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# The role of oxidants and reactive nitrogen species in irritable bowel syndrome: A potential etiological explanation

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**Background:** The aim of this study was to evaluate the plasma concentrations of malondialdehyde (MDA) and nitric oxide (NO) and the plasma activities of oxidant and antioxidant enzymes in patients with IBS.





**Material/Methods:** A total of 36 patients with IBS were included in the study. Thirty-five healthy subjects were selected to form the control group. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase (XO), adenosine deaminase (AD) activities, and malondialdehyde (MDA) and nitric oxide (NO) concentrations were studied in the serum samples of all patients and controls.

**Results:** Plasma XO and AD activities, and MDA and NO concentrations were significantly higher in IBS patients than in controls. The SOD, CAT, and GSH-Px activities in the serum of patients with IBS were significantly lower than that of controls.

**Conclusions:** These results suggest that lipid peroxidation and alterations in the oxidant-antioxidant enzymatic system may play a role in the pathogenesis of IBS. Increased lipid peroxidation in IBS may be related to an increase in NO level and XO activity and a decrease in antioxidant enzymes activities. In addition, increased AD activity may have a role in immunological changes of IBS patients.

**Key words:** irritable bowel syndrome • malondialdehyde • nitric oxide • oxidant enzyme • antioxidant enzymes

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## Background

Irritable bowel syndrome (IBS or spastic colon) is a common gastrointestinal (GI) disorder that affects 5–20% of individuals worldwide [1]. It occurs more often in women than in men, and is more commonly diagnosed in patients younger than 50 years of age [2]. IBS is a symptom-based diagnosis characterized by chronic abdominal pain, diarrhea, constipation, discomfort, bloating, and a change in bowel habit. Some patients complain of daily symptoms, while others present with intermittent symptoms at intervals of weeks or months. IBS is a disease with a high social cost, as it considerably reduces the quality of life. Although environment, social learning, diet, intestinal microbiota, low-grade inflammation and disturbances in the enterochromaffin cell function, and genetical factors (especially some gene polymorphism) are considered as etiopathogenic causes of IBS, the exact cause remains unknown [3,4].

Reactive oxygen species (ROS), including superoxide, hydroxyl, hydrogen peroxide, singlet oxygen, and nitric oxide are generated by cells in some physiological and pathological circumstances. ROS can react with all macromolecules, such as lipids, proteins, nucleic acids, and carbohydrates, particularly polyunsaturated fatty acids on the cell membrane. After the beginning of an initial reaction with ROS, a continuing chain reaction is started and cell injury and, ultimately, cell death, occurs. ROS-initiated oxidative stress can be regulated by the antioxidant defense system, which includes enzymatic and nonenzymatic antioxidants. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase are enzymes that have antioxidant properties. Vitamin E, vitamin C, and flavonoids are nonenzymatic antioxidants. Clinical and experimental studies have reported the role of oxidative stress in inflammatory bowel disorders [5].

To our knowledge, there are no clinical studies in the literature about oxidative stress in patients with IBS. In the present study, we evaluated the plasma concentrations of malondialdehyde (MDA) and nitric oxide (NO) and the plasma activities of oxidant and antioxidant enzymes in patients with IBS and in age- and sex-matched healthy controls to investigate the role of oxidative stress in the pathogenesis of IBS.

## Material and Methods

### Patients and controls

The study included 36 patients (19 females, 17 males; age range: 18–62 years) who had applied to Namık Kemal University Faculty of Medicine, Department of Gastroenterology, and were diagnosed with IBS using the Rome III criteria and had no alarm symptoms, no chronic disease history, and had macroscopically

normal colonoscopy/upper gastrointestinal system endoscopic findings. There were 35 control patients (19 females, 16 males; age range: 18–63 years) who had no history of chronic disease or drug use, who were undergoing colonoscopy for reasons not related to IBS (familial colorectal cancer screening or investigation of anemia), and who had macroscopically normal colonic mucosa. The study was carried out in accordance with the Declaration of Helsinki II with approval of the ethics committee of Namık Kemal University Faculty of Medicine. All participants were informed about the study protocol and written consent was obtained from each of them.

