ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/medsci.2020-79596

Oxidant-Antioxidant Balance and Trace Elements in Children with Functional Dyspepsia

Fonksiyonel Dispepsili Çocuklarda Oksidan-Antioksidan Denge ve Eser Elementler

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Some of the preliminary data of this study were presented as a summary in 5th Gevher Nesibe International Health Sciences Congress, 24-25 April 2020, Ankara, Turkey.

ABSTRACT Objective: Functional dyspepsia (FD) is the most common functional abdominal pain disorder among children and adolescents according to the Rome IV criteria. The aim of the study was to determine the possible effect of the equilibrium of oxidant and antioxidant and trace elements in pediatric FD. **Material and Methods:** The patient group consisted of 23 children who were diagnosed with FD according to the Rome IV Questionnaire on Pediatric Gastrointestinal Disorders. The control group consisted of 23 children aged 11-17 who did not meet the Rome IV criteria and had no chronic disease. Measurements of zinc, copper levels and recently developed new generation oxidant-antioxidant balance markers were performed spectrophotometrically using a commercial kit. Routinely measured hemogram and vitamin B12 and vitamin D test results were obtained from the patient files retrospectively. **Results:** In comparison of the control group, the pediatric FD group had significantly higher levels of oxidized thiol, ischemia-modified albumin, neutrophil and neutrophil/lymphocyte, a significantly lower lymphocyte count and reduced thiol ratio, and significantly lower vitamin D, native thiol, and copper levels. **Conclusion:** FD is potentially related to inflammation, serum copper content, and oxidative stress. However, the relationship between FD and inflammation, copper levels, and oxidative stress has not been adequately investigated in children. As a conclusion of this clinical trial, thiol balance, ischemia modified albumin, neutrophil/lymphocyte ratio, vitamin D and copper content will likely be essential for diagnosis and follow-up in pediatric FD.

Keywords: Dyspepsia; child; trace elements; oxidative stress; antioxidants

ÖZET Amaç: Fonksiyonel dispepsi (FD), Roma IV kriterlerine göre çocuklar ve adölesanlar arasında en sık görülen fonksiyonel karın ağrısı bozukluğudur. Çalışmanın amacı, pediatrik FD'de oksidan ve antioksidan dengenin ve eser elementlerin olası etkisini belirlemektir. **Gereç ve Yöntemler:** Hasta grubu, Roma IV Pediatrik Gastrointestinal Bozukluklar Anketi'ne göre FD tanısı alan 23 çocuktan oluşturuldu. Kontrol grubu, Roma IV kriterlerini karşılamayan ve kronik hastalığı olmayan 11-17 yaş arası 23 çocuktan oluştu. Çinko, bakır seviyeleri ve yakın zamanda geliştirilen yeni nesil oksidan-antioksidan denge belirteçlerinin ölçümleri, ticari bir kit kullanılarak spektrofotometrik olarak yapıldı. Rutin ölçülen hemogram ve B12 ile D vitamini tetkik sonuçları, hasta dosyalarından retrospektif olarak alındı. **Bulgular:** Kontrol grubu ile karşılaştırıldığında, pediatrik FD grubunda anlamlı biçimde daha yüksek seviyelerde okside tiyol, iskemi modifiye albumin, nötrofil ve nötrofil/lenfosit, anlamlı biçimde daha düşük lenfosit sayısı ve azalmış tiyol oranı ve önemli ölçüde daha düşük D vitamini, doğal tiyol ve bakır seviyeleri bulundu. **Sonuç:** FD, potansiyel olarak inflamasyon, serum bakır içeriği ve oksidatif stres ile ilişkilidir. Ancak çocuklarda FD ile inflamasyon, bakır seviyeleri ve oksidatif stres arasındaki ilişki yeterince araştırılmamıştır. Bu klinik çalışmanın bir sonucu olarak tiyol dengesi, modifiye edilmiş albumin, nötrofil/lenfosit oranı, D vitamini ve bakır içeriği, pediatrik FD'de tanı ve takip için muhtemelen gerekli olacaktır.

