Cytokine 93 (2017) 34–43

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Inflammatory cytokines and oxidative stress biomarkers in irritable bowel syndrome: Association with digestive symptoms and quality of life

Razieh Choghakhori[a], Amir Abbasnezhad[a,b,*], Amin Hasanvand[c], Reza Amani[d,e]

[a] Nutritional Health Research Center, Department of Nutrition, Lorestan University of Medical Sciences, Khorramabad, Iran
[b] Nutrition and Metabolic Diseases Researcher Center, Department of Nutrition, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran
[d] Health Research Institute, Diabetes Research Center, Department of Nutrition, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[e] Food Security Research Center, Health Research Institute, Department of Clinical Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran

ARTICLE INFO

Keywords:
Inflammatory cytokines
Oxidative stress biomarkers
Irritable bowel syndrome
Digestive symptoms
Quality of life

ABSTRACT

A growing body of evidence suggests a possible role for low-grade inflammation in the pathogenesis of irritable bowel syndrome (IBS). The objectives of this study were to measure serum levels of tumor necrosis factor (TNF)-α, interleukin (IL)-17, interleukin (IL)-10, malondialdehyde (MDA) and total antioxidant capacity (TAC) in IBS patients and healthy controls (HCs), and to evaluate possible correlations of such markers with gastrointestinal (GI) symptoms and quality of life (QoL). Ninety Rome III positive IBS patients and 90 sex and age matched HCs were recruited. GI symptoms, IBS-QoL, IBS severity score system (IBSSS), and the serum levels of inflammatory cytokines and oxidative stress biomarkers were evaluated. In IBS patients, TNFα, IL-17 and MDA cytokines were significantly ($P < 0.05$) higher, and IL-10 cytokine and TAC were significantly ($P < 0.05$) lower vs. HCs. When comparing IBS subtypes, TNFα and IL-17 were significantly ($P < 0.05$) higher, and IL-10 was significantly ($P < 0.05$) lower in diarrhea predominant IBS (IBS-D) compared to HCs, whereas the inflammatory cytokine profile of other subtypes more closely resembled that of HCs. The serum levels of MDA and TAC were significantly different ($P < 0.05$) in all the subtypes vs. HCs. All the inflammatory cytokines had significant ($P < 0.05$) correlations with GI symptoms, IBSSS and IBS-QoL, whereas no significant association was found between oxidative stress biomarkers and these symptoms. IBS-D patients display increased pro-inflammatory cytokines and decreased anti-inflammatory cytokines. Present study demonstrated a correlation between inflammatory cytokines and both IBS symptoms and QoL.

1. Introduction

The irritable bowel syndrome (IBS) is a painful chronic functional bowel disorder which is usually associated with altered bowel habit [1]. Recent studies have provided evidence of immune cell infiltration and activation in the intestinal mucosa of IBS patients, and suggest a possible role for low-grade inflammation in the pathogenesis of IBS [1]. Inflammation or injury to tissues leads to the visceral hypersensitivity which is thought to play an important role in the development of chronic pain and discomfort in IBS patients [2]. There is a growing body of evidence demonstrating that IBS patients show altered cytokine profiles as compared to healthy groups [3]. Pro-inflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin (IL)-6 and IL-8 have been shown to be elevated in IBS patients [3,4]. Previously, it has been shown that IBS patients may be genetically predisposed to produce lower amounts of the anti-inflammatory cytokine interleukin 10 [5]. Furthermore, pro-inflammatory cytokines directly affect the hypothalamus, and activate hypothalamic-pituitaryadrenal axis (HPA), the core endocrine stress system [6]. Corticotropin-releasing hormone (CRH), is the primary regulatory peptide produced in the hypothalamus in response to the inflammation [7,8]. There is an exaggerated HPA response to the inflammation, accompanied by an increased intestinal response to CRH in IBS patients, which results in generating digestive symptoms [9]. Nevertheless, a limited number of studies have assessed the relationships between inflammation and clinical symptoms of IBS. In addition, Psychological stressors have a role in activation of the HPA [10]. Psychiatric comorbidities seem to be common in IBS patients, which results in impaired quality of life (QoL), and there is an association between IBS symptoms and these comorbidities [2].

Abbreviations: IBS, irritable bowel syndrome; QoL, quality of life; IBSSS, IBS severity score system; IBS-QoL, IBS specific QoL; GI, gastrointestinal; IBS-D, diarrhea-predominant; IBS-C, constipation-predominant; IBS-A, alternating bowel habits; TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; SD, standard deviation; VAS, visual analogue scale

* Corresponding author at: Goledasht Blvd, Khorramabad PO Box: 6813833946, Iran.
E-mail address: abbasnezhad.a@ajums.ac.ir (A. Abbasnezhad).

http://dx.doi.org/10.1016/j.cyto.2017.05.005
Received 26 January 2017; Received in revised form 4 May 2017; Accepted 4 May 2017
Available online 12 May 2017

It is well documented that oxidative stress and inflammation are inevitably linked together, as leukocytes activation can produce a reactive oxygen species (ROS) by resident cells such as vascular smooth muscle and endothelial cells [11]. Enzymatic and nonenzymatic antioxidant's defense system can regulate ROS-initiated oxidative stress [12]. In 2013, a study indicated that antioxidant status against ROS is impaired in IBS patients, and alterations in the oxidant-antioxidant enzymatic system may play a role in the pathogenesis of IBS and its symptoms [12].

In the present study, inflammatory cytokines such as IL-10, IL-17 and TNFα were assessed in IBS patients. IL-17 members have an important role in inflammatory and autoimmune diseases and are potential targets for future pharmacotherapy [13]. IL-17 induces several genes associated with inflammation and mediates inflammatory responses in various tissues [14]. Moreover, we assessed oxidative stress biomarkers such as serum malondialdehyde (MDA) levels and total antioxidant capacity (TAC). The aim of the current study was to compare the inflammatory cytokines and oxidative stress biomarkers in IBS patients with healthy controls and in different subtypes of IBS. Furthermore, the relationships between inflammation and clinical symptoms were evaluated.

2. Materials and methods

2.1. Subject population

A total of 90 IBS patients consist of both genders aged between 18 to 70 were recruited by an attendant gastroenterologist through medical examination based on the Rome III Diagnostic Criteria for Functional GI Disorders for the diagnosis of IBS [15]. All the participants were elicited from the outpatient Clinic of the Jundishapur University Hospital, in February and March 2015. Patients with IBS were sub-classified as diarrhea-predominant (IBS-D), constipation-predominant (IBS-C), and those with alternating bowel habits (IBS-A) [16].

Any evidence of abdominal surgery, celiac disease, or other primary GI illnesses, GI infection, pregnancy, lactation and alcohol consumption were considered as exclusion criteria. Additional exclusion criteria consist of concurrent chronic diseases such as diabetes and diagnosed and/or treated malignancy in the past 5 years and any usage of anti-inflammatory drugs (including nonsteroids, steroids, antihistaminics, and mast cell stabilizers). A total of 90 sex and age matched healthy controls (HCs) were used for comparison. After allocation, all participants were asked to refer to the lab for blood sampling the next day.

Anthropometric measures, clinical history, demographic data and a complete physical examination of each subject were assessed. The study protocol was approved by the Medical Ethics Committee at the Jundishapur University of Medical Sciences (Registration No. ir.ajums.rec.1394.306). Approved informed consent was obtained from all the patients involved in the study.

2.2. Laboratory analyses

Blood samples were collected after an 8–12 h overnight fasting. For each sample, 5 mL of blood was drawn. Serums were frozen at −20 °C immediately and then stored at −80 °C until further laboratory analyses were carried out. Serum levels of TNFα, IL-10 and IL-17 were measured using an enzyme-linked immunosorbent assay (ELISA) (BOSTER BIOLOGICAL TECHNOLOGY Co., Ltd., USA). Tiobarbituric acid method was used for measuring serum malondialdehyde (MDA) levels [17]. For analyzing the serum total antioxidant capacity (TAC), the ferric reducing ability of plasma (FRAP) method was used [18].