Exclusion criteria were alcohol and substance abuse or dependence, presence of severe organic disorders, use of any antioxidants like vitamin E or C, presence of gastroenterological disorder, presence of infectious and viral disease, and excessive obesity.

### Biochemical analysis

#### *Measurement of plasma XO activity*

XO activity was assayed spectrophotometrically at 293 nm and 37°C with xanthine as substrate [6]. The formation of UA from xanthine results in increase in absorbency. One unit of activity was defined as 1 mol of UA formed per minute at 37°C, pH 7.5, and expressed in U·l<sup>-1</sup>.

#### *Measurement of plasma AD activity*

AD activity was estimated spectrophotometrically by the method of Giusti [7], which is based on the direct measurements of the formation of ammonia, which is produced when AD acts in excess of adenosine, and expressed in U·l<sup>-1</sup>.

#### *Measurement of plasma NO level*

As NO measurement is very difficult in biologic specimens, sample nitrite and nitrate concentrations are used as an index of NO production. The method was based on the Griess reaction [8]. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured after conversion of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions of sodium nitrite. Linear regression was performed by using the peak area from the nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results are expressed as μmol·l<sup>-1</sup>.

#### *Measurement of plasma MDA level*

In the samples, MDA concentrations were determined using the method of Draper and Hadley [9], based on the reaction

**Table 1.** XO activity, and NO, and MDA levels of serum in IBS patients and controls.

	n	Age	MDA ( $\mu\text{mol/l}$ )	NO ( $\mu\text{mol/l}$ )	XO (U/l)
IBS	36		2.05 $\pm$ 0.9	13.53 $\pm$ 2.38	1.67 $\pm$ 0.41
Control	35		1.55 $\pm$ 0.5	11.90 $\pm$ 1.71	1.25 $\pm$ 0.49
<i>p</i> value		<i>p</i> >0.05	<i>p</i> <0.01	<i>p</i> <0.05	<i>p</i> <0.01

Values are given as mean  $\pm$ s.d. MDA – malondialdehyde; NO – nitric oxide; XO – xanthine oxidase; IBS – irritable bowel syndrome.

**Table 2.** Antioxidant enzymes and AD activities of serum in IBS patients and controls.

	n	SOD (U/ml)	CAT (k/l)	GSH-Px (U/l)	AD (U/l)
IBS	36	11.62 $\pm$ 3.29	6.27 $\pm$ 1.49	49.22 $\pm$ 12.57	18.13 $\pm$ 5.11
Control	35	14.94 $\pm$ 3.03	7.13 $\pm$ 1.07	59.06 $\pm$ 11.08	14.48 $\pm$ 3.33
<i>p</i> value		<i>p</i> <0.001	<i>p</i> <0.01	<i>p</i> <0.01	<i>P</i> <0.01

Values are given as mean  $\pm$ s.d. SOD – superoxide dismutase; CAT – katalase; GSH-Px – glutathion peroxidase; AD – adenosine deaminase; IBS – irritable bowel syndrome.

of MDA with thiobarbituric acid (TBA) at 95°C. In the TBA test reaction, MDA and TBA react to form a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 95°C for 15 min. The sample was mixed with 2.5 volumes of 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of supernatant was reacted with 0.67% TBA in a boiling water bath for 15 min. After cooling, the absorbance was read at 532 nm. Arbitrary values obtained were compared with a series of standard solutions (1,1,3,3 tetramethoxypropane). Results are expressed as  $\mu\text{mol}\cdot\text{l}^{-1}$ .

#### Measurement of plasma SOD activity

Total SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al [10]. The principle of the method is based on the inhibition of nitroblue tetrazolium reduction by the xanthine – xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the sample after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U ml<sup>-1</sup>.