Anahtar Kelimeler: Dispepsi; çocuk; eser elementler; oksidatif stres; antioksidanlar

Clinical pediatrics has widely experienced a practical issue, called functional abdominal pain disorders (FAPDs). Functional dyspepsia (FD) is the most common FAPD among children and adolescents according to the Rome IV criteria (3-7.6%). Patients may present with epigastric pain, postprandial fullness, early satiation, or burning.[1,2] The etiology of FAPDs is attributed to different potential factors. Genetics, dysbiosis, early life stress, psychosocial distress, post-infectious physiology, gut inflammation,

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Peer review under responsibility of Turkiye Klinikleri Journal of Medical Sciences.

Received: 15 Oct 2020 ***Received in revised form:*** 30 Nov 2020 ***Accepted:*** 28 Dec 2020 ***Available online:*** 14 Jan 2021

and diet are potentially responsible factors.[3,4] Oxidative stress is also responsible for the development of FD.[5] However, there are no data on oxidative stress markers in children and adolescents diagnosed with FD. The relationship between functional pediatric gastrointestinal diseases and organic gastrointestinal diseases in adulthood is also unclear.

Oxidative stress constantly occurs during metabolism. As a result of the reactions of oxygen in routine metabolic processes, free radicals are constantly formed in organisms as a result of radiation exposure, drugs, xenobiotics, and the effects of harmful chemicals. All released free radicals combine to promote oxidative stress. Oxidative stress has cytotoxic and genotoxic effects. For this reason, there is a requirement for a balance system between oxidants and antioxidants to neutralize the continuous oxidative stress components and sustain the life of the organism. Many enzymes and nonenzyme structures in the antioxidant system neutralize many free radicals, making it possible to maintain balance and the life of the organism. Oxidative damage occurs when oxidative stress increases and the antioxidant system cannot compensate for this condition. Oxidative damage is included in the etiologies of more than 100 serious diseases. Thiol balance, ischemia-modified albumin (IMA) levels, antioxidant enzyme paraoxonase 1 and arylesterase 1 activities are new and effective biomarkers that may be used to measure the oxidant-antioxidant balance as well as the total antioxidant status (TAS) and total oxidant status (TOS).[6-9] Considering the current research in the literature, oxidative stress in children has not been adequately studied other than, more specifically, the relationship between pediatric FD and trace elements and antioxidant status. However, zinc and copper are trace elements that have important biological functions and act as cofactors for hundreds of enzymes.[10] Many methods have been developed to measure oxidative stress and antioxidant status. Measuring the new generation oxidant and antioxidant parameters, which have been recently developed in our study, increases the original value of our study. The aim of the study was to determine the possible linearity between pediatric FD and serum zinc and copper levels, oxidant-antioxidant balance, and routine biochemical tests.

MATERIAL AND METHODS

RESEARCH DESIGN

The patient group included 23 children (16 girls and 7 boys) aged 11-17 years who were diagnosed with FD based on the Rome IV Questionnaire on Pediatric Gastrointestinal Disorders.[11] The exclusion criterion was described as being malnourished catching celiac or inflammatory bowel disease or another organic disease. The control group included 23 children aged 11-17 years who did not meet the Rome IV criteria and had no chronic diseases. Blood samples were taken from the patients who applied to Alanya Training and Research Hospital and the volunteer control group between 8.10.2019 and 8.2.2020. The consents of the patients and their parents were obtained. Hemogram and biochemistry analyzes were performed in the central laboratory of Alanya Training and Research Hospital. It was then retrospectively taken from their files and evaluated. Oxidative stress, antioxidant status and trace element analyzes were performed in Alanya Alaaddin Keykubat University Faculty of Medicine Research Laboratories.

ETHICAL CONSIDERATION

The study was conducted with the approval of the Alanya Alaaddin Keykubat University Clinical Research Ethics Board meeting dated 29.3.2019 and numbered 10354421-2019/5. Written consent was obtained from the parents of the patients. During the clinical research, the principles of the Declaration of Helsinki were followed. The authors declare having followed the protocols in use at their working center regarding patients' data publication.

