



Inappropriate Total Oxidant/Antioxidant Status, Nitric Oxide Oxidation End Products and Trace Element Levels in Patients with Inflammatory Bowel Disease

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Abstract

Background: This study aimed at evaluating oxidant and antioxidant markers (including nitrite, nitrate, total antioxidant capacity (TAC), malondialdehyde (MDA), iron, selenium, glutathione peroxidase (GPx), and glutathione reductase (GR) in patients with inflammatory bowel disease (IBD) and compare them with healthy controls.

Methods: Serum samples were obtained from 35 patients (19 ulcerative colitis (UC) and 16 Crohn's disease (CD) in the active phase of the disease) and 30 healthy controls. Serum levels of nitrite and nitrate, TAC, MDA, iron, selenium, glutathione peroxidase, and glutathione reductase were measured. The results were compared between the two groups using independent t-student test. The Pearson's correlation coefficient (for continuous data) was performed using the SPSS software.

Results: Serum levels of nitrite, nitrate, and MDA were significantly higher ($P = 0.001$) in patients with IBD, while the levels of TAC, trace elements, glutathione peroxidase (GPx), and Glutathione Reductase (GR) levels were lower ($P < 0.05$) in patients with IBD. However, when females were considered separately, Gpx and GR activities were not significantly different in those with and without IBD. The present results showed that nitrite, MDA, GPx, GR, and Se: MDA ratio had the strongest correlation with disease activity score.

Conclusions: There is an imbalance between oxidant and antioxidant properties in patients with IBD, which highlights the role of oxidative stress in the pathogenesis of this disease.

Keywords: Nitrites, Nitrates, Antioxidants, Malondialdehyde, Iron, Selenium, Glutathione Peroxidase, Glutathione Reductase

1. Background

The general term of IBD represents chronic, relapsing, and remitting disorders, associated with uncontrolled continuous or episodic inflammation of the gastrointestinal (GI) tract, and is classified to two major groups: CD and UC (1). These diseases influence over one million individuals in the United States, with prevalence rates close to 200 per 100,000 in some populations (2). Over the past two decades, the occurrence of IBD has increased, for example it has increased in some countries, that have rarely been reported amongst Asian countries (India, South Korea, China, and Thailand), especially in the Mediterranean area (Iran and Lebanon) (3-6). Although the causes of the increase and etiology of IBD are not clear, both diseases are

considered to be multifactorial and result from the interplay of genetic, dietary habits and environmental factors, and immunological responses (6-9).

During the last two decades, nitric oxide (NO), an unstable free radical, has been known as one of the most versatile players in immune responses. The level of NO oxidation end products, nitrite, and nitrate, is considered as an index of NO production. Also, this level reflects NO-Synthases (NOS) activity, such as the inducible form of NOS (iNOS), which synthesizes NO in high (micro molar) amounts (10, 11). Enhanced NO production and positive immune reactivity for iNOS expression in colonic mucosa of UC patients was previously reported (12). In addition, a significant increase in colonic NO from children with both UC and CD (13) and in adults (14) was demonstrated compared

to healthy controls.

Both reactive oxygen and nitrogen species (ROS/RNS) attack on the polyunsaturated lipids (particularly membrane lipids and lipoproteins), leading to the production of highly reactive molecule, MDA, that can induce cell death through membrane impairment and damage to other macromolecules (15, 16). According to a recent study, higher level of serum MDA was observed in UC patients (17) and experimental model of colitis (18), yet Ahmet Tuzunet al. reported no significant differences between active IBD patients and the control group (19).

In the 21st century, selenium (Se), as a vital trace mineral element, received ample attention because of its crucial role in antioxidant enzymes and anti-inflammatory effects via seleno-methionine and seleno-cysteine forms in seleno-proteins, particularly the glutathione peroxidase family (20, 21). Reduced synthesis of seleno-proteins, mainly GPx, associated with low selenium supply, has been observed (22). Also, the decrease in serum GPx activity in IBD patients was reported in a study by D. Achiteiet al. (23), yet Ahmet Tuzunet al. (19) recorded an increase in GPx activity in patients compared to controls.

Moreover, excessive production of ROS and the up-regulation of pro-inflammatory genes, such as the iNOS gene in RAW 264.7 macrophages with Se deficiency, were illustrated (24). In addition to selenium, iron is an important immunomodulator and is thereby crucial to suppress inflammation and effective response to infection. Iron is essential for redox reactions, oxygen delivery to tissues, gene regulation, and cell growth (25-27).

The aim of this study was to evaluate serum nitrite and nitrate levels, redox body status (serum Total antioxidant capacity (TAC) as an indicator of synergistic or antagonistic effect of antioxidant compounds in serum and MDA as a general indicator of oxidative stress were chosen to estimate redox body status) and the trace elements, Fe and Se, with relative enzymes (GPx and glutathione reductase activity) and their correlation in IBD patients.