2.3. Digestive symptoms, severity and quality of life

For evaluating clinical symptoms, patients' abdominal pain, dissatisfaction with bowel habits and overall GI symptoms were assessed, using a self-reporting 100-mm visual analogue scale (VAS), where 0 indicated no symptoms and 100 represented the worst symptoms ever experienced. The IBS severity score system (IBSSS) [19] was used for evaluating IBS severity. The IBSSS, which has been accredited for IBS patients, included 5 clinically applicable items over a 10-day period: (1) severity of abdominal pain, (2) frequency of abdominal pain, (3) severity of abdominal distention or tightness, (4) dissatisfaction with bowel habits, and (5) interference of IBS with life in general [19]. Each item was scored on a scale from 0 to 100, and the sum of the 5 items was considered as the rate of IBS severity (range 0–500). Quality of life was assessed via a self-report measure specific to IBS (IBS-QoL) with 34 items [20]. The individual responses to 34 items were summed and averaged for a total score and then transformed to a 0–100 scale. Higher scores indicating better IBS specific quality of life [20].

2.4. Statistical analysis

All statistical analyses were carried out using SPSS version 16 statistical software (SPSS Inc., Chicago, Ill). For all tests, two-sided P values < 0.05 were considered statistically significant unless otherwise stated. The normal distribution of data related to normality was assessed using the Kolmogorov–Smirnov test. Data were reported as mean ± standard deviation or median (25th, 75th percentile) for parametric and nonparametric data, respectively. Student's t test, χ^2-test, or Fisher's exact test were used for between the groups comparisons, when appropriate. One-way analysis of variance (ANOVA) was used for comparison of the biochemical factors in different IBS subtypes. Linear regression was used for determining the possible correlation between biochemical factors and clinical symptoms and IBS-QoL.

3. Results

3.1. Baseline Characteristics

The mean age of the subjects in the IBS group and the HCs were 37.66 (range, 21–59) and 38.69 (range, 23–59), respectively (Table 1). Among the 90 IBS patients enrolled in the study, 33.3% were IBS-C, 26.7% were IBS-D and 40% were IBS-A (Table 1). As Table 1 indicates, there were no significant differences between the IBS and HCs groups regarding the age, education, smoking, anthropometric measures and daily energy intake.

Table 1
Subject characteristics.

Characteristics	IBS patients (n = 90)	Healthy controls (n = 90)	P value
Age, years	37.66 ± 8.84	38.69 ± 9.21	0.44
Female, n (%)	61 (67.8)	61 (67.8)	
IBS subtypes, n (%): [a]			
IBS-C	30 (33.3)	–	
IBS-D	24 (26.7)	–	
IBS-A	36 (40)	–	
Education level, n (%): [a]			0.36
None/Primary	20 (22.2)	28 (31.1)	
Middle/High school	39 (43.3)	37 (41.1)	
University or higher	31 (34.4)	25 (27.8)	
Smoking status, n (%): [a]			0.12
Never	61 (67.8)	69 (76.7)	
Smoking/Ex-Smoker	29 (32.2)	21 (23.3)	
BMI, kg/m^2	25.12 ± 2.78	24.56 ± 3	0.2
Body fat percentage	28.13 ± 6.38	27.33 ± 6.1	0.4
Daily Energy Intake	1804.11 ± 188.321	1782.08 ± 187.31	0.43

IBS, irritable bowel syndrome; BMI, body mass index; IBS- C, constipation subtype; IBS-D, diarrhea subtype; IBS-A, alternating subtype.
All data are shown as mean ± standard deviation, and analyzed by two-sample t test unless otherwise indicated. P values < 0.05 were considered statistically significant.
[a] Data are numbers (%), and were analyzed by χ^2 test or Fisher's exact test. P values < 0.05 were considered statistically significant.

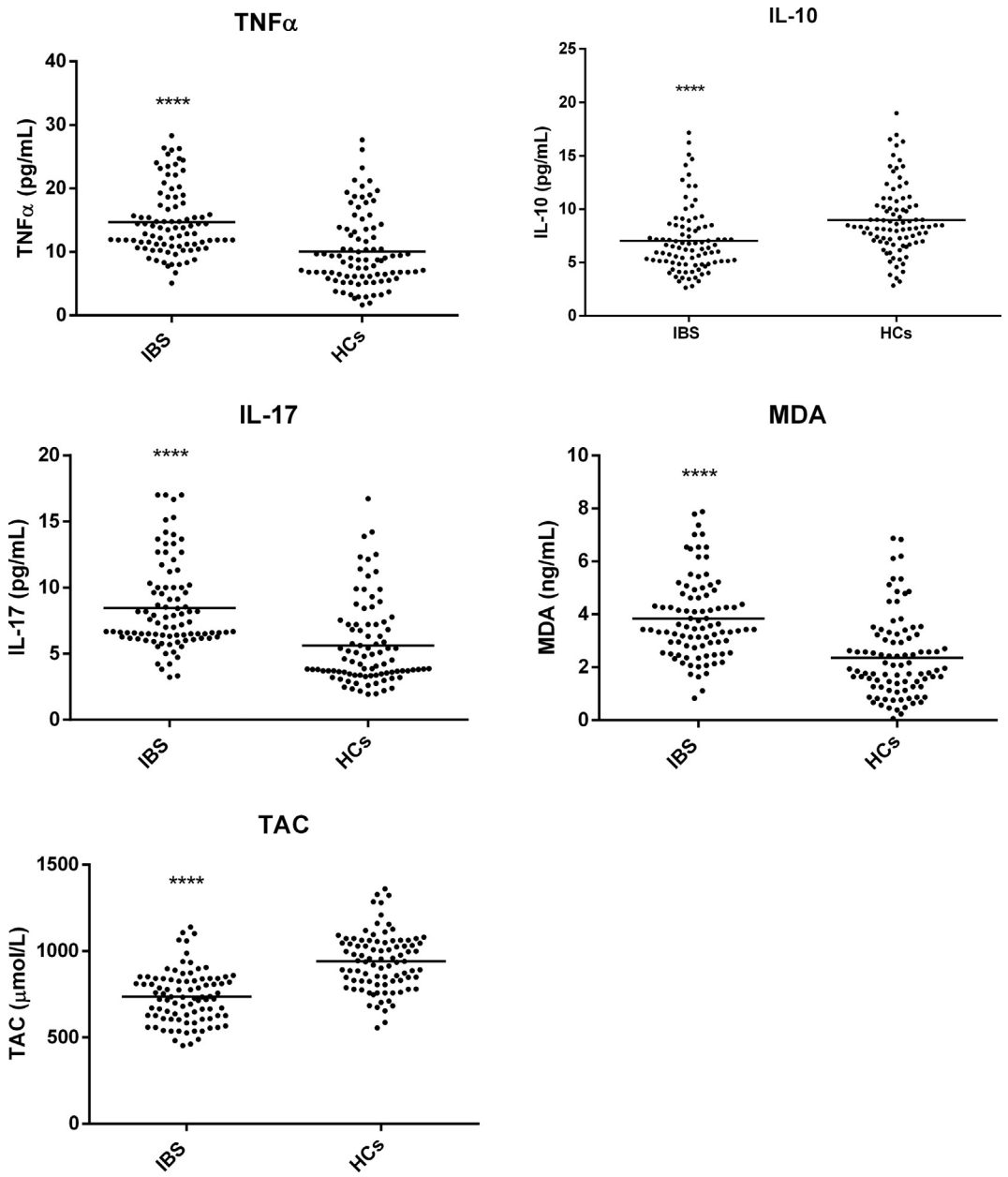

Fig. 1. Mean serum levels of inflammatory cytokines and oxidative stress biomarkers in patients with IBS and HCs. (**** $P < 0.05$). IBS, irritable bowel syndrome; HCs, healthy controls; TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

3.2. Biochemical factors between IBS patients and healthy controls

As Fig. 1 shows, there was a significant increase in TNFα (14.7 ± 5.27 vs. 10.04 ± 5.75 pg/mL, $P < 0.001$), IL-17 (8.46 ± 3.32 vs. 5.63 ± 3.15 pg/mL, $P < 0.001$) and MDA (3.85 ± 1.53 vs. 2.36 ± 1.54 ng/mL, $P < 0.001$) in the serum of IBS patients compared with the HCs. Moreover, there was a significant decrease in the serum levels of the IL-10 (7.02 ± 3.07 vs. 9 ± 3.23 pg/mL, $P < 0.001$) and TAC (736.87 ± 151.74 vs. 941.11 ± 166.22 μmol/L, $P < 0.001$) in the IBS group as compared to HC group.