LABORATORY ANALYSES

Hemogram analyzes were performed in the Central Laboratory of Alanya Training and Research Hospital in whole blood taken into EDTA tubes. The blood collected in biochemistry tubes with gel was centrifuged at 4,000 g for 10 minutes under cooling for other biochemical analyses in centrifuge device (Thermo Scientific SL 40R, USA). Results of hemogram and vitamin B_{12}, D levels were then retrospectively taken from their files and evaluated. The serum that was obtained was portioned in Eppendorf

tubes. The serum samples were frozen in a laboratory-type deep freezer set at -80°C (Thermo Scientific Forma 88000, USA) and taken out to thaw under room conditions after the specimina were completed. They were mixed with a vortex device (Thermo Scientific, USA). Vitamin B_{12} and D levels were measured in an Siemens Advia Centaur XPT autoanalyzer device, using commercial kits (Siemens Healthcare Diagnostics Muenchen, Germany). Afterwards, commercial kits (Rel Assay Diagnostics, Turkey) and the spectrophotometric method were used to measure the TOS, TAS, thiol balance, IMA levels, paraoxonase 1 activity, arylesterase activity, and zinc and copper levels. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA).

PARAOXONASE 1 ACTIVITY

Nonmanual technique was applied to the serum samples to measure the paraoxonase 1 activity. Commercial kits were used to determine the paraoxonase 1 activities (Relassay Diagnostics, Turkey). The measurements were completed with media for basal paraoxonase activity without NaCl and for salt-stimulated paraoxonase activity with NaCl. The hydrolysis of paraoxon (diethyl-p-nitrophenyl phosphate) was monitored with a tag after an observation of higher absorbance quality at 37°C and 412 nm to quantify p-nitrophenol yielded from hydrolysis. The net rate of enzymatic activity was calculated by subtracting the basal activity rate from the salt-stimulated activity rate. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The results are expressed in units per liter, which is equal to the hydrolysis of 1 micromole of substrate in 1 liter in 1 minute.[12]

ARYLESTERASE ACTIVITY

Nonmanual technique was applied to the serum samples to measure the arylesterase activity. Commercial kits were used to determine the arylesterase activities (Relassay Diagnostics, Turkey). The activity was estimated using a substrate (phenylacetate), and the hydrolysis of phenylacetate yielded phenol and acetic acid. The resulting phenol joined 4-aminoantipyrine and potassium ferricyanide, and it was measured with the colorimetric method. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The results are expressed in units per liter, which is equal to the hydrolysis of 1 micromole of phenylacetate in 1 liter in 1 minute.[13]

TOTAL ANTIOXIDANT STATUS

Commercial kits were used to determine the TAS levels (Relassay Diagnostics, Turkey). The fiction-automated method involved using antioxidants to bleach the typical color of the more stable 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation. Ideal tender values (<0.03) are found based on the trial. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The experimental results are expressed in μmol Trolox equivalent/L.[14]

TOTAL OXIDANT STATUS

Commercial kits were used to determine the TOS levels (Relassay Diagnostics, Turkey). The recent method allows for the pattern oxidants to oxidize the ferrous ion-o-dianisidine complex to ferric ions. The glycerol molecules, which were found in the reaction medium, boosted the oxidation reaction. The acidic medium hosts a colorful mixture of the ferric ion and xylenol orange. It was observed that overall oxidant molecules present in the pattern could change the color intensity that was observable on spectrophotometry. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The results are expressed in micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 equivalent/L) since hydrogen peroxide was used to calibrate the dissection.[15]

OXIDATIVE STRESS INDEX

The oxidative stress index (OSI) is equivalent to the ratio of TOS/TAS. The resulting unit for TAS was transformed into μol/L, and OSI values were calculated as follow: OSI (arbitrary unit)=TOS (μmol H_2O_2 equivalent/L) / TAS (μmol Trolox equivalent/L).[16]

ISCHEMIA-MODIFIED ALBUMIN MEASUREMENT

The serum IMA levels were determined based on the Albumin Cobalt Binding Test principles. The measurements were performed in the serum. Commercial kits were used to determine the IMA levels

(Relassay Diagnostics, Turkey). After all samples were collected, they were transported under cold chain, thawed, and studied. The serum IMA values were found using a admixture of cobalt chloride (5 µL) to patient serum (95 µL) following five-minute incubation. During incubation, the cobalt chloride concentration was 0.58 mmol/L. Due to ischemia, a tiny part of cobalt bonds with albumin. To determine the amount of cobalt that did not bond with albumin, we blended the concentration with 25 µL of dithiothreitol in the measurement cuvette after being incubated to reach up to 1.67 mmol/L, and consequently, a colorful mix was created with the unbound cobalt by dithiothreitol. The colored complex measurements were conducted on spectrophotometry (WL: 500 nm). After a 5-point calibration curve for the interval of 5-180 U/mL was drawn, the absorbance values were assessed on this calibration curve. Thus, the IMA levels were calculated based on the calibration curve. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The unit for IMA levels was g/L.[17,18]