2. Methods

2.1. Materials

In this case, sodium acetate, acetic acid, nitric acid, Thiobarbituric acid (TBA), (FeCl_3), TPTZ (2,4,6-tripyridyl-s-triazine), ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) selenium standard (SeO_2) (Merck, Darmstadt, Germany), hydrochloric acid, and trichloroacetic acid (TCA), were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2. Patients

The prospective mean age of patients and controls was 33.2 ± 9.0 and 33.4 ± 9.0 years, respectively.

Patient and healthy control groups were chosen among the people admitted to the endoscopic ward of Ayatollah Rouhani hospital of Babol University of Medical Sciences, Babol, Iran (January 2014 to February 2015). The diagnosis of IBD was based on clinical, endoscopic, and histological findings. All patients were in the active phase of the disease, having proctosigmoiditis, left colitis, extensive or pancolitis in the case of UC and ileitis or ileocolitis in the case of CD. The disease activity was scored for UC and CD patients, respectively, based on the studies of Rachmilewitz (28) and Harvey-Bradshaw (29). The locations of CD disease were the ileum, colon, small bowel, and the gastroduodenal region. The location of UD disease was the rectum. Assessing the disease activity was based on clinical symptoms, quality of life score, and endoscopic indices.

The diagnosis of UC was based on the history of diarrhea or blood or pus in the stool; macroscopic appearance on endoscopy of continuous mucosal inflammation affecting the rectum in continuity with some or all of the colon. Endoscopic findings were edematous mucosa, erythema, loss of vascular markings, and mucosal friability.

The diagnosis of CD was based on abdominal pain and stool frequency, intestinal longitudinal ulcer or deformity, induced by a longitudinal ulcer or cobblestone pattern. Endoscopic findings were discontinuous distribution of longitudinal ulcers.

The inclusion criteria were having a diagnosed IBD (including CD and/or UC) for the patient group and consenting to participate in the study for patient and healthy control groups. Exclusion criteria for patient and healthy control groups were as follows: being pregnant, malignancy, severe co-existing diseases, history of any chronic disease of upper gastrointestinal system (such as acid peptic ulcer), history of GI surgery, having infectious disease, diabetes, severe cardiovascular disease, renal failure, taking medicine, use of antioxidant supplements, supplementation of micronutrient, and smoking.

2.3. Healthy Controls

Healthy volunteers were enrolled in the study from the general population of Babol as the healthy control group. Health was defined as physical, mental, and social well-being of a person and lack of any acute or chronic diseases, history of chronic disease, or acute or chronic medication and the absence of any health problems in that course of life (30).

Written informed consent was obtained from the participants. All protocols involving patients and control subjects were approved by the ethics committee of Babol University of Medical Sciences with code number P/J/30/4332, 93/3/19.

2.4. Sampling and Assay

To assess the biochemical parameters, peripheral (cephalic or basilica) venous blood samples (5 mL) were collected in the morning (between 8:00 and 11:00) after overnight fasting. Samples were immediately transferred to acid washed serum-separator tubes and after 20 minutes were incubated at room temperature followed by centrifugation at 2000 rpm for 10 minutes. After serum separation, aliquots were transferred to five micro-tubes, and stored at -80°C until the time of assays. Hemolyzed specimens were excluded.

For the assessment of blood redox status, the levels of thiobarbituric acid reactive substances (TBARS) and TAC of the serum were measured. To evaluate MDA (TBARS) level, a spectrophotometric method was used, based on published data (31).

The TAC was determined using the colorimetric method of plasma ability to reduce ferric ion by Ferric reducing antioxidant power (FRAP). Based on this method, the antioxidant ability of serum/plasma to reduce ferric ion at low pH leads to produce Fe (II)-IPTZ blue colored complex that has λ max at 593 nm was evaluated (32).

Serum nitrite and total NO (nitrite + nitrate) concentrations were measured spectrophotometrically (Microplate reader, model: RT 2100C, Hamburg, Germany), using human nitric oxide assay Kit (lot: NO1471), provided by Biocore Diagnostik Ulm GmbH (Zell Bio GmbH, Germany), and according to the manufacturer's instructions. For measuring nitrite alone, nitrate reduction was prevented by deleting nitrate reductase (reagent type 4 in the kit) from the assay.

Serum glutathione peroxidase (GPx) and Glutathione Reductase (GR) activity were measured spectrophotometrically (Microplate reader, stat fax-2100, USA) with human GPx (lot: ZB-A71621) and GR (lot: ZB-A2151101) activity assay kit produced by Biocore Diagnostik Ulm GmbH (ZellBio GmbH, Germany), based on the manufacturer's instructions. The assay and the outcomes of assaying oxidative markers had been validated against other methods (33-37).