3.3. Biochemical factors in different bowel habit subtypes of IBS

The serum levels of TNFα were not different in IBS-C and IBS-A patients as compared to HCs (12.2 ± 2.91 and 12.13 ± 2.8 vs. 10.04 ± 5.75 pg/mL, $P > 0.05$, respectively) (Fig. 2). However, TNFα levels were significantly higher in IBS-D patients in comparison to HCs (21.7 ± 3.96 vs. 10.04 ± 5.75 pg/mL, $P < 0.001$). In addition, the serum levels of IL-17 were not different in IBS-C and IBS-A patients vs. HCs (6.9 ± 1.61 and 6.91 ± 1.85 vs. 5.63 ± 3.15 pg/mL, $P > 0.05$, respectively), whereas the IL-17 levels were significantly higher in IBS-D (12.76 ± 2.8 vs. 5.63 ± 3.15 pg/mL, $P < 0.001$). Likewise, in IBS-C and IBS-A, the serum levels of IL-10 were not different when compared with HCs (7.55 ± 3.5 and 7.48 ± 3.24 vs. 9 ± 3.23 pg/mL, $P > 0.05$, respectively), however, in IBS-D patients the serum levels of IL-10 were significantly lower (5.65 ± 1.62 vs. 9 ± 3.23 pg/mL, $P < 0.001$) (Fig. 2).

As Fig. 2 shows, the serum levels of MDA were significantly higher in IBS-C, IBS-D and IBS-A as compared with HCs (3.71 ± 1.73, 3.74 ± 1.07 and 4.03 ± 1.62 vs. 2.36 ± 1.54 ng/mL, $P < 0.001$; respectively), and the TAC levels were significantly lower in IBS-C, IBS-D and IBS-A vs. HCs (729.39 ± 121.33, 754.57 ± 167.63 and 731.31 ± 166.11 vs. 941.11 ± 166.22 μmol/L, $P < 0.001$; respectively).

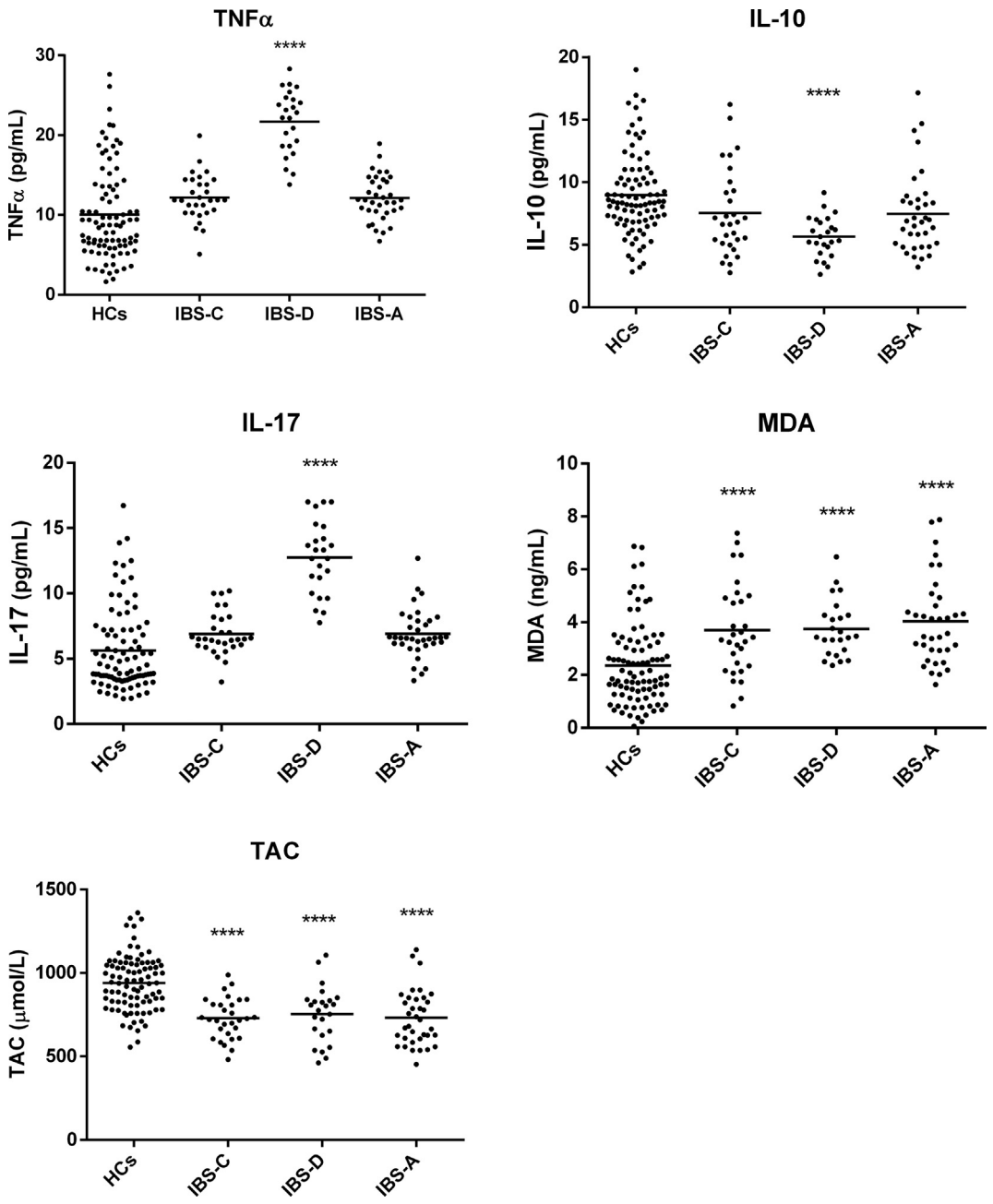

Fig. 2. Mean serum levels of inflammatory cytokines and oxidative stress biomarkers in different bowel habit subtypes of IBS in comparison to HCs. (**** $P < 0.05$). IBS-D, diarrhea predominant IBS; IBS-C, constipation-predominant IBS; IBS-A, alternating bowel habits IBS; HCs, healthy controls; TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

3.4. Associations between biochemical factors, clinical symptoms and quality of life

In the IBS patients, serum levels of the TNFα and IL-17 had a significant positive correlation with the severity of pain, dissatisfaction with bowel habits, overall GI symptoms and IBSSS, and negative association with IBS-QoL (Figs. 3–7). Additionally, lower serum IL-10 levels correlated with higher severity of pain, dissatisfaction with bowel habits, overall GI symptoms and IBSSS, and there was a positive correlation between serum IL-10 levels and IBS-QoL (Figs. 3–7). MDA and TAC had no significant correlation with the digestive symptoms and IBS-QoL (Figs. 3–7).