THIOL BALANCE MEASUREMENT

The serum thiol-disulfide balance was spectrophotometrically studied with newly developed methods. The measurement method was a previously defined standard colorimetric method that has been frequently applied in the literature. Commercial kits were used to determine the total and natural thiol levels (Relassay Diagnostics, Turkey). In summary, functional thiol groups formed by reduction of disulfide bonds. The reduced but unused sodium borohydride was exhausted and formaldehyde cleared it away, and due to being reacted with 5,5-dithiobis-2-nitrobenzoic acid, all thiol groups that included reduced to create native ones. The quantity of dynamic disulfide is equivalent to the difference between the halves of overall thiol and natural thiol. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The following ratios were subsequently calculated: reduced thiol=(natural thiol/overall thiol)*100; oxidized thiol=(disulfide/overall thiol)*100; and thiol oxidation reduction ratio=(natural thiol/disulfide)*100. The corresponding units are as follows: disulfide levels to µmol/L, and overall thiol, natural thiol and oxidized thiol to µmol/L/alb (g/L).[19]

ZINC LEVELS

Commercial kits were used to determine the zinc levels by spectrophotometrically method (Relassay Diagnostics, Turkey). In alkaline conditions the color of 5-Br-PAPS turned from red-orange to light pink due to the zinc content in the specimina. At 548 nm absorbance alters in proprotion to the total zinc amount per specimen. Zinc sulfate was useful in the experimental calibration through dissolution in deionized water. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The corresponding units are µg/dL.[20]

COPPER LEVELS

Commercial kits were used to determine the copper levels by spectrophotometrical method (Relassay Diagnostics, Turkey). In acidic conditions the color of DiBr-PAESA turned from red-orange to violet due to the copper content in the specimina. At 572 absorbance alters in proportion to the total copper amount per specimen. Copper sulfate was useful in the experimental calibration through dissolution in deionized water. Absorbance measurement was made in a microplate reader device (Biotek Synergy H1, USA). The corresponding units are µg/dL.[21]

LYMPHOCYTE, LEUKOCYTE AND NEUTROPHIL COUNTS

Lymphocyte, leukocyte and neutrophil counts were measured in the hemogram autoanalyzer device. Sysmex branded reactives and Sysmex XN-1000 hematology autoanalyzer device (Sysmex Corporation, Kobe, Japan) were used. Measurements were made on whole blood taken into an EDTA tube.[22]

25-OH VITAMIN D MEASUREMENT

In our laboratory, the 25-OH vitamin D test was measured in a fully automated, highly efficient immunoassay test system in serum samples. Siemens branded kit and Siemens Advia Centaur XPT autoanalyzer device (Siemens Healthcare Diagnostics Muenchen, Germany) were used. The assay range of the 25-OH vitamin D kit is 4.20-150.0 ng/mL.[23,24]

VITAMIN B_{12} MEASUREMENT

In our laboratory, the vitamin B_{12} parameter was measured in a fully automated, highly efficient immunoassay test system in serum samples. Vitamin B_{12} was assayed by chemiluminescent paramagnetic microparticle immunoassay method. Siemens branded kit and Siemens Advia Centaur XPT autoanalyzer device (Siemens Healthcare Diagnostics Muenchen, Germany) were used. The assay range of the vitamin B_{12} kit is 45-2,000 pg/mL.[23,25]

STATISTICAL ANALYSIS

Categorical variables were assigned the computed frequencies and percentages, and continuous variables the means, standard deviations, and medians. Normal distribution testing of the continuous variables was performed using the Kolmogorov-Smirnov test. Significance level was identified as p value <0.05. The intergroup comparison of laboratory results were made with an ANOVA.

RESULTS

Relative to the control, the pediatric FD group had significantly higher oxidized thiol (p=0.036), IMA (p=0.006), neutrophil (p=0.037), and neutrophil/ lymphocyte levels (p=0.001), a significantly lower lymphocyte count (p=0.009) and reduced thiol ratio (p=0.036), and significantly lower 25-OH vitamin D (p=0.007), natural thiol (p=0.024), and copper content (p<0.001). Although there are numerical differences between the groups in terms of leukocytes, vitamin B_{12}, paraoxonase 1, arylesterase, TAS, TOS, OSI, total thiol, disulfide, thiol oxidation reduction ratio and zinc levels, there is no statistically significant difference (p>0.05). The results are shown in detail in Table 1 below. ROC curve and area under ROC curve were shown in Figure 1 and Table 2.