Human iron assay kit to measure iron concentration (Pars Azmon, Iran) was used, and finally Se was detected using atomic absorption spectrophotometer graphite furnace (model: PG990, Beijing, China). The following precautions were taken to minimize adventitious oxidation of biochemical factors under these conditions: (i) the working solutions and buffers were prepared immediately before use; (ii) buffers and solutions were freshly prepared in metal-free de-ionized water; and (iii) adventitious catalytic metals displayed in laboratory equipment were removed by washing with 0.1 M-HCl, followed by repeated washing. In addition, Fe from clotted blood, red cell lysis and therefore release of Fe and heme that causes oxidation in the

serum were involved. Previously, evaluation of some other serum trace element levels and superoxide dismutase activity in patients with inflammatory bowel disease was reported (36).

2.5. Statistical Analysis

The means and Pearson's correlation coefficient (for continuous data) were performed using the SPSS software, version 19.0. To compare groups, independent-samples t-test was performed and P values of < 0.05 were considered for statistical significance. All values were expressed as mean \pm standard deviation (SD). Pearson's correlation test and matrix scatter plot were performed to evaluate the correlation between parameters.

3. Results

The prospective mean age of patients and controls was 33.2 ± 9.0 and 33.4 ± 9.0 years, respectively. Comparison of biochemical parameters (Nitrite, Nitrate, Nitrate/Nitrite ratio, FRAP, MDA, Selenium, Selenium/MDA ratio, Iron, GPx activity, GR activity) between IBD patients and healthy individuals demonstrate a statistical significance (Table 1). The clinical characteristics of the studied groups are demonstrated in Table 2. Although the GPx and GR activity did not show any significant difference in females with IBD compared to controls (P value > 0.05); in males and in all patients, it decreased significantly (P value < 0.05) in comparison with controls. Among the other biochemical parameters, nitrite, nitrate, and MDA increased significantly in the patient group compared with the controls. However, the levels of FRAP and trace elements (Se and Fe) concentration were reduced significantly in patients compared to controls. Also, total antioxidant status FRAP, MDA, selenium, Se:MDA ratio, GPx and GR activity in healthy, UC and CD individuals are shown as mean \pm SD in Figures 1 to 2. The correlation analysis between Disease Activity (DA) score and biochemical parameters is illustrated in Figures 3 to 4, using matrix scatter plots. Nitrite, nitrate, MDA (TBARS), Se:MDA ratio, GPx, and GR activity indicated a significant correlation with the DA score, yet nitrite, MDA, Se:MDA ratio, and GPx and GR activity had the strongest correlation with the DA score. There was no significant correlation between DA score and trace elements.

4. Discussion

In this study, it was observed that serum NO metabolites (nitrite and nitrate) increased significantly in the patient group compared to controls. Serum nitrite and nitrate were 41.6% and 9.9% higher in IBD patients compared

Table 1. Comparison of Nitrite, Nitrate, Nitrate/Nitrite Ratio, FRAP, MDA, Selenium, Selenium/MDA ratio, Iron, GPx Activity, GR Activity of Patients with IBD and Healthy Control Subjects

