SHORT COMMUNICATION

Evaluation of Paraoxonase and Arylesterase activities in patients with irritable bowel syndrome

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Abstract

The aims of the present study were to evaluate oxidative status, by investigating the serum Paraoxonase/ Arylesterase (PON/ARE) activities along with conjugated dienes in patients with IBS and controls and to confirm the link between oxidative stress and IBS.

Thirty IBS patient and 30 healthy subjects were recruited. Total serum cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), PON and ARE activities and conjugated dienes levels were measured.

Mean serum PON1 activity was lower in IBS group compared to that of the control group whereas there was no significant difference in ARE activity between IBS and control groups ($p<0.000$, $p<0.716$, respectively). Serum conjugated diene levels of the IBS group was significantly higher than that of the control group ($p<0.01$).

The drop in PON activity accompanied with an increase in conjugated diene levels indicate the presence of oxidative stress, a disturbance in prooxidant - antioxidant balance and increased inflammation in IBS patients.

Keywords: IBS, PON1, ARE, Conjugated Diene.

Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder among people worldwide. The pathophysiology of IBS remains incompletely defined. Microscopic inflammation has been considered as a primary factor in the pathogenesis of IBS.[1] The inflammatory cascade begins by inflammatory cell infiltration of the mucosa, and pro-inflammatory mediators such as reactive oxygen metabolites (ROM) are released. Antioxidant defenses are overwhelmed by ROM, leading to cell damage and lipid peroxidation. It has been shown that increased colonic inflammation induces cell membrane lipid peroxidation and expression of interleukin-1α messenger RNA (IL-1α mRNA) in mucosal biopsies of IBS patients.[2]

Paraoxonase 1(PON1) is a calcium-dependent esterase, hydrolyses lipid peroxides and acts in conjunction with another esterase, Arylesterase (ARE) to form an important component of the enzymatic antioxidant system. Furthermore, it was proposed that PON1 could play a role in anti-inflammatory response, and reduced levels of PON and ARE were related with increased inflammation.[3]

To our knowledge, there is no data on PON/ARE activities in patients with IBS. The aims of the present study were to evaluate oxidative status, by investigating the serum PON/ARE activities along with conjugated dienes, the indicator of lipid peroxidation,[4] in patients with IBS and controls and to confirm the link between oxidative stress and IBS.

Patients and Methods

Thirty consecutive patients, aged 42±10 years, with known or suspected IBS (9 males and 21 females) were invited to participate in this cross sectional study. The ROME III criteria were used to diagnose IBS, and all patients underwent colonoscopy to rule out other organic colon diseases. Exclusion criteria were alcohol and substance abuse or dependence, presence of GI disorder (peptic ulcer disease, gallbladder disorder, pancreatitis, liver disease, inflammattory bowel disease, or malignancy), diabetes, hypertension, infectious, rheumatologic and cardiovascular diseases, previous surgery of the GI tract, use of any NSAIDs, prokinetics, antihistamines, corticosteroids or immunosuppressive agents. Thirty healthy volunteer subjects, aged 41±8 years (10 male, 20 female) were recruited from the general population as controls. Control subjects were also screened for participation eligibility using exclusion criteria similar to those for the IBS patients. Demographic characteristics and body mass index (BMI) of all subjects were recorded. Routine blood analyses including complete blood count, electrolytes, fasting glucose, kidney and liver function tests, C-reactive protein and erythrocyte sedimentation rates of all participiants were obtained. Only subjects with normal biochemical analysis results were included in the study. Informed consent was

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obtained from participants before the study. The study protocol and study procedures were approved by the Ethics Committee of Namik Kemal University.

Blood samples were obtained from all participants in the morning after an overnight fast. Enzymatic methods were used in the determination of total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG). The sera were separated by centrifugation at 3.000 x g for 10 minutes and stored frozen at -70°C until assay

Paraoxonase and ARE activities were measured using synthetic paraoxon and phenylacetate substrates, respectively. PON activity was determined by measuring the rate of paraoxon hydrolysis (diethyl p-nitrophenylphosphate) to yield p-nitrophenol. The rate of paraoxon hydrolysis was measured by monitoring the increase in absorbance at 412nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17,000 M^{-1} cm^{-1}.[19] PON activity was expressed as U/L serum. Enzymatic activity of ARE was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of ARE activity was defined as 1μmol phenol generated/min under the above conditions and expressed as U/L serum. Serum conjugated dienes were determined by the modified method of John and Steven.[5]

Data were analyzed using the Statistical Package for the Social Sciences for Windows, version 16.0 (SPSS, Chicago, IL). A confidence interval (CI) of 95% and a 2-tailed P value of less than 0.05 were considered to be statistically significant for all analyses. Variables were tested for homogeneity of variance using the Levene test and for normality of distribution by utilizing the Kolmogorov-Smirnov test. The group means were compared by using Independent Sample T test. Differences in gender was assessed by a χ^2 test. All numerical data are expressed as mean ± standard deviation (mean ± SD).