4. Discussion

In the present study, the results relating to the comparison of the inflammatory cytokines between IBS patients and HCs indicate that serum levels of the pro-inflammatory cytokines (TNFα and IL-17) increased, and the serum levels of IL-10, as an anti-inflammatory cytokine, decreased in IBS patients. To the best of our knowledge, this is the first time that the serum levels of IL-17 has been assessed in IBS patients. It is notable that, IL-17 has an important role in the pathogenesis of gut inflammation by the activation of NF-κB and MAP kinase in intestinal subepithelial myofibroblasts [21]. In several tissues, IL-17 mediates inflammatory responses and induces a number of genes associated with inflammation such as IL-8, IL-6 [22,23]. As seen in Fig. 2, the serum levels of IL-17 increased in IBS-D, but not in other subtypes. Correspondingly, the serum levels of TNFα were

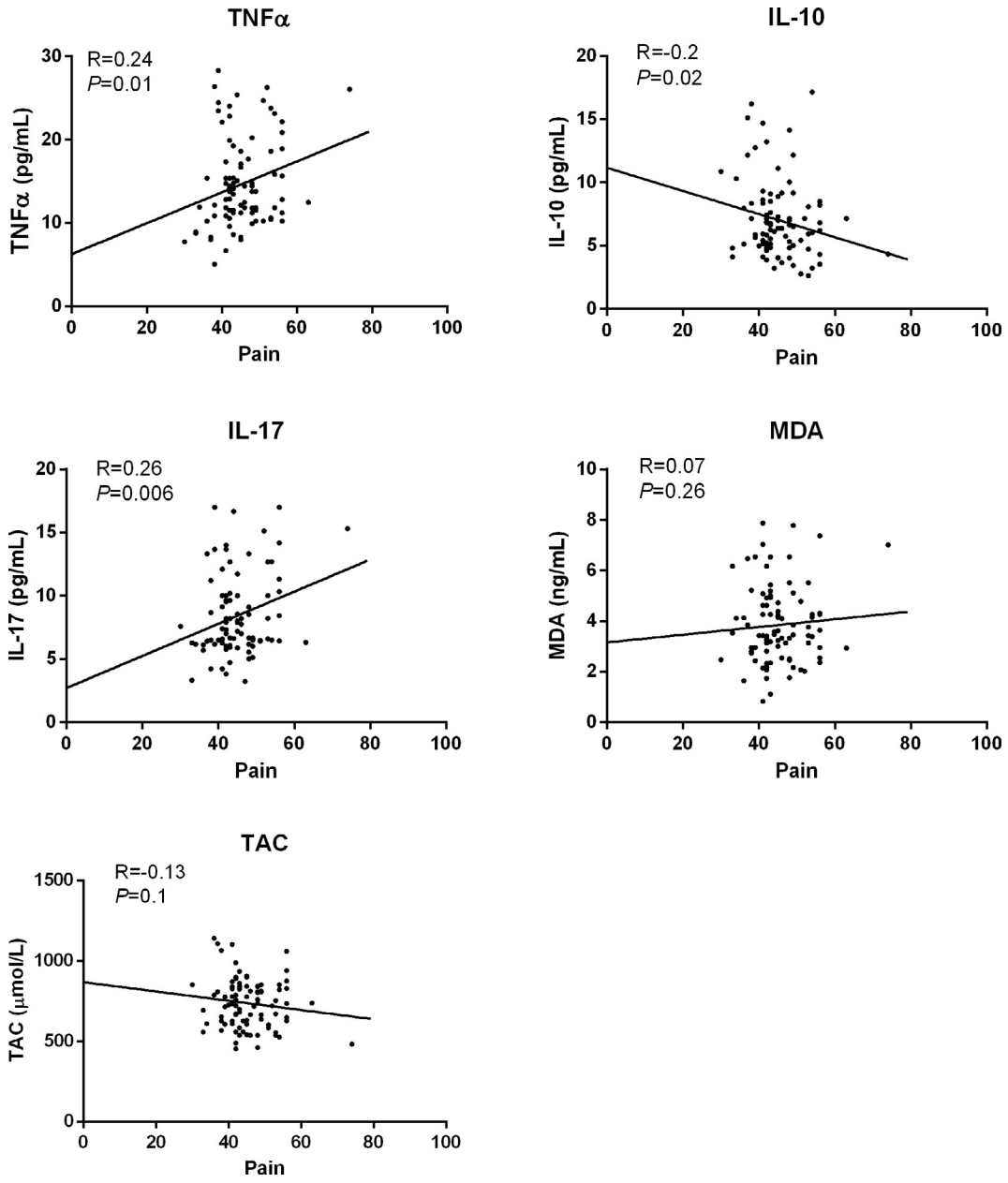

Fig. 3. The correlation of the severity of abdominal pain with inflammatory cytokines and oxidative stress biomarkers in patients with IBS. *P* values < 0.05 were considered statistically significant. TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

significantly higher ($P < 0.05$), and the serum levels of IL-10 were lower ($P < 0.05$) in IBS-D patients, whereas the serum levels of TNFα and IL-10 in other subtypes more closely resembled that of HCs. A growing body of evidence supports the increase in inflammatory cytokines in IBS patients [4,24,25]. A study of 37 IBS patients and 37 healthy volunteers, demonstrated that plasma levels of IL-6 ($P = 0.003$) and IL-8 ($P = 0.001$) were higher in IBS than in controls [24]. Another study indicated that the serum levels of IL-10 was significantly ($P < 0.001$) lower, and the serum levels of TNFα was significantly ($P = 0.010$) higher in IBS patients vs controls [25]. Furthermore, recent studies have suggested that, some IBS patients may be genetically susceptible to inflammation, and there are associations between various candidate genes such as polymorphisms of the IL-10, and TNFα genes, and IBS [26]. Gonsalkorale et al. reported that, frequencies of the high producer genotype for anti-inflammatory cytokine interleukin 10 had significantly reduced in IBS patients [5].

In this context, it is noteworthy that the comparison of the immune profile between different subtypes of IBS is rarely done, but when this is done clear differences are seen [4,27,28]. In the study of 55 IBS patients, TNFα, IL-1β and IL-6 cytokine levels were significantly ($P < 0.017$) higher in IBS patients compared to HCs [4], and when IBS subtypes analyzed, all cytokine levels were significantly ($P < 0.05$) higher in IBS-D patients [4]. Furthermore, in the study of Liebregts et al., [27] patients with IBS-D showed significantly increased TNFα ($P < 0.001$), IL-1 β ($P = 0.003$), IL-6 ($P = 0.02$), and IL-10 levels ($P = 0.011$) compared with HCs, whereas no significant differences were observed for IBS-C. Likewise, in the study of Hughes et al., [29] IBS-D patients had significantly ($P < 0.05$) elevated concentrations of IL-1b, IL-10, TNFα and IL-6 compared to HCs. Additionally, Schmulson et al. [25] demonstrated that women with IBS-D had the lowest IL-10 ($P < 0.001$) and highest TNFα ($P = 0.021$) vs. other subtypes, however, in men with IBS there were no significant ($P > 0.05$) difference between subtypes.

