemoglobin Levels, MPV: Mean Platelet volume, PLT: Serum Platelet*

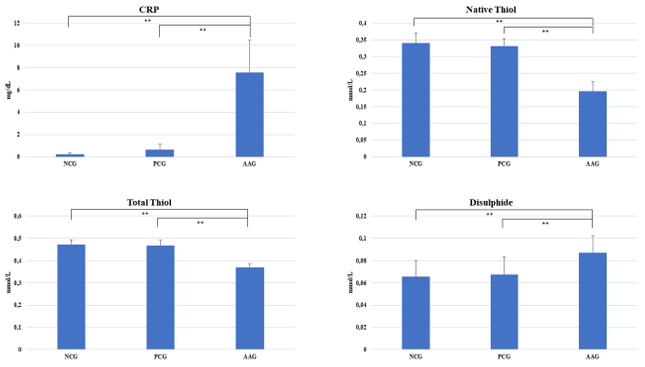

Figure 3. *Comparison serum CRP, native thiol, total thiol and introduction *p<0.05, **p<0.001. CRP: C-Reactive Protein, NCG: Negative Control Group, PCG: Positive Control Group, AAG: Acute Appendicitis Groups.*

4. DISCUSSION

In clinical practice, the diagnosis AA is still a big challenge for physicians. A definitive diagnosis would prevent unnecessary surgery (20-23). Even with sophisticated diagnostic methods, the rate of misdiagnosis of AA and abdominal pain in children varies between 28 % to 100 % (21). The importance of oxidative stress markers has an increasing interest in addition to inflammatory markers in AA diagnosis. Therefore, oxidative markers such as thiol and disulphide levels could be used as promising indicators for AA in children (24–26, 28-29).

In literature, some researchers have pointed out AA patients have decreased levels of thiol groups when compared with control (8,24,25,30). Oxidative stress causes an increase in DS levels and decrease in thiol levels. Recent studies investigated this change and compared with healthy individuals as a control group, which might be misleading. In this case control study of AA, AP, and control group, we tried to discriminate the difference of similar cases among 3 groups.

Elmas et al. has investigated the evolvement of thiol/disulphide ratio in AA group and control and found a significant difference between control and AA group (p<0.05) (30). Our findings were coherent with results presented by Elmas et al. (30). However, in our study, AP, and AA, were investigated through thiol/disulphide homeostasis perspective. Abdominal pain is one of the most common clinical findings of AA and discrimination of AP and AA is a challenge for physicians. According to our results, there is statistically significant difference between AP and AA groups in terms of thiols/disulphide levels (Figure 3).

Also, Yilmaz et al., Dumlu et al. and Ozyazici et al. reported that thiol/disulphide homeostasis was shifted towards disulphide side in AA patients (8,24,25). Our results showed that TT, NT, and DS levels were also impaired, and statistically significant difference has been present among the groups (p<0.001), which was favorable to findings in the literature.

Ozyazici et al. stated that increase of DS/NT ratio is related to the severity of inflammation, and it is possible to conclude that this ratio could be related with the progression of AA and could be used as a marker of AA together with other commonly used markers.

In our study the levels of NT and TT and the NT /TT ratio are lower in patients with AA as compared to AP and healthy individuals. Besides, it is also investigated for the first time that DS level and DS/NT and DS/TT ratios in AA, AP, and healthy patients. In other words, thiol/disulphide homeostasis was found to shift towards disulphide side in AA group.

There are several limitations to our work. We evaluated a relatively small sample size in one center. On the other hand, common diagnostic tools used for a scoring system such as procalcitonin, hs-CRP, ultrasonography, computerized Tomography, etc. have not been compared with thiol/disulphide assay. Studies which investigate the relationship between thiol/disulphide assays and other diagnostic tools should be accomplished for in future studies.

5. CONCLUSION

The early diagnosis acute appendicitis in pediatric population is hard to achieve in every cases due to the different feature of the disease. Novel instruments or biomarkers may prevent complications and give clinicians early notification about red flags. Our results revealed that thiol/disulphide homeostasis disturbed in children with AA. This shift towards the formation of disulphide may serve as a novel biomarker in AA. However, further studies are required to optimize this assay.

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