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RESEARCH ARTICLE

Evaluation of Some Oxidative Stress Parameters in Iraqi Patients with Inflammatory Bowel Disease

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Abstract

Objectives: Inflammatory bowel disease (IBD) refers to chronic inflammations that affect any part of the gastrointestinal tract (GIT). This study aimed to evaluate of some oxidative stress parameters (TOC, TAC, OSI and MDA) in sera of Iraqi patients with IBD. Methods: Three main groups were included in this study; 30 patients (15male &15female) with Crohn's disease (CD group), 30 patients (15male & 15female) with ulcerative colitis (UC group) and 30 (15female & 15female) apparently healthy controls (HC group) comparable for age and gender without a history of IBD were involved in the current study. Results: TOC in the sera of both patient groups (CD&UC) was a highly significant increase ($p<0.001$) when compared to the (HC group) while significant decrease ($p<0.05$) in the TAC of (CD group), as well as highly significant decrease ($p<0.001$) in the TAC of (UC group) was observed in comparison with their levels in corresponding (HC group). A highly significant increase ($p<0.001$) in serum (OSI) of two patient groups (CD & UC) in comparison to that of (HC group).Meanwhile, significant increase ($p<0.05$) in serum MDA concentration of both patient groups (CD & UC) when compared to that of the (HC group) was observed. Conclusion: We found a significant increase in TOC, OSI and MDA while a significant decrease in TAC of patient groups. Therefore, CD and UC subjected to an increased in oxidative stress situation.

Keywords: *Inflammatory bowel disease, Crohn's disease, Ulcerative colitis, Oxidative stress.*

Introduction

Inflammatory bowel disease (IBD) refers to chronic inflammations that affect any part of the gastrointestinal tract (GIT) [1]. The major phenotypes of IBD are Crohn's disease (CD) and ulcerative colitis (UC) [2]. UC is characterized by superficial inflammation that beginning in the rectum and can expand proximally to affect the whole colon, while CD can affect any part of the GIT from mouth to anus [3, 4]. Both UC and CD share a number of symptoms and signs such bloody diarrhea, abdominal pain, urgency, fever, weight loss, and tenesmus [5].

The pathogenesis of IBD remains uncertain and involves a complex interaction between genetic threat, environmental factors, gut microbiota and mucosal immune response [6]. Oxidative stress (OS) is an imbalance between oxidants molecule and antioxidant molecules [7]. OS is associated with several chronic diseases in terms of etiology [8].

It is among the immune-regulatory factors, has been proposed as one of the major mechanism involved in the pathophysiology of IBD [9]. Substantial evidence suggests that IBD is associated with an imbalance between Reactive oxygen species (ROS) and antioxidant activity which creates OS as the result of either ROS overproduction or a decreased antioxidant activity [10]. CD patients show decreased main cellular antioxidant enzymes such as Superoxide dismutase (SOD) and Glutathione Peroxidase (GPx)) activities in intestinal mucosa [11].

Likewise, Nitric oxide synthase (NOS) is thought to be the main producer of nitric oxide (NO) in UC, the induction of colonic NOS may be involved in the mucosal vasodilation and increased vascular permeability of active UC, and could also contribute to the impaired motility that accompanies toxic dilatation [12].

Following the oxidative stress, the lipid peroxidation {Malondialdehyde (MDA) level is also increased. MDA is the most important studied product as a result of peroxidation of unsaturated fatty acids. MDA is considered to be the most important biochemical marker for determining the lipid peroxidation. In this process, free radicals damage the saturated fatty acids, which are in fact the main components of the cell membrane, and lead to the formation of MDA following the lipid peroxidation. In addition, free radicals can produce MDA by directly affecting the DNA and its oxidation [13]. The current study was aimed to evaluate of some oxidative stress parameters in sera of Iraqi patients with IBD.

Materials and Methods

Three main groups were included in this study; 30 patients (15male &15female) with Crohn's disease (CD group), 30 patients (15male &15female) with ulcerative colitis (UC group) and 30 (15male &15female) apparently healthy controls (HC group) comparable for age and gender without a history of IBD were involved in the current study. The working period was from October (2018) to March (2019). The samples were collected from patients who attending to Gastroenterology and Hepatology teaching Hospital in Baghdad, Iraq.

Then the sample was centrifuged at 3000xg for five minutes. The obtained serum was stored at (-20°C) for determination total antioxidant capacity (TOC), total oxidant capacity (TAC) and MDA concentrations. The exclusion criteria involved smocking, pregnancy, liver diseases, colon cancer and autoimmune diseases. This study protocol was approved by the Ethics Committee of the College of Science/ University of Baghdad.

Serum Total Antioxidant Capacity and Total Oxidant Capacity: (TOC) and (TAC) in the serum of the study groups were measured according to methods developed by Erel [14, 15].

Oxidative stress index (OSI): The OSI value was calculated according to the formula:

OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq / L) / TAS (μmol Ascorbic acid Eq / L).

Serum Malondialdehyde Concentration

MDA concentration was determined in serum of CD, UC and HC groups by the modified method of Satoh [16].

The data were analyzed using SPSS by licensed materials version 20 computer software. The data in this study were presented as a Mean± Standard deviation (Mean± SD) using independent-samples t-Test to compare mean. The range test at ($p<0.05$) is accepted as statistically significant, while it's highly significant when ($p<0.001$).