It is well known that IBS symptoms can be triggered by gastrointestinal infections [30,31]. Inflammation and immune activation have a non-negligible role in the pathogenesis of IBS and initiate the onset of

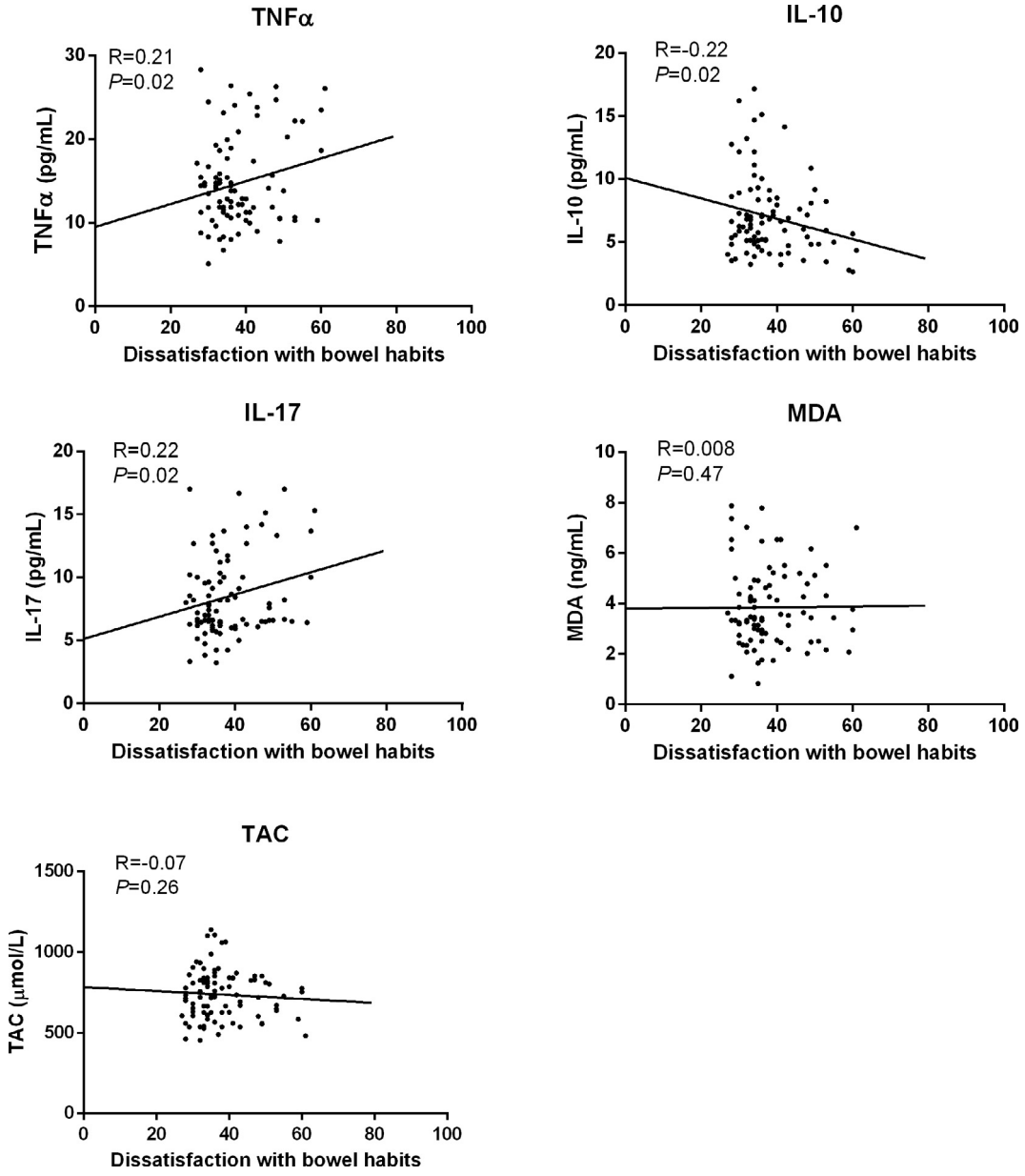

Fig. 4. The correlation of the severity of dissatisfaction with bowel habits with inflammatory cytokines and oxidative stress biomarkers in patients with IBS. *P* values < 0.05 were considered statistically significant. TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

GI symptoms [1]. IBS symptoms are generated by gut dysfunction, visceral hypersensitivity and, in some cases, epithelial dysfunction [2]. Visceral hypersensitivity has an important impact on the generation of the chronic pain and discomfort in IBS patients [2]. Inflammatory mediators such as cytokines affect nociceptor terminals, and activate intracellular signaling pathways, which in turn heighten sensitivity of both the peripheral and the central nervous system, and lead to pain hypersensitivity [2]. Furthermore, inflammatory cytokines such as TNFα could affect the release of corticotrophin-releasing hormone (CRH), the primary hypothalamic regulatory peptide of the HPA axis, and studies indicated that the increase in inflammatory cytokines cause an exaggerated response of the HPA axis in IBS patients as compared to the healthy subjects [7,9]. These phenomena accompanied by an increased intestinal response to CRH, leads to generation of digestive symptoms of IBS [9]. Indeed, HPA axis dysfunction and immune activation may be implicated in digestive symptoms generation in IBS patients. Accordingly, there might be possible correlations between the inflammation and the severity of symptoms; nevertheless, a limited number of studies have assessed these correlations. The results of our study demonstrate that there were weak but significant correlations between the serum levels of cytokines (TNFα, IL-17 and IL-10), and the intensity of clinical symptoms such as abdominal pain, dissatisfaction with bowel habits, overall GI symptoms and IBSSS. Similar to our findings, Hughes et al. demonstrated that the serum levels of TNFα and IL-1β correlated significantly with self-reported symptoms of pain frequency and pain intensity in IBS patients [29]. In 2011, a negative correlation between the serum IL-10 levels and the bowel symptoms intensity in IBS patients has been demonstrated; however, there were no significant correlations between other cytokines such as IL-1β, IL-6, IL-8, IL-12 and TNFα, and the bowel symptoms [32].

Furthermore, we have found that there was a significant correlation between inflammatory cytokines and quality of life of IBS patients. The lower TNFα and IL-17 serum levels were significantly correlated with higher IBS-QoL, and the lower IL-10 serum level was significantly correlated with lower IBS-QoL. The quality of life is an important measure of the impact of IBS, and requires tailored treatment and monitoring. Psychological distress such as anxiety and depression which are common in IBS patients, leads to impaired quality of life