Biochemical Parameters	Mean \pm SD		Mean Difference, % ^a	P Value
	IBD Patients	Controls		
Men				
Nitrite, nM/L	13.5 \pm 0.8	9.7 \pm 0.5	39.1	0.001
Nitrate, nM/L	16.4 \pm 0.6	15.2 \pm 0.7	7.9	0.001
Nitrate/Nitrite ratio	1.2 \pm 0.05	1.5 \pm 0.09	-20.0	0.001
FRAP, nM/L	0.79 \pm 0.8	1.10 \pm 0.8	-28.1	0.001
MDA, μ m/L	4.1 \pm 0.6	1.8 \pm 0.5	127.8	0.001
Selenium, μ g/L	77.1 \pm 5.9	92.8 \pm 9.1	-16.9	0.001
Selenium/MDA ratio	19.0 \pm 3.7	54.3 \pm 20.0	-65.0	0.001
Iron, μ g/dL	61.76 \pm 39.1	99.1 \pm 24.1	-37.7	0.003
GPx activity, U/mL	221.3 \pm 54.8	312.6 \pm 32.7	-29.2	0.001
GR activity, U/L	45.8 \pm 10.9	74.2 \pm 21.3	-38.2	0.001
Women				
Nitrite, nM/L	13.7 \pm 0.7	9.5 \pm 0.7	44.2	0.001
Nitrate, nM/L	16.8 \pm 1.0	15.0 \pm 0.6	12.0	0.001
Nitrate/Nitrite ratio	1.2 \pm 0.06	1.5 \pm 0.1	-20.0	0.001
FRAP, nM/L	0.77 \pm 0.1	1.13 \pm 0.9	-31.8	0.001
MDA, μ m/L	4.4 \pm 1.0	2.0 \pm 0.6	120.0	0.001
Selenium, μ g/L	77.6 \pm 4.4	95.8 \pm 8.5	-18.9	0.001
Selenium/MDA ratio	18.4 \pm 4.9	53.2 \pm 23.0	-65.4	0.001
Iron, μ g/dL	56.4 \pm 28.6	92.1 \pm 32.3	-38.76	0.002
GPx activity, U/mL	301.0 \pm 65.4	311.6 \pm 37.8	-3.4	0.174
GR activity, U/L	62.7 \pm 19.8	65.8 \pm 11.6	-4.7	0.109
All				
Nitrite, nM/L	13.6 \pm 0.7	9.6 \pm 0.6	41.6	0.001
Nitrate, nM/L	16.6 \pm 0.8	15.1 \pm 0.6	9.9	0.001
Nitrate/Nitrite ratio	1.2 \pm 0.06	1.5 \pm 0.09	-20.0	0.001
FRAP, nM/L	0.78 \pm 0.09	1.12 \pm 0.08	-30.3	0.001
MDA, μ m/L	4.3 \pm 0.9	1.9 \pm 0.5	126.3	0.001
Selenium, μ g/L	77.4 \pm 5.1	94.3 \pm 8.8	-17.9	0.001
Selenium/MDA ratio	18.7 \pm 4.3	53.7 \pm 21.2	-65.1	0.001
Iron, μ g/dL	59.0 \pm 33.7	95.6 \pm 28.2	-38.2	0.001
GPx activity, U/mL				0.006
GR activity, U/L	262.3 \pm 72.0	303.6 \pm 35.9	-13.6	0.027
	54.5 \pm 18.0	65.0 \pm 19.3	-16.1	

Abbreviations: FRAP, status of total antioxidant; MDA, Malondialdehyde, lipid per-oxidative index.

^aMean difference (%) = (patients mean-controls mean)/(controls mean) \times 100.

to controls, respectively. The results demonstrated an association between NO production index and disease severity. Both nitrite and nitrate had a significant correlation with

inflamed tissues, and also nitrite, particularly, showed the strongest positive correlation (Figure 3) with disease activity (DA) score. These findings are consistent with the