DISCUSSION

Dyspepsia frequently includes bloating of the upper gastrointestinal tract, epigastric pain, nausea, and restlessness. It is defined by one or more of the following complaints: pain, nausea, bloating, early satiety, indigestion, belching, and intermittent vomiting in the stomach. However, in the majority of patients with dyspepsia, the cause cannot be determined after examinations and is referred to as FD. If a pathological condition cannot be detected from gastric mucosa examinations performed in these patients, the etiology of the complaints is considered to be a functional disorder. Dyspepsia caused by a functional disorder is FD. It has been argued that FD was related to inflammation and oxidative stress.[3-5] This relationship, however, has not been adequately investigated in children. The IMA level is a marker of ischemia rather than tissue damage. IMA levels have been associated with increased reactive oxygen species levels. If higher IMA levels in the FD group in the current study (p=0.006) are supported by new research, the IMA levels may be a valuable laboratory parameter in the diagnosis of FD.[17,18] Since our study is a clinical human study, it is not possible to take tissue samples and measure oxidative stress from tissues. Increased serum IMA levels indicate increased oxidative stress in the blood. Increased oxidative stress in the blood affects the tissues in a long time. Although the gastric mucosa structure is expected to be normal in FD, the increase in oxidative stress in the blood suggests that stomach damage and other related diseases may be caused in the long term. The control group had significantly higher natural thiols (p=0.024) and reduced thiols (p=0.036), while the pediatric FD group had significantly higher oxidized thiols (p=0.036), which indicates that the thiol balance is skewed toward oxidation in pediatric FD patients, and this may be a significant laboratory parameter in the diagnosis of the disease.[19] Relative to the control, the FD group had significantly lower serum copper levels (p<0.001). In an experimental animal study in the literature, high serum copper levels were shown to increase the activity of lipase and amylase, serum gastrin concentrations, and antioxidant capacity. In this case, it may be effective for enzymes to use metal ions as cofactors.[26] In our study, the low serum copper concentration in children diagnosed with FD showed that copper levels should be investigated in the diagnosis, mechanism, and treatment of this disease.

IMA levels come to the fore as an important and new biochemical indicator for many diseases caused by oxidative damage.[18] If the increase in oxidative

TABLE 1: Evaluation of various laboratory parameters in children diagnosed with pediatric functional dyspepsia.

Parameters	Unit	Control group (n=23) mean±SD	Functional dyspepsia group (n=23) mean±SD	p value
Lymphocytes	Cells per microlitre of blood	3,383.04±1,516.93	2,316.09±1,079.56*	0.009
Neutrophils	Cells per microlitre of blood	3,159.57±1,041.22	4,020.87±1,615.19*	0.037
Neutrophils/lymphocytes	Ratio	1.16±0.65	1.94±0.80*	0.001
Leukocytes	Cells per microlitre of blood	7,376.96±1,480.71	7,080.87±1,952.62	0.565
Vitamin B_{12}	pg/mL	433.22±113.74	455.61±242.11	0.690
25-OH vitamin D	ng/mL	19.26±5.40	14.39±6.29*	0.007
Paraoxonase 1	U/L	617.30±155.73	637.61±93.07	0.594
Arylesterase	U/L	759.17±108.22	776.61±52.85	0.491
Total antioxidant status	µmol Trolox equivalent/L	1.43±0.25	1.43±0.20	0.948
Total oxidant status	µmol H_2O_2 equivalent/L	3.14±1.56	3.53±1.21	0.349
Oxidative stress index	Ratio	2.32±1.41	2.57±1.14	0.520
Total thiol	(µmol/L)/	344.03±31.37	338.24±43.56	0.608
Natural thiol	(µmol/L)/	277.53±23.10	258.36±31.99*	0.024
Disulfide	(µmol/L)/	33.25±11.85	39.94±14.72	0.097
Reduced thiol ratio	Ratio	80.93±6.02	76.73±7.09*	0.036
Oxidized thiol ratio	Ratio	9.53±3.01	11.64±3.54*	0.036
Thiol oxidation reduction ratio	Ratio	982.47±484.46	769.54±403.69	0.113
Ischemia modified albumin	g/L	0.99±0.29	1.16±0.05*	0.006
Zinc	µg/dL	96.13±24.71	102.53±38.31	0.505
Copper	µg/dL	107.77±32.36	77.58±19.85*	<0.001