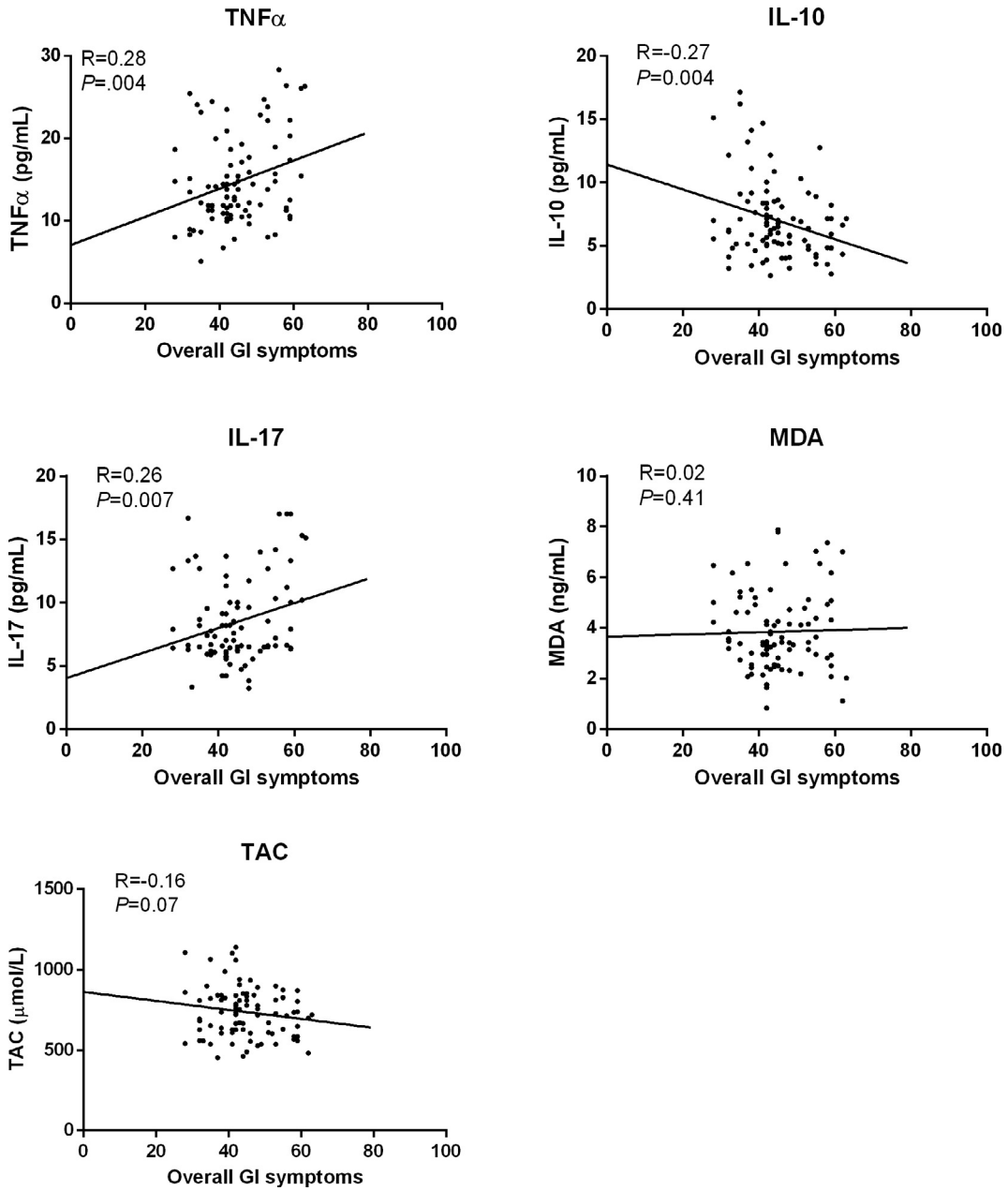

Fig. 5. The correlation of the severity of overall GI symptoms with inflammatory cytokines and oxidative stress biomarkers in patients with IBS. *P* values < 0.05 were considered statistically significant. GI, gastrointestinal; TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

[2], and research has identified a strong correlation between the quality of life score and both anxiety and depression in IBS patients [33]. Furthermore, psychological stressors can activate HPA axis, the core endocrine stress system [34], which provides an important connection between the brain and the gut immune system [2]. High rates of stressful life events and other psychosocial trauma affect symptom severity in IBS patients, and most of these patients report that stress changes stool pattern and leads to acute abdominal pain [2]. In 2007, Liebregts et al. indicated that LPS-induced TNFα production has a significant positive association with anxiety score in IBS patients [4]. Furthermore, in the study of Chang et al., the serum levels of IL-1β, IL-6, IL-8, IL-10, IL-12, and TNFα were assessed in IBS patients, and there was no significant correlation between the immune markers and IBS quality of life [32]. Our results relating to the correlations between cytokines and quality of life were in contrast to the results of Chang et al. [32]. A limitation of our study is that, psychological factors such as depression and anxiety were not assessed.

Moreover, the results of the present study demonstrate that, the serum levels of MDA increased and the serum levels of TAC decreased in IBS patients; although, there were no significant correlations between these oxidative stress biomarkers and both the digestive symptoms and quality of life. To our knowledge, there are limited numbers of study, which assessed the oxidative stress in patients with IBS. Oxidative stress and inflammation are inevitably linked and may act in tandem to form a circuit. Immune cells activation and infiltration, can produce the ROS by resident cells [11]. Furthermore, Schwartz et al. indicated that ROS contribute to the increased expression of inflammatory cytokines such as IL-1β and TNFα [35]. In the study of 36 IBS patients, the plasma concentrations of MDA and nitric oxide (NO), and the plasma activities of oxidant such as xanthine oxidase and adenosine deaminase increased and antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase decreased in IBS patients compared to the healthy controls [12]. Another limitation of our study is that, the tiobarbituric acid method was used

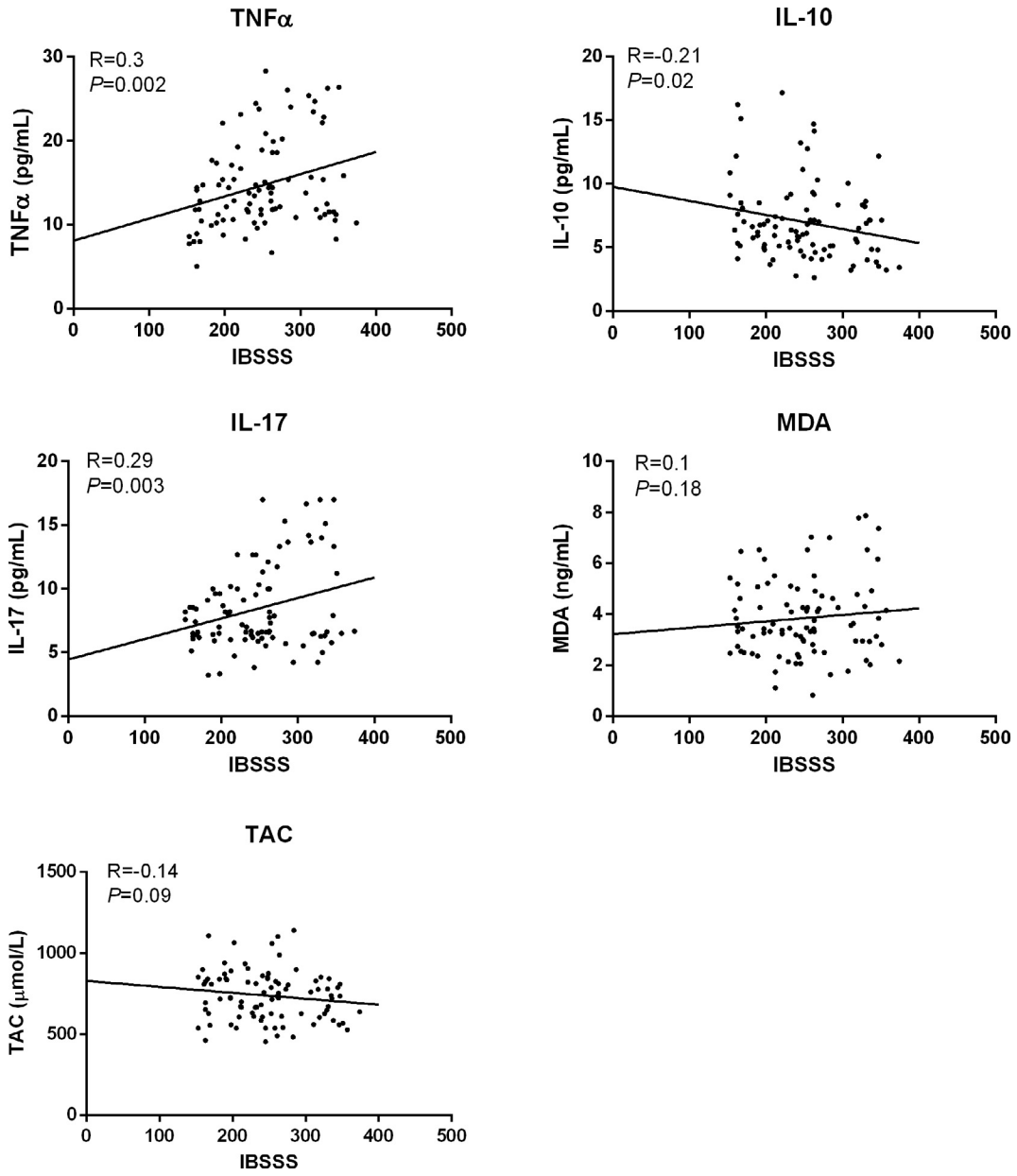

Fig. 6. The correlation of the IBSSS with inflammatory cytokines and oxidative stress biomarkers in patients with IBS. *P* values < 0.05 were considered statistically significant. IBSSS, IBS symptoms severity score; TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

for measuring serum MDA levels, which has the problems of specificity and variability of data [36,37].