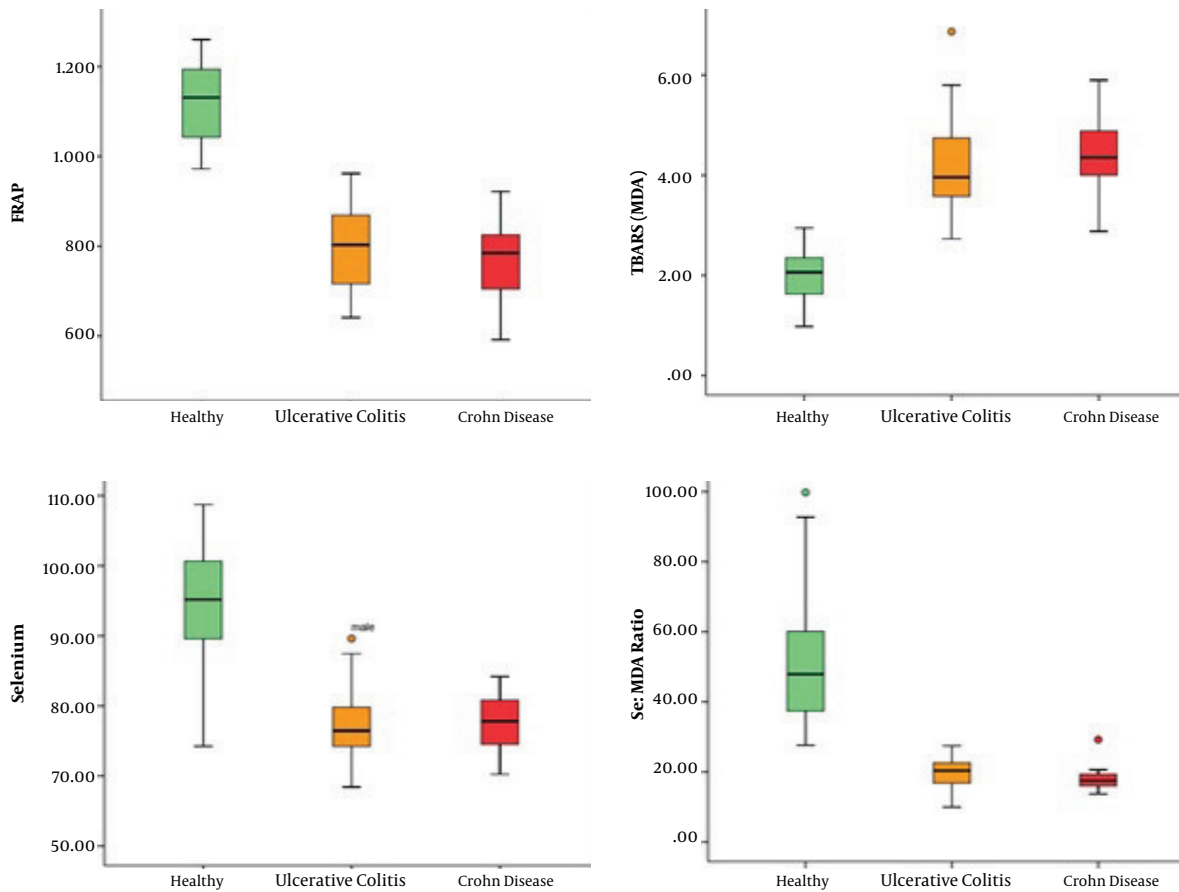


Figure 1. Comparison of biochemical parameters (selenium, FRAP) in healthy controls, ulcerative colitis and crohn's disease. FRAP: total antioxidant status; TBARS(MDA): Malondialdehyde, lipid per-oxidative index. To compare groups, Independent-samples t test was performed and P value < 0.05 was considered for statistical significance. (Boxplot 1, healthy control; boxplot 2, ulcerative colitis, and boxplot3, crohn's disease). In these boxplots, the data for Se:MDA ratio in healthy and crohn's disease appeared skewed. Also, the data for Se and MDA in ulcerative colitis was skewed.

Table 2. Clinical Characteristics of the Studied Groups

Clinical Characteristics/Groups	Healthy Control	CD Patients	UC Patients
Disease duration, y	-	8.6 ± 1.2	7.3 ± 1.1
Active disease (endoscopic/histologic)	-	15	18
Clinical remission	-	16	19
Disease location	-	Ileum and colon, colon only, small bowel only and gastroduodenal region	Rectum

study of Saijo et al. (10), which revealed the increase in plasma nitrite and nitrate in acute rodent model of colitis, yet in the chronic model, only nitrite had a significant increase. Also, nitrite elevation was observed to be much more than nitrate. Based on the fact that the usual range of plasma nitrite is much lower than the normal range of nitrate, plasma nitrite could be considered as a better index of inflammation than nitrate. Depending on the site of induction, iNOS stimulation can be beneficial or detrimental leading to both protective and harmful effects and the paradoxical role of NO in physiological processes in the GI tract (10, 38-43).

In this study, it was observed that serum iron concentration decreased significantly, which was 38.2% lower in IBD patients compared to controls. This result is in agreement with the findings of other investigations (44, 45). The