*This value showed that the inter-group difference was statistically significant (p<0.05). Formulas: oxidative stress index=total oxidant status/total antioxidant status; reduced thiol=(natural thiol/overall thiol)*100; oxidized thiol=(disulfide/overall thiol)*100; and thiol oxidation reduction=(natural thiol/disulfide)*100.

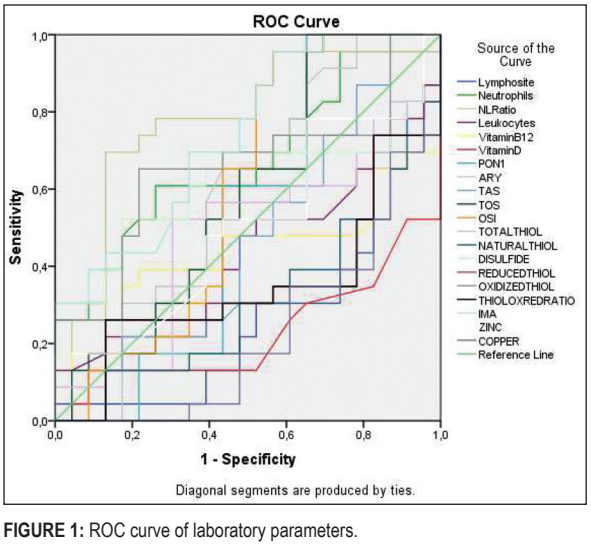

FIGURE 1: ROC curve of laboratory parameters.

stress cannot be balanced with antioxidant capacity, the damage that occurs may impair tissue and organ functions, as it affects the structure of cells. The structure of the cell and nucleus membrane can be disrupted. Genetic material may be damaged. Lipid and protein molecules can be oxidized. The vascular endothelial layer may lose its integrity.[27-30] Conflicting information has been reported in the literature regarding the relationship between vitamin B_{12} and stomach symptoms.[31] In our study, no significant difference was found between the groups in terms of B_{12} levels. Thus, it can be said that no change in vitamin B_{12} levels is expected in patients with FD. Various nutritional problems and malabsorption also occur in stomach and intestinal diseases. For this reason, it has

TABLE 2: Area under the ROC curve for laboratory parameters.

				Asymptotic 95% confidence interval	
Test result variable(s)	**Area**	**Std. error[a]**	**Asymptotic sig[b]**	**Lower bound**	**Upper bound**
Lymphosite	0.265	0.074	0.006	0.119	0.411
Neutrophils	0.669	0.081	0.049	0.511	0.827
Neutr/lymph. ratio	0.781	0.071	0.001	0.642	0.920
Leukocytes	0.431	0.087	0.423	0.261	0.601
Vitamin B_{12}	0.432	0.090	0.429	0.255	0.609
25-OH vitamin D	0.228	0.073	0.002	0.086	0.370
Paraoxonase 1	0.518	0.092	0.835	0.338	0.698
Arylesterase	0.587	0.087	0.312	0.417	0.757
Total antioxidant status	0.464	0.087	0.676	0.294	0.635
Total oxidant status	0.584	0.086	0.328	0.415	0.754
Oxidative stress index	0.599	0.089	0.249	0.425	0.774
Total thiol	0.480	0.088	0.818	0.307	0.653
Natural thiol	0.310	0.079	0.027	0.156	0.464
Disulfide	0.635	0.086	0.116	0.467	0.803
Reduced thiol	0.346	0.084	0.073	0.181	0.511
Oxidized thiol	0.654	0.084	0.073	0.489	0.819
Thiol oxid. red. ratio	0.346	0.084	0.073	0.181	0.511
Ischemia modified albumin	0.737	0.072	0.006	0.595	0.879
Zinc	0.505	0.087	0.956	0.334	0.676
Copper	0.223	0.068	0.001	0.091	0.356