In conclusion, the present study demonstrates that pro-inflammatory cytokines including TNFα and IL-17 increased, and in contrast, anti-inflammatory cytokine IL-10 decreased in patients with IBS. Moreover, there were significant correlations between inflammatory cytokines and intensity of symptoms as well as quality of life. The results of our study provide further evidence to support the view that pro-inflammatory cytokines have an important role in the pathogenesis of IBS, and also, in generation of digestive symptoms. Additionally, the serum levels of antioxidants decreased and the serum levels of MDA increased in IBS patients; hence, this alteration in the oxidant-antioxidant system may be implicated in the pathogenesis of IBS. Furthermore, when comparing bowel habit subtypes, clear differences in inflammatory cytokines were observed with increased concentrations of TNFα and IL-17 cytokines, and decreased IL-10 cytokine, in IBS-D. Nevertheless, further studies are needed to provide additional evidence.

Conflict of interest

None.

Author's contribution

AA: is the guarantor of the manuscript; AA, RA and RC: provided the overall concept, framework for the manuscript and involved in the drafting and critical revision of the manuscript; AA: was responsible for the statistical analyses; All authors contributed to data interpretation, critically reviewed the manuscript and approved the final version of the manuscript, including the authorship list. None of the authors reported a conflict of interest related to the study.

Acknowledgements

This work was financially supported by a Grant (No. NRC-9410) from the Vice-Chancellor for Research at the Jundishapur University of

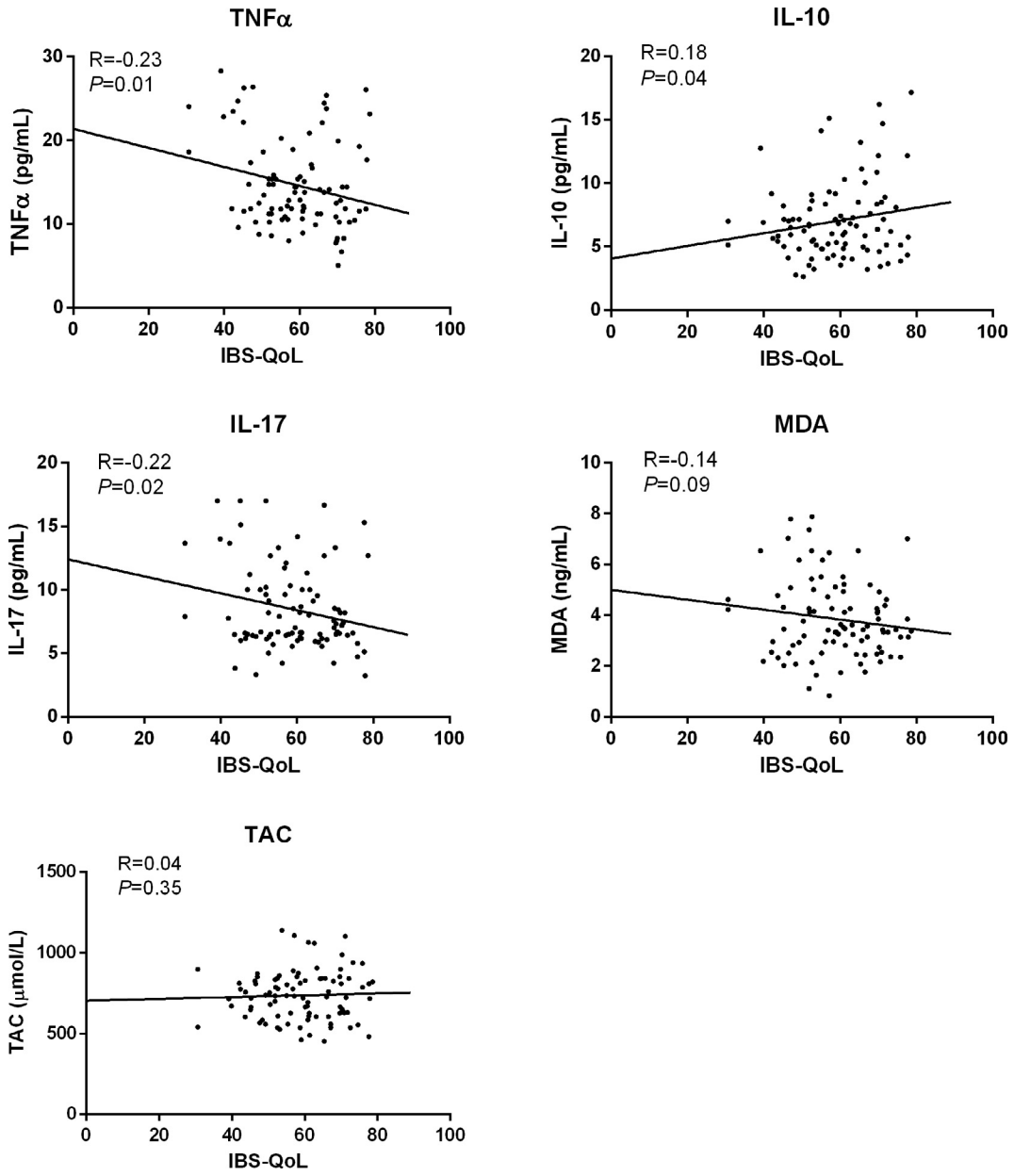

Fig. 7. The correlation of the IBS-QoL with inflammatory cytokines and oxidative stress biomarkers in patients with IBS. *P* values < 0.05 were considered statistically significant. IBS-QoL, IBS quality of life; TNFα, tumor necrosis factor α; IL-10, interleukin 10; IL-17, interleukin 17; MDA, malondialdehyde; TAC, total antioxidant capacity.

Medical Sciences and approved by the Nutrition and Metabolic Diseases Research Center, Jundishapur University of Medical Sciences, Ahvaz, Iran.

References

[1] S. Collins, T. Piche, P. Rampal, The putative role of inflammation in the irritable bowel syndrome, Gut 49 (6) (2001) 743–745.

[2] R. Spiller, Q. Aziz, F. Creed, A. Emmanuel, L. Houghton, P. Hungin, R. Jones, D. Kumar, G. Rubin, N. Trudgill, Guidelines on the irritable bowel syndrome: mechanisms and practical management, Gut 56 (12) (2007) 1770–1798.

[3] P. Scully, D.P. McKernan, J. Keohane, D. Groeger, F. Shanahan, T.G. Dinan, E.M. Quigley, Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity, Am. J. Gastroenterol. 105 (10) (2010) 2235–2243.

[4] T. Liebregts, B. Adam, C. Bredack, A. Röth, S. Heinzel, S. Lester, S. Downie-Doyle, E. Smith, P. Drew, N.J. Talley, Immune activation in patients with irritable bowel syndrome, Gastroenterology 132 (3) (2007) 913–920.

[5] W. Gonsalkorale, C. Perrey, V. Pravica, P. Whorwell, I. Hutchinson, Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? Gut 52 (1) (2003) 91–93.

[6] A.V. Turnbull, C.L. Rivier, Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action, Physiol. Rev. 79 (1) (1999) 1–71.