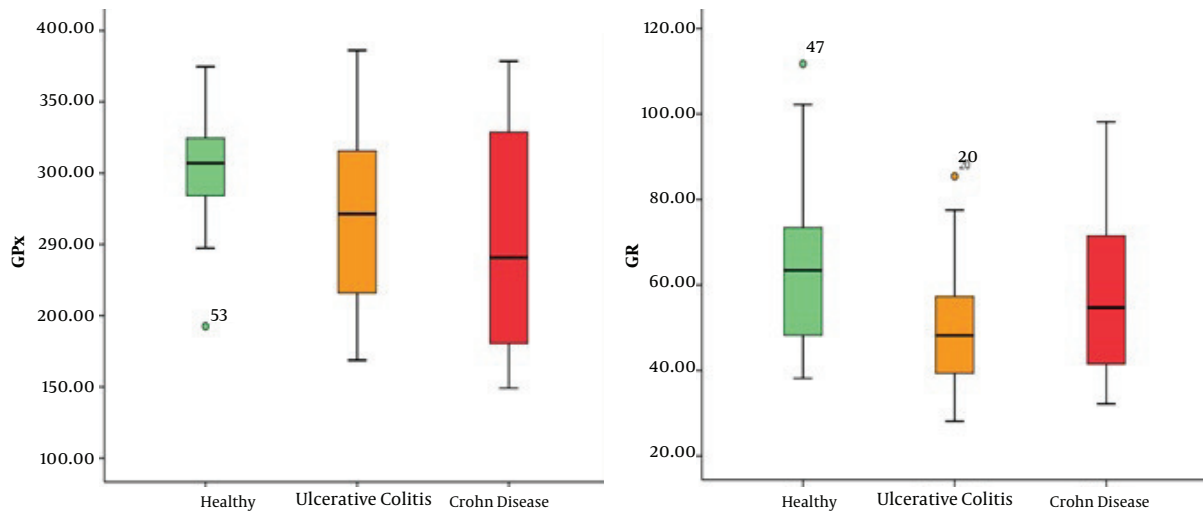


Figure 2. Comparison of antioxidative enzymes activity (GPx and GR) in healthy controls, ulcerative colitis and crohn's disease. GPx: glutathione peroxidase activity; GR: glutathione reductase activity. To compare groups, Independent samples t-test was performed and P value < 0.05 was considered for statistical significance. (Boxplot 1, healthy control; box plot 2, ulcerative colitis, and boxplot3, crohn's disease). GPx activity was skewed in healthy controls, but GR activity was skewed in both healthy control and ulcerative colitis groups.

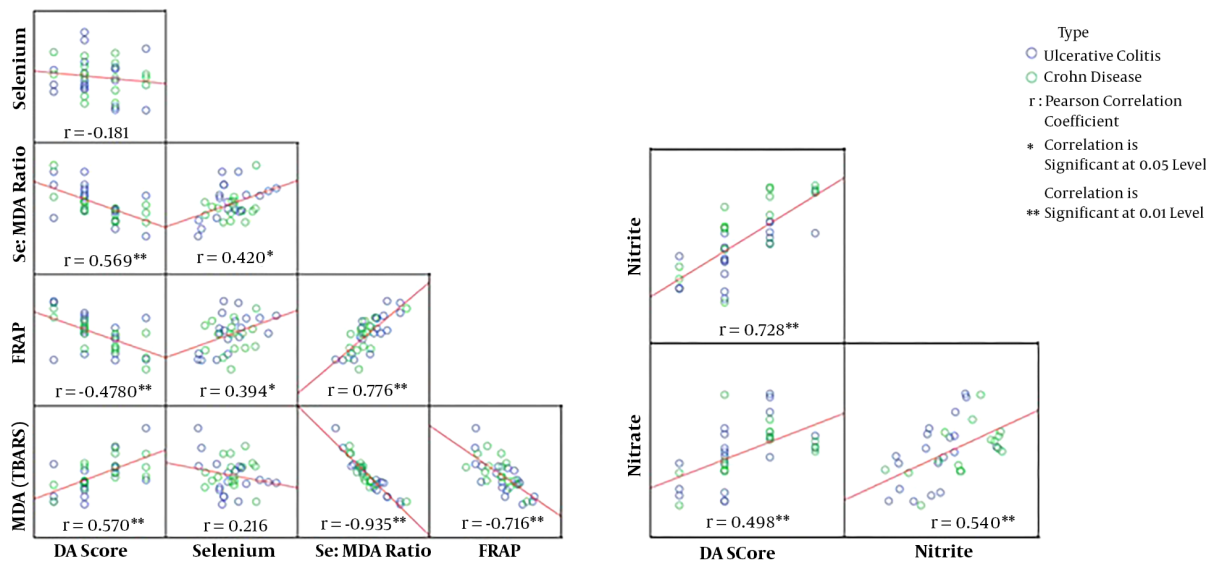


Figure3. The correlation analysis between MDA, FRAP, selenium levels, Se/MDA ratio, and DA score in IBD patients. All values were expressed as mean \pm SD. Pearson's correlation test and matrix scatter plot was performed to evaluate the correlation between parameters.

cause of anemia in patients with IBD is multifactorial, yet the most probable origins are blood loss through the ulcerations of the intestinal mucosa, reduced iron absorption and intake, and anemia of chronic disease (ACD) (46).

Iron (Fe), as the most abundant trace mineral, is critical to biochemical reactions. Iron is crucial for oxidation-

reduction reactions, oxygen delivery to tissues, gene regulation, and cell growth. Iron maintains the immune system through its involvement with peroxide- and NO-producing enzymes, cytokine production and function, and lymphocyte proliferation (16, 27-29, 47, 48).

In this study, the antioxidant level was found to be

30.3% lower in patients compared with healthy controls. Also, MDA level was 126.3% higher in patients compared with the control group. The current results are in agreement with those reporting that MDA level increased in patients with ulcerative colitis (49). A positive correlation between TBARS and disease activity and a negative correlation between FRAP and disease activity expressed the oxidant/antioxidant imbalance and oxidative stress. In a published study by Laurens Kruidenier et al. 3-Nitro-L-tyrosine (3-NT), an indicator of peroxynitrite-mediated protein nitration, NOS expression and MDA level, were significantly higher in inflamed IBD mucosa compared to controls or non-inflamed mucosa, and this fact shows the importance of oxidative stress in tissue damage of IBD patients (50).