Results

There was no significant difference between the groups in terms of age and gender (p<0.545 and p<0.874, respectively). The mean values of BMI in control and IBS groups were 29.2±4.6 kg/m^2 and 28.3±4.4kg/m^2, respectively and no difference was found between the groups (p<0.441). The mean serum levels of total cholesterol, triglyceride, HDL and LDL in control group were 188±29 mg/dL, 136±42 mg/dL, 49.5±10 mg/dL, and 111±33 mg/dL, respectively. The mean serum levels of total cholesterol, triglyceride, HDL and LDL in IBS group were 194±27 mg/dL, 129±69 mg/dL, 49.7±9 mg/dL and 118±27 mg/dL, respectively. The lipid profile of groups was found to be similar (all p>0.05) (Table-1).

Table-1: Demographics of irritable bowel syndrome patients and controls.

Parameters	Control	IBS	p
Mean Age, years	41.0±8	42.5±10	0.545
BMI, kg/m2	29.2±4.6	28.3±4.4	0.441
Gender(male/female), n/n	9/21	10/20	0.874
Total Cholesterol, mg/dL	188±29	194±27	0.431
Triglyceride, mg/dL	136±42	129±69	0.648
HDL, mg/dL	49.5±10	49.7±9	0.941
LDL, mg/dL	111±33	118±27	0.384

IBS: Irritable bowel syndrome; BMI: Body mass index; HDL: High-density lipoprotein; LDL; Low-density lipoprotein.

Table-2: The comparison of serum PON/ARE activities and conjugated diene levels of the groups.

Parameters	Control	IBS	p
PON, U/mL	134.5±62	68.5±54	0.000
ARE, mU/mL	79.7±40.9	83.3±34.5	0.716
Conjugated dienes, nmol/mL	77.9±9.4	87.1±12.7	0.003

IBS: Irritable bowel syndrome; PON: paraoxonase; ARE: arylesterase.

Mean serum PON1 activity was 134.5±62U/mL in control group and 68.5±54U/mL in IBS group. Mean serum PON1 activity was significantly lower in IBS group (p<0.001). Mean serum ARE activity of the control group was 79.7±40.9mU/mL, whereas mean serum ARE activity of IBS group was 83.3±34.5mU/mL. There was no statistically significant difference between groups with regard to mean serum ARE activity (p<0.716). Serum conjugated diene levels of the IBS group and control group were 87.1±12.7nmol/mL and 77.9±9.4nmol/mL, respectively. Serum conjugated diene level was significantly higher in IBS group than that of the control group (p<0.01) (Table-2).

Discussion

In spite of numerous clinical research studies, the etiology of IBS remains obscure. Recently, some studies have demonstrated increased intestinal permeability and increased numbers of lymphocytes in colonic biopsies of some IBS patients.[6] This might be a mechanism for maintenance of the condition by exposing the gut to luminal antigen and activating the mucosal immune system.[7] Colonic inflammation and increased permeability lead to an increase in cell membrane lipid peroxidation in mucosal biopsies. Therefore, in IBS patients it is reasonable to examine the activities of antioxidant enzymes and levels of lipid peroxidation products such as PON and ARE and conjugated dienes, respectively.

PON1, a HDL associated esterase/lactonase, prevents oxidative stress and fight inflammation. It provides protection against free radicals by limiting the oxidation of phospholipids, but this activity disappears in oxidative environments and inflammatory disorders such as inflammatory bowel diseases.[8] The present study demonstrated, for the first time, that serum PON activities were decreased in patients with IBS which is complicated with microinflammation and oxidative stress. However, there was no significant difference between groups with respect to ARE activity. Genetic differences may be one of the probable explanations for this discrepancy.

Reactive oxygen metabolites are involved in many inflammatory disorders, including those of the gastrointestinal tract.[9] Oxidative injury caused by free radicals is recognized to occur in inflammation and lipid peroxidation. Conjugated dienes are known to be the first products of lipid peroxidation. Dormandy et al. established that the determination of diene conjugation level was a reliable marker of free-radical activity in biological systems.[10] In the present study, the levels of conjugated dienes were measured to evaluate lipid peroxidation. Conjugated diene levels, an indicator of increased inflammation and lipid peroxidation, in the patient group were found to be higher than that of the control group. This result concludes that oxidative stress and inflammation may play a role in IBS.

To sum up, the drop in PON activity accompanied with an increase in conjugated diene levels indicate the presence of oxidative stress, a disturbance in prooxidant - antioxidant balance and increased inflammation in IBS patients.

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