#### Measurement of plasma GSH-Px activity

GSH-Px (EC 1.6.4.2) activity was measured by the method of Paglia and Valentine [11]. The enzymatic reaction in the tube, containing NADPH, reduced glutathione (GSH), sodium azide, and glutathione reductase, was initiated by addition of H<sub>2</sub>O<sub>2</sub> and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity is given as U·l<sup>-1</sup>.

#### Measurement of plasma CAT activity

CAT (EC 1.11.1.6) activity was determined according to Aebi's method [12]. The principle of the assay is based on the determination of the rate constant (s<sup>-1</sup>, k) of the H<sub>2</sub>O<sub>2</sub> decomposition at 240 nm. Activities are expressed as k (rate constant) per l (k/L).

#### Statistical analysis

All values are expressed as mean  $\pm$  standard deviation. SPSS for Windows 11.0 was used for statistical analysis. The t test was used to estimate the significance between parameters. *P*<0.05 was considered to be significant.

The Minitab program was utilized for power calculation. In the sample patient population, n=36 and the impact factor for d=4 was found to be 80%.

## Results

In patients with IBS, significantly higher concentrations of plasma MDA and NO concentrations were found compared to the control group (*P*<0.01, and *p*<0.05, respectively) (Table 1). Oxidant enzyme XO activity was increased in the IBS group compared to the control group (*p*<0.01) (Table 1). AD activity in patients with IBS was found to be increased compared to controls (*P*<0.01) (Table 1). There was a remarkable decrease in SOD, CAT, and GSH-Px activities in patients with IBS compared to control subjects (*p*<0.001, *p*<0.01, and *p*<0.01, respectively) (Table 2).

## Discussion

To date, measurement/evaluation of antioxidant and antioxidant enzyme activities, and nitric oxide and lipid peroxidation product have not been studied in samples of IBS patients associated with oxidative stress. In this study, plasma XO activity was measured and higher levels of the enzyme activity were found in patients with IBS in comparison with the control group. XO, in the presence of its substrate hypoxanthine or xanthine, reduces molecular oxygen to superoxide anion radical and hydrogen peroxide, which can further react to form the more reactive hydroxyl radical, termed ROS. XO-derived ROS have been suggested to be critical factors in several mechanisms of tissue pathophysiology. IBS represents a common digestive system mucosal disease with altered humoral and cellular immunities. Recently, studies have shown that inflammatory cytokine levels increased [13] and levels of mucosal-soluble mediators such as ZO-1 and levels of adhesion molecules I [14] decreased and also extracellular matrix components, and matrix receptors were reduced [15] in the samples of patients with IBS. Schwartz et al. [17] reported that XO-derived ROS contribute to the increased expression of mRNA for interleukin 1 beta (IL-1 $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which are both found to be increased in IBS samples [13]. In several studies of inflammatory bowel disease, the role of inflammation as a causative factor of abnormal intestinal motor function has been reported. Inflammatory mediators such as IL-1 and H<sub>2</sub>O<sub>2</sub> have been shown to be associated with altered sigmoid motor dysfunction in ulcerative colitis [16]. We suggest that a similar situation is present in the intestines of patients with IBS.

In the purine metabolic pathway, AD is an important deaminating enzyme, which converts adenosine and 2'-deoxyadenosine to inosine and 2'-deoxyinosine, respectively. AD is not only a cytosolic enzyme, but it can also be found as an ecto-enzyme. Plasma AD activity has physiologic functions thought to be responsible for cellular immunity. AD activates T cells by binding to surface receptors. In our study, plasma AD activity was found to be high, supporting the opinion that cellular immunity plays an important role in the pathogenesis of IBS [13]. Furthermore, AD activity can be accepted as an important factor in some other alterations observed in cellular immunity, such as elevation of T cell fractions in peripheral blood, and elevation of T cell activity and expression in mucosa in IBS patients [18]. Increased AD activity, by inducing T cell subtypes, may play an important role in the synthesis of proinflammatory cytokines.