These findings were supported by the study of Rezaie et al. which indicated oxidative stress in essential organs during inflammation. Also, an increase in lipid peroxidation in serum/plasma and a decrease in TAC were seen (51). The present study is in line with the study by Kruidenier et al. that reported the significant elevation of serum MDA in IBD patients versus the control group (50). However, Tuzun et al. (19) reported non-significant differences in plasma MDA level between IBD patients and the control group. Additionally, they reported an increased level of plasma glutathione peroxidase (GPx), activity as an indicator of response to free radical over production, yet the current study found a decrease in both serum GPx and GR activity in patients compared to controls. Even though the high activity of GPx to delete free radicals is expected, its activity depends on GR activity and glutathione (GSH) regeneration. In this study, the GSH level was not measured yet low level of TAC and a higher level of MDA indicated low serum reduction ability and lower antioxidant profile. In addition, this study indicated a 17.9% decrease in patient's serum selenium levels in comparison with healthy controls. Selenium, as a vital component for GSH-Px activity, plays a central role in hydro-peroxide scavenging and illustrates a relationship between Se balance and antioxidant activity. In line with the present study, low serum GPx activity, associated with low selenium level in CD patients (35, 52) and in mice (53), has been reported previously. Moreover, a strong positive correlation was found between Se concentration and GPx activity and total antioxidant status (Figures 3 and 4), which reflects the antioxidant effect of selenium. Besides, GPx activity has a significant negative correlation with DA score and positive correlation with GR activity. The current results are not in agreement with those reporting that the serum concentration of GPX showed a positive correlation with disease histopathologic activity index in ulcerative colitis patients (54). Nevertheless, the present study showed a significant decrease in GPx and GR activity in all patients compared to controls yet in females,

the decrease was not significant. The major finding of the present study was the detection of reduced GPx and Gr activity in male patients with IBD, yet the data indicate that the GPx and Gr activity was not significantly different in females with and without IBD. These data indicate that gender may have an impact on the antioxidant system. The results demonstrate that females have higher resting antioxidant levels than males. This result may be associated with the interference of estrogens and micronutrient with gut antioxidant capacity by acting at the active site of Gpx and Gr enzymes, or efflux mechanism of intracellular oxidant in females. The reason behind GPx and Gr activity not being significantly different in females with and without IBD might be interpreted as a possible secondary adaptive mechanism to the long term inflammatory process.

In agreement with the current findings, Gentschew et al. reported a positive correlation between the high occurrence rate of CD and low Se status among New Zealanders. In addition, they found low serum Se level in CD patients against controls (55). In a study done by Tomoko Johtatsu et al. serum Se level was found to be significantly lower in CD patients compared to the control group (56). Involvement of selenium in antioxidant system has been reported yet its role in modulating oxidative stress and preventing chronic diseases is controversial (57). Se deficiency leads to a large reduction in Se-GSH-Px, thus, ROS formation, such as H₂O₂, is increased. Reactive oxygen metabolites up-regulate pro-inflammatory genes, including iNOS and COX-2, which lead to both oxidative and nitrosative stress (24, 56). In line with this study, it has been reported that high Se treatment in TNBS-induced colitis reduces both inflammation and cell necrosis in rat colon via improvement of mitochondrial tissue function (58). Furthermore, Colleen Rock et al. pointed out the decrease in lipid hydroperoxide in HEK-293 cell line when treated with selenium sufficient medium after exposure to 15-HpETE (59).

In this study, it was observed that in IBD patients, selenium concentration was reduced while MDA level was enhanced, thus a new parameter, Se: MDA ratio, was calculated for the first time, which was 65.1% lower in IBD patients versus the control group. Additionally, a significant negative correlation between Se: MDA ratio and disease activity was observed. These observations suggest that Se: MDA ratio can be considered as a potential parameter in screening IBD patients. The correlation analysis between biochemical parameters and DA score in IBD patients is novel of this study. In addition, the correlation analysis between trace elements, antioxidative enzymes, and DA score in IBD patients is another novel aspect of this study. Nitrate/nitrite ratio, selenium/MDA ratio, GPx activity, and GR activity have not been presented elsewhere. These findings will be added to the large body of results in the literature.