The test result variable(s): Neutrophils, leukocytes, vitamin B_{12}, 25-OH vitamin D, arylesterase, total antioxidant status, ischemia modified albumin, Zinc has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a]Under the nonparametric assumption; [b]Null hypothesis: true area=0.5.

been described in the literature that the risk of vitamin D deficiency is high in gastroinestinal diseases. Low vitamin D levels have been described in gastroesophageal reflux, irritable bowel syndrome and Celiac disease.[32] There is not sufficient data in the literature to show low vitamin D levels in FD patients. In our study, a significant decrease in 25-OH vitamin D was shown in the FD group compared to the control group (p=0.007). For this reason, our results showing the difference of 25-OH vitamin D are unique and will become more valuable if supported by new research. The presence of *Helicobacter pylori* has been suggested as one of the possible factors of increased oxidative stress level in patients with FD.[31,33] Since our study is restrospective, we do not have any information showing the presence of *Helicobacter pylori*. New comprehensive experimental and clinical studies can be planned to demonstrate this situation. The ratio of neutrophil to lymphocyte indicates systemic inflammation.[34] In our study, the pediatric FD group had significantly higher neutrophil counts (p=0.037) and neutrophil to lymphocyte ratio (p=0.001) and significantly lower lymphocyte counts (p=0.009) than the control group. Micro-inflammation has been shown in the literature in patients with FD.[35] However, there are no similar studies showing this situation on the neutrophil/lymphocyte ratio. According to our results, the increased neutrophil/lymphocyte ratio can be used as a clinical marker for FD. Increased oxidative stress may adversely affect the gastric mucosa structure and functions and lead to atherosclerosis. It is thought that many diseases, including FD, may be associated with increased oxidative stress. In increased oxidative stress, the increase in ROS triggers the inflammatory response. As a result of increased inflammation, it can disrupt the tissue structure by affecting the DNA inside the cell. In our re-

search results, it can be thought that the increased inflammation and oxidative stress indicators emerging in the FD group may lead to FD.[5,36] FD disease has a high incidence in children. On the other hand, there is still a need for a laboratory indicator in the diagnosis of FD. Although there are numerical differences between the groups in terms of leukocytes, vitamin B_{12}, paraoxonase 1, arylesterase, TAS, TOS, OSI, total thiol, disulfide, thiol oxidation reduction ratio and zinc levels, there is no statistically significant difference in our study ($p>0.05$). If the number of patients is increased, significant results may be obtained for other oxidative stress and antioxidant system parameters. According to the results of this study, there is a need for follow-up in these children and adolescents in terms of the development of organic gastrointestinal diseases in adulthood. Our data will be strengthened if the results of this study are supported by new research.

CONCLUSION

FD is potentially related to inflammation, serum copper content, and oxidative stress. However, the relationship between FD and inflammation, copper levels, and oxidative stress has not been adequately investigated in children. As a result of this clinical trial, thiol balance, ischemia modified albumin, neutrophil/lymphocyte ratio, vitamin D and copper content will likely be essential for diagnosis and follow-up in pediatric FD.

STRENGTHS

Our paper contains a research of high original value. The changes in pediatric FD patients were investigated using the new generation oxidative stress and antioxidant balance parameters. There is very limited data in this area in the literature. This original research paper will make an important scientific contribution by pioneering new research on FD in pediatric patients.

LIMITATIONS

The total number of children in the patient and control group included in the study was limited to 46, because the study was conducted on children, complying with ethical restrictions, and requiring consent. For this reason, although the number of patients in the article is sufficient for statistical evaluation, it limits the value of the study. The balance between oxidative stress and antioxidant status may need to be taken into account in the diagnosis of FD and treatment follow-up in pediatric patients, if more extensive experimental studies are conducted and our data in this article are supported.

MAIN POINTS

According to the research results in this paper it was determined that:

- The deterioration resulting from oxidation in **native-total thiol balance** may be a laboratory indicator for pediatric FD patients.
- **IMA**, which is the indicator of the deterioration caused by oxidation of albumin may be a laboratory indicator for pediatric FD patients.
- **Vitamin D** deficiency should be considered as a laboratory indicator in pediatric FD patients.
- The lowness of **copper**, which is an important trace element for digestive functions should be considered as a laboratory indicator in pediatric FD patients.
- The increase in the **neutrophil/lymphocyte ratio**, which is a routine laboratory parameter as an indicator of systemic inflammation, may be significant for pediatric FD patients.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

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