A strict correlation is observed between NO production and gastrointestinal diseases and it is suggested that elevation of NO plays an important role in the pathogenesis of these diseases [19,20]. In our study, we found significantly higher

plasma NO concentrations in IBS patients compared with the controls. There are not enough studies in the literature on the relation between IBS and NO, and there is no consensus between the few clinical studies on this subject. While Dykhuizen et al. [21] reported that there is no significant change in NO level between IBS patients and control subjects, Yazar et al. [22] reported an increase in (with constipation) IBS patients. Reinders et al. [23], using chemiluminescence technique, also found an increase in NO concentrations in rectal mucosa of patients with IBS. Although it is not known if AD activity has a direct effect on NO synthesis, it is claimed that there is a synergistic relationship between adenosine and NO production. Because increase in the amount of substrate is one of the significant factors increasing enzyme activity, increase in AD activity may suggest an increase in the amount of adenosine, which is an AD substrate. Therefore, increase in the amount of adenosine might have stimulated NO synthesis. In addition, many studies in the literature show that an increase in the amount of NO is accompanied by an increase in AD activity [25]. NO is a strong inhibitor of smooth muscle contraction, and high local concentrations of NO has been hypothesized to play an important role in the development of toxic mega colon. Hence, high NO concentrations may be one of the factors disrupting normal bowel movements in the setting of IBS [24]. In our study, it was observed that SOD, CAT, and GSH-Px activities were significantly lower in the IBS group than in the control group. These results suggest that antioxidant status against ROS is impaired in IBS. We considered that the decreased antioxidant capacities of cells could be responsible for the increase in ROS, which may explain the increased level of MDA. A search in PubMed did not reveal any clinical studies that investigated antioxidant enzyme activities in samples of IBS patients, and there is no consensus between the few experimental studies on this subject. While Mozaffari et al. [26] showed by experimental study decreased antioxidant power of the samples in IBS rats in comparison with the controls, Ding et al. [27], in a proteomic analysis of colonic mucosa in a rat model of IBS, reported that antioxidant activity (peroxiredoxin-6) is over-expressed.

## Conclusions

These results suggest that lipid peroxidation and alterations in the oxidant-antioxidant enzymatic system may play a role in the pathogenesis of IBS. Increased lipid peroxidation in IBS may be related to an increase in NO level and XO activity, and a decrease in antioxidant enzyme activities. We believe our study provides further evidence of the role of oxidative and nitrosative pathophysiology in development of irritable bowel syndrome and encourages further research for the treatment of this disorder with agents targeting these complex pathways.