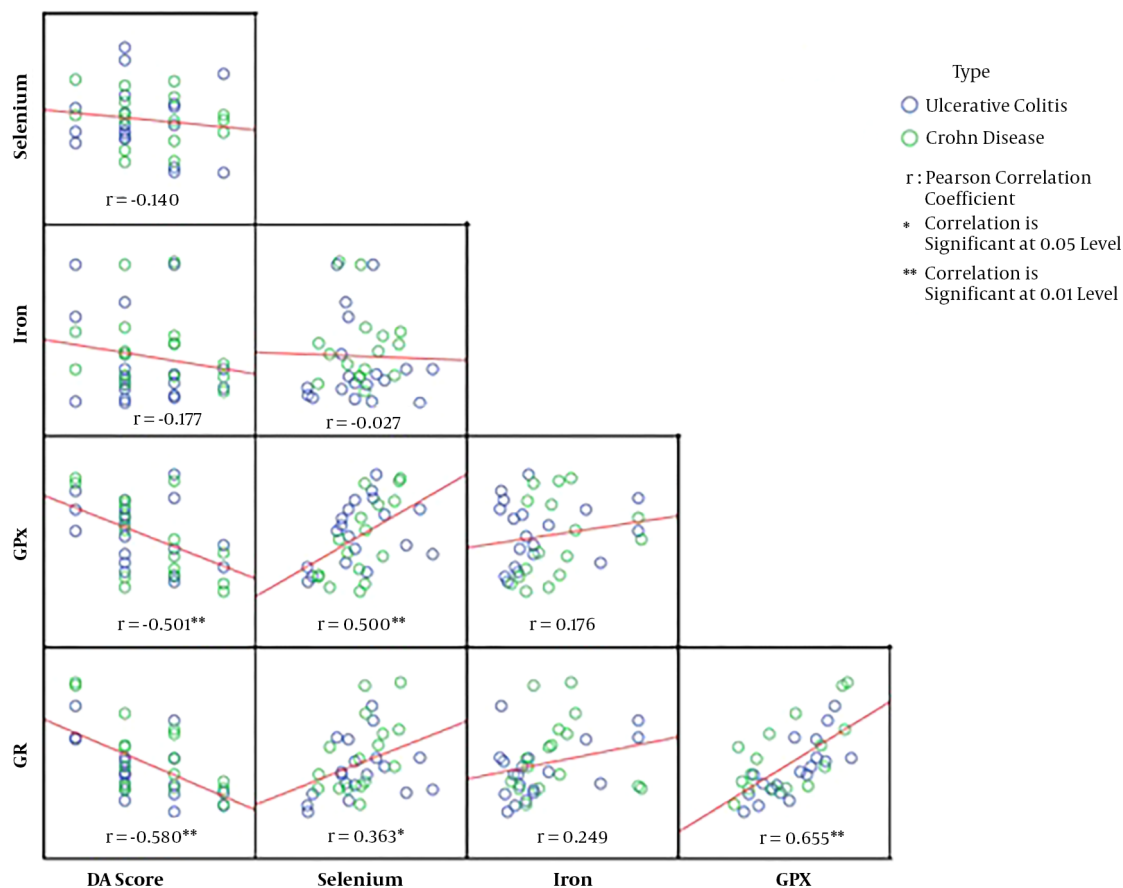


Figure 4. The correlation analysis between GR, GPX, iron, selenium levels and DA score in IBD patients. All values were expressed as mean \pm SD. Pearson's correlation test and matrix scatter plot were performed to evaluate the correlation between parameters.

Overall, among the experimental and human IBD studies, the most common finding is the increase in ROS generation and an elevation in NO derived from iNOS. The exact role of these reactive species in the pathophysiology of IBD is unknown yet if, in fact, ROS or RNS have a role in tissue damage and pathophysiology of IBD then, antioxidant therapy should be useful and can reduce disease severity (60). As seen in a published studies, GSH administration in TNBS-induced experimental colitis restores mucosal thiol content, reduces lipid peroxidation (MDA), and improves colonic damage (61). Furthermore, it has been reported that antioxidant-treatment in DSS-induced colitis in mice significantly improved hematocrit and colon length with higher levels of blood reduced GSH versus the control group (62).

5. Limitation

The number of patients included in this study was small and it is important to include a much larger cohort of patients.

6. Conclusions

The findings suggest that NO, ROS, RNS, and generally oxidative stress play an important role in IBD pathophysiology. The issue that oxidative stress is the result of inflammation or the cause of it is unknown. Nonetheless, their exact mechanisms are complex and ill-defined. Taken together, further prospective experimental studies under controlled conditions (diet, drug and other environmental factors) and larger sample sizes for evaluating both serum and colon biomarkers and their related mechanisms are needed.

Based on the results of this research, available serum level of nitrite and nitrate, TAC, MDA, and Se provide reproducible, quantitative tools that can complement clinical assessment to aid clinicians in IBD management.

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Footnotes

Authors' Contribution: Durdi Qujeq prepared the manuscript, Erfan Mohammadi conducted the experiments and performed the analysis. Hassan Taheri was involved in conception and design, Karimollah Hajian-Tilaki performed the data analysis and interpretation.

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Conflict of Interest: The authors had no conflict of interest.

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