[7] T.G. Dinan, E.M. Quigley, S.M. Ahmed, P. Scully, S. O'Brien, L. O'Mahony, S. O'Mahony, F. Shanahan, P.N. Keeling, Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? Gastroenterology 130 (2) (2006) 304–311.

[8] A.J. Dunn, Systematic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism parallelling the increased plasma corticosterone, Life Sci. 43 (5) (1988) 429–435.

[9] S. Fukudo, T. Nomura, M. Hongo, Impact of corticotropin-releasing hormone on gastrointestinal motility and adrenocorticotropic hormone in normal controls and patients with irritable bowel syndrome, Gut 42 (6) (1998) 845–849.

[10] T.G. Dinan, Glucocorticoids and the genesis of depressive illness. A psychobiological model, Brit. J. Psychiat. 164 (3) (1994) 365–371.

[11] N.D. Vaziri, B. Rodríguez-Iturbe, Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension, Nat. Clin. Practice Nephrol. 2 (10) (2006) 582–593.

[12] R. Mete, F. Tulubas, M. Oran, A. Yilmaz, B.A. Avci, K. Yildiz, C.B. Turan, A. Gurel, The role of oxidants and reactive nitrogen species in irritable bowel syndrome: a potential etiological explanation, Med. Sci. Monit.: Int. Med. J. Exp. Clin. Res. 19 (2013) 762.

[13] J.K. Kolls, A. Lindén, Interleukin-17 family members and inflammation, Immunity 21 (4) (2004) 467–476.

[14] R.E. Anglin, Z. Samaan, S.D. Walter, S.D. McDonald, Vitamin D deficiency and depression in adults: systematic review and meta-analysis, Brit. J. Psychiat. 202 (2)

(2013) 100–107.
[15] F. Rome, Guidelines-Rome III diagnostic criteria for functional gastrointestinal disorders, J. Gastr. Liver Dis.: JGLD 15 (3) (2006) 307.
[16] G.F. Longstreth, W.G. Thompson, W.D. Chey, L.A. Houghton, F. Mearin, R.C. Spiller, Functional bowel disorders, Gastroenterology 130 (5) (2006) 1480–1491.
[17] N.A. Botsoglou, D.J. Fletouris, G.E. Papageorgiou, V.N. Vassilopoulos, A.J. Mantis, A.G. Trakatellis, Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples, J. Agric. Food Chem. 42 (9) (1994) 1931–1937.
[18] I.F. Benzie, J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1) (1996) 70–76.
[19] C. Francis, J. Morris, P. Whorwell, The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress, Aliment. Pharmacol. Ther. 11 (2) (1997) 395–402.
[20] D.A. Drossman, D.L. Patrick, W.E. Whitehead, B.B. Toner, N.E. Diamant, Y. Hu, H. Jia, S.I. Bangdiwala, Further validation of the IBS-QOL: a disease-specific quality-of-life questionnaire, Am. J. Gastroenterol. 95 (4) (2000) 999–1007.
[21] K. Hata, A. Andoh, M. Shimada, S. Fujino, S. Bamba, Y. Araki, T. Okuno, Y. Fujiyama, T. Bamba, IL-17 stimulates inflammatory responses via NF-κB and MAP kinase pathways in human colonic myofibroblasts, American Journal of Physiology-Gastrointestinal and Liver, Physiology 282 (6) (2002) G1035–G1044.
[22] S. Fujino, A. Andoh, S. Bamba, A. Ogawa, K. Hata, Y. Araki, T. Bamba, Y. Fujiyama, Increased expression of interleukin 17 in inflammatory bowel disease, Gut 52 (1) (2003) 65–70.
[23] J.-S. Feng, Z. Yang, Y.-Z. Zhu, Z. Liu, C.-C. Guo, X.-B. Zheng, Serum IL-17 and IL-6 increased accompany with TGF-β and IL-13 respectively in ulcerative colitis patients, Int. J. Clin. Exp. Med. 7 (12) (2014) 5498.
[24] T.G. Dinan, G. Clarke, E.M. Quigley, L.V. Scott, F. Shanahan, J. Cryan, J. Cooney, P. Keeling, Enhanced cholinergic-mediated increase in the pro-inflammatory cytokine IL-6 in irritable bowel syndrome: role of muscarinic receptors, Am. J. Gastroenterol. 103 (10) (2008) 2570–2576.
[25] M. Schmulson, D. Pulido-London, O. Rodriguez, N. Morales-Rochlin, R. Martinez-García, M.C. Gutierrez-Ruiz, J.C. López-Alvarenga, G. Robles-Díaz, G. Gutiérrez-Reyes, Lower serum IL-10 is an independent predictor of IBS among volunteers in Mexico, Am. J. Gastroenterol. 107 (5) (2012) 747–753.
[26] M. Park, M. Camilleri, Genetics and genotypes in irritable bowel syndrome: implications for diagnosis and treatment, Gastroenterol. Clin. North Am. 34 (2) (2005) 305–317.
[27] T. Liebregts, B. Adam, C. Bredack, M. Gururatsakul, K.R. Pilkington, S.M. Brierley, L.A. Blackshaw, G. Gerken, N.J. Talley, G. Holtmann, Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia, Am. J. Gastroenterol. 106 (6) (2011) 1089–1098.
[28] P.A. Hughes, H. Zola, I.A. Penttila, L.A. Blackshaw, J.M. Andrews, D. Krumbiegel, Immune activation in irritable bowel syndrome: can neuroimmune interactions explain symptoms & quest, Am. J. Gastroenterol. 108 (7) (2013) 1066–1074.
[29] P.A. Hughes, A.M. Harrington, J. Castro, T. Liebregts, B. Adam, D.J. Grasby, N.J. Isaacs, L. Maldeniya, C.M. Martin, J. Persson, Sensory neuro-immune interactions differ between irritable bowel syndrome subtypes, Gut (2012) gutjnl-2011-301856.
[30] M. McKendrick, N. Read, Irritable bowel syndrome—post salmonella infection, J. Infect. 29 (1) (1994) 1–3.
[31] S.M. Bennet, A. Polster, H. Törnblom, S. Isaksson, S. Capronnier, A. Tessier, B. Le Nevé, M. Simrén, L. Öhman, Global cytokine profiles and association with clinical characteristics in patients with irritable bowel syndrome, Am. J. Gastroenterol. (2016).
[32] L. Chang, M. Adeyemo, I. Karagiannidis, E.J. Videlock, C. Bowe, W. Shih, A.P. Presson, P.-Q. Yuan, G. Cortina, H. Gong, Serum and colonic mucosal immune markers in irritable bowel syndrome, Am. J. Gastroenterol. 107 (2) (2012) 262–272.
[33] N.G. Rey de Castro, V. Miller, H.R. Carruthers, P.J. Whorwell, Irritable bowel syndrome: a comparison of subtypes, Am. J. Gastroenterol. 30 (2) (2015) 279–285.
[34] D. de Wied, M. Diamant, M. Fodor, Central nervous system effects of the neurohypophyseal hormones and related peptides, Front. Neuroendocrinol. 14 (4) (1993) 251–302.
[35] M.D. Schwartz, J.E. Repine, E. Abraham, Xanthine oxidase-derived oxygen radicals increase lung cytokine expression in mice subjected to hemorrhagic shock, Am. J. Respir. Cell Mol. Biol. 12 (4) (1995) 434–440.
[36] H. Kosugi, T. Kato, K. Kikugawa, Formation of yellow, orange, and red pigments in the reaction of alk-2-enals with 2-thiobarbituric acid, Anal. Biochem. 165 (2) (1987) 456–464.
[37] M. Kadiiska, B. Gladen, D. Baird, D. Germolec, L. Graham, C. Parker, A. Nyska, J. Wachsman, B. Ames, S. Basu, Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl 4 poisoning? Free Radical Biol. Med. 38 (6) (2005) 698–710.