## References:

1. El-Salhy M: Irritable bowel syndrome: Diagnosis and pathogenesis. *World J Gastroenterol*, 2012; 18(37): 5151-63
2. Agréus L, Svärdsudd K, Nyrén O, Tibblin G: Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. *Gastroenterology*, 1995; 109: 671-80
3. Matricon J, Meleine M, Gelot A et al: Review article: associations between immune activation, intestinal permeability and the irritable bowel syndrome. *Aliment Pharmacol Ther*, 2012; 36(11-12): 1009-31
4. Mazur M, Furgała A, Jabłoński K et al: Autonomic nervous system activity in constipation-predominant irritable bowel syndrome patients. *Med Sci Monit*, 2012; 18(8): CR493-99
5. Zhu H, Li YR: Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence. *Exp Biol Med* (Maywood), 2012; 237(5): 474-80
6. Prajda N, Weber G: Malign transformation-linked imbalance: decreased XO activity in hepatomas. *FEBS Lett*, 1975; 59: 245-49
7. Giusti G: Adenosine deaminase. In Bergmeyer MV, ed. *Methods of enzymatic analysis*, 2nd edn. Academic Press, New York, 1974; 1092-98
8. Cortas NK, Wakid NW: Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem*, 1990; 36: 1440-43
9. Draper H, Hadley M: Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*, 1990; 86: 421-31
10. Sun A, Wang JT, Chia JS, Chiang CP: Levamisole can modulate the serum tumor necrosis factor- $\alpha$  level in patients with recurrent aphthous ulcerations. *J Oral Pathol Med*, 2006; 35: 111-16
11. Paglia DE, Valentine WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*, 1967; 70: 158-69
12. Aebi H: Catalase. In Bergmeyer HU, ed: *Methods of Enzymatic Analysis*. New York, Academic Press, 1974; 673-77
13. McKernan DP, Gaszner G, Quigley EM et al: Altered peripheral toll-like receptor responses in the irritable bowel syndrome. *Aliment Pharmacol Ther*, 2011; 33: 1045-52
14. Ohman L, Isaksson S, Lundgren A et al: A controlled study of colonic immune activity and beta7+ blood T lymphocytes in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol*, 2005; 3: 980-86
15. Ohman L, Lindmark AC, Isaksson S et al: B-cell activation in patients with irritable bowel syndrome (IBS). *Neurogastroenterol Motil*, 2009; 21: 644-50
16. Cao W, Fiocchi C, Pricolo VE: Production of IL-1, hydrogen peroxide, and nitric oxide by colonic mucosa decreases sigmoid smooth muscle contractility in ulcerative colitis. *Am J Physiol Cell Physiol*, 2005; 289: C1408-16
17. Schwartz MD, Repine JE, Abraham E: Xanthine oxidase-derived oxygen radicals increase lung cytokine expression in mice subjected to hemorrhagic shock. *Am J Respir Cell Mol Biol*, 1995; 12: 434-40
18. Ohman L, Isaksson S, Lindmark AC et al: T-cell activation in patients with irritable bowel syndrome. *Am J Gastroenterol*, 2009; 104(5): 1205-12
19. Chadwick VS, Chen W, Shu D et al: Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology*, 2002; 122(7): 1778-83
20. Rivera LR, Poole DP, Thacker M, Furness JB: The involvement of nitric oxide synthase neurons in enteric neuropathies. *Neurogastroenterol Motil*, 2011; 23(11): 980-88
21. Dykhuizen RS, Masson J, McKnight G et al: Plasma nitrate concentration in infective gastroenteritis and inflammatory bowel disease. *Gut*, 1996; 39(3): 393-95
22. Yazar A, Büyükaşar K, Polat G et al: The urinary 5-hydroxyindole acetic acid and plasma nitric oxide levels in irritable bowel syndrome: a preliminary study. *Scott Med J*, 2005; 50(1): 27-29
23. Reinders CI, Herulf M, Ljung T et al: Rectal mucosal nitric oxide in differentiation of inflammatory bowel disease and irritable bowel syndrome. *Clin Gastroenterol Hepatol*, 2005; 3(8): 777-83
24. Cao W, Vrees MD, Kirber MT et al: Hydrogen peroxide contributes to motor dysfunction in ulcerative colitis. *Am J Physiol Gastrointest Liver Physiol*, 2004; 286: G833-43
25. Yağcı R, Gürel A, Ersöz I et al: The activities of paraoxonase, xanthine oxidase, adenosine deaminase and the level of nitrite in pseudoexfoliation syndrome. *Ophthalmic Res*, 2009; 42(3): 155-59
26. Mozaffari S, Esmaily H, Rahimi R et al: Effects of Hypericum perforatum extract on rat irritable bowel syndrome. *Pharmacogn Mag*, 2011; 7(27): 213-23
27. Ding Y, Lu B, Chen D et al: Proteomic analysis of colonic mucosa in a rat model of irritable bowel syndrome. *Proteomics*, 2010; 10(14): 2620-30