Results and Discussion

Three main groups were included in this study CD, UC and HC. The mean ± SD ages of the three groups (CD, UC and HC) were 27.2±6.75, 29.13±8.97 and 27.82±5.76 years respectively,. TOC in the sera of both patient groups (CD&UC) was a highly significant increase ($p<0.001$) when compared to the (HC group) while significant decrease ($p<0.05$) in TAC in (CD group), as well as highly significant decrease ($p<0.001$) in TAC of (UC group) was observed in comparison with their levels in corresponding (HC group) as shown in Table 1. A highly significant increase ($p<0.001$) in serum OSI of two patient groups (CD & UC) in comparison to that of the (HC group) as shown in (Table 1).

The results in (Table 1) show a significant increase ($p<0.05$) in serum MDA concentration of both patient groups (CD & UC) when compared to that of the (HC group). The current study was in agreement with several authors for the TOC and TAC systems in IBD. They found that patients with IBD had significantly higher TOC and OSI and lower TAC than controls group [17, 18]. The observed increase in serum concentration of TOC may be due to increased oxidant molecules. In the course of chronic inflammation as in the bowel it was known that the reactive oxygen species may be produced in excess [19].

The main source of oxidant molecules in human gut is phagocytic leukocytes which accumulate within mucus of patients with IBD during active colitis [20]. In order to destroy and engulf invading microorganisms, Neutrophils, monocytes, eosinophils and macrophage release large amounts of ROS [21]. The other source of these oxidant molecules is the types of enzymes found in the colon, which including aldehyde oxidase,

xanthine oxidase, lipoxygenase and prostaglandin synthase, which have the potential to generate large quantities of ROS [22]. The third source of free radicals in IBD is inducible nitric oxide synthase (iNOS) which can expressed at high levels in activated macrophages, leukocytes and epithelial cells in the intestinal mucosa to liberation high amount of reactive nitrogen species (RNS) [23].

Another source of free radicals is mitochondria due to dysregulation of the mitochondrial electron transport chain leading to increased generation of mitochondrial reactive oxygen species (mROS) during chronic inflammation such as IBD [24]. The decrease the TAC can be explained as the presence a high amount of free radicals in chronic inflammation, which lead to depleting of the antioxidant system.

During chronic inflammation activated inflammatory cells (lymphocytes, neutrophils and macrophage) lead to producing high levels of ROS and RNS [25]. The presence of high amount of free radicals may play a major role in the depletion of antioxidant system defense in the intestinal mucosa leading to macromolecules damage.

The other three factors may lead to reduced antioxidant levels are the inadequate intake of dietary antioxidants, mutations in enzymatic antioxidant genes and depletion of cellular glutathione as a result of detoxification of a huge amount of xenobiotics [26]. Our study was in agreement with several authors for the MDA in inflammatory bowel disease. They found that patients with IBD had significantly higher MDA levels than the control group [27, 28, 29].

The results of the presented study were also consistent with some previous studies which found that there was a significant increase in MDA levels in sera of patients with IBD when compared to healthy subjects when using erythrocyte and tissue models [30, 32].

The results of serum MDA disagree with some studies, such as the study by Barbosa et al in which the plasma MDA in ulcerative colitis was measured and reported to be normal and not different from those in the healthy control group [33]. As well as, disagree with a study in Turkey, by Tuzun et al., when they measured plasma MDA concentration in patients with IBD and observed there are no significant difference comparisons to that of the healthy control group [34].

These alterations in results may be due to difference of samples (serum and plasma) and duration of this disease. This increase in concentration of malodialdehyde may be resulted from increased lipid peroxidation due to increased free radicals that have the ability to attack phospholipids in the cell membrane [35].

Our study suggested that the main reason for increasing the concentration of MDA is oxidative stress, where we found a significant decrease in TAC and at the same time there was a significant increase in TOC, which lead to the inability of antioxidants to control the oxidizing molecules and this caused a large increase in the free radicals which have the ability attack of phospholipids in the cell membrane and thus cause lipid peroxidation.

Table 1: mean ± SD of total oxidant capacity, total antioxidant capacity, oxidative stress index and malondialdehyde in sera of CD and UC patient groups comparison to that of healthy control group (HC)

Parameters	Group	Mean ± SD	Groups	Mean ± SD	P-Value
TOS (μmol/L)	HC	21.97±3.66	CD	34.21±5.75	0.000**
			UC	36.88±7.72	0.000**
TAS (μmol/L)	HC	590.91 ±41.89	CD	547.06± 59.27	0.002*
			UC	479.61±76.58	0.000**
OSI	HC	0.0372±0.0064	CD	0.0630±0.0126	0.000**
			UC	0.0790±0.0221	0.000**
MDA (nmol/L)	HC	5.55±0.69	CD	6.54±0.99	0.049*
			UC	6.77±0.98	0.032*

TOC (total oxidant capacity), TAC (total antioxidant capacity), OSI (oxidative stress index), MDA (malondialdehyde), $p<0.05$ significant, $p<0.001$ highly significant

Conclusion

We measured total oxidant capacity, total antioxidant capacity and malondialdehyde concentration in inflammatory bowel disease patient groups (CD & UC) and healthy control group (HC). We found a significant increase in TOC, OSI and MDA while a significant decrease in TAC. Therefore, CD and UC subjected to an increased in oxidative stress situation, this can result from an imbalance between oxidants and antioxidants as clear from the results of the present study.

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