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ORIGINAL ARTICLE

Serum immune biomarkers in irritable bowel syndrome

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Summary
Background and objectives: Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal (GI) disorder, which presents with abdominal pain and changes in the bowel habits. Although the exact cause of IBS remains uncertain, some studies have shown that the inflammation and cytokine imbalance may act as potential etiological factors. The aim of our study is to compare the serum levels of interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor-alpha (TNF-α) in patients with IBS with the healthy controls. The other aim of this study is to evaluate possible association between above-mentioned cytokines and IBS subtypes.
Methods: Seventy-four IBS patients diagnosed based on Rome III criteria and 75 gender and age-matched healthy controls were included in this study. Cytokines were measured in the serum using enzyme-linked immunosorbent assays (ELISA).
Results: Patients were classified into groups of IBS with diarrhea (IBS-D): 34, IBS with constipation (IBS-C): 29, and IBS with mixed symptoms (IBS-M): 11. The serum levels of IL-6, IL-8 and TNF-α were significantly higher in patients with IBS as compared to controls ($P < 0.001$). There was no difference in serum levels of cytokines based on IBS subtypes.

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Conclusions: Higher serum level of IL-6, IL-8 and TNF-α in IBS suggests an important role of cytokines as immune mediators in the pathogenesis of this functional GI disorder. To understand any association between cytokines and IBS subtypes, further investigations with larger sample sizes are desired.

Introduction

Irritable bowel syndrome (IBS) is the most prevalent functional gastrointestinal (GI) disorder, which presents with abdominal pain and changes in bowel habits and manifests as diarrhea and/or constipation [1]. The exact cause of IBS is unknown, but a variety of factors such as changes in the normal microbiota of the GI tract, inflammatory mediators, food allergies, and abnormality in the gut—brain axis are proposed to play a role [2—7]. Several lines of evidence show that inflammation and cytokine imbalance may act as potential etiological factors in IBS [8,9]. Cytokines are soluble extracellular proteins or glycoproteins with immunological functions that are produced by a broad range of cells, including T- and B-lymphocytes, macrophages, mast cells, etc. [10]. Interleukin 6 (IL-6), interleukin 8 (IL-8), and the tumor necrosis factor-alpha (TNF-α) are important functional cytokines in the immune system. IL-6 is a multifunctional cytokine that is produced by immune cells, adipocytes, fibroblasts, endothelial cells and macrophages. IL-6 has effects that vary from tissue damage, to suppress the inflammation and regulation of neural and metabolic processes [11]. IL-8 is a proinflammatory cytokine that is produced by neutrophils, T lymphocytes, natural killer cells, endothelial cells and fibroblasts. IL-8 is a universal biomarker for various diseases [12]. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine produced mainly by macrophages and monocytes. This cytokine has a key role in the pathogenesis of many inflammatory diseases [13]. Alterations in the mucosal and systemic levels of cytokines in patients with IBS have been highlighted in several studies, although the findings are controversial [14,15]. Therefore, we aim to investigate the serum levels of IL-6, TNF-α, and IL-8 in patients with IBS and compare them with healthy individuals. Furthermore, we aim to study the correlation of the above-mentioned cytokines with the IBS subtypes.

Materials and methods

Ethics statements

All the participants were selected from Kerman, a city in southeast Iran. All subjects gave a written informed consent for the enrollment in this study. The research was performed between 2013 and 2014 and approved by the Ethical Committee of the Kerman University of Medical Sciences. The approval number is K/92/247.

Selection of patients and controls

Seventy-five healthy controls from the Kerman Blood Transfusion Center and 74 patients with IBS were enrolled in our study. The diagnosis of IBS was based on Rome III. Patients were screened by a gastroenterologist to rule out organic GI diseases. Irritable bowel syndrome was classified into IBS with diarrhea (IBS-D), IBS with constipation IBS (IBS-C), and IBS with mixed symptoms (IBS-M), according to the Rome III criteria [16]. Controls did not have any history of chronic GI, autoimmune, allergic, or infectious diseases and had no active GI symptoms during recruitment. The demographic data for controls was as follows: males (frequency = 36%, $n = 27$), females (frequency = 64%, $n = 48$). The mean age for patients and controls was 35.52 ± 11.72 and 37.37 ± 12.5 years, respectively. The controls and patients with IBS were age and sex-matched. The demographic and clinical characteristics of patients are given in Table 1. All samples were collected non-fasting.

Cytokine assay

From each subject, 5 ml of blood was collected in plain tubes. The serum was separated and archived at −80 °C until further analysis. Serum levels of IL-6, IL-8, and TNF-α were measured by the enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (Bosterbio, China). The assay range of Bosterbio ELISA kits were 7.8—500 pg/ml for TNF-α and IL-8 and 4.69—300 pg/ml for IL-6 measurement in the serum samples. As the declaration of Bosterbio Company, the mentioned ELISA kits were validated via Inter-Assay and Intra-Assay precision and the results of validations are available online at the following website: http://www.bosterbio.com/products/picokine-tm-elisa-kits.html.

Statistics

Statistical analyses, such as, descriptive statistics, one-way analysis of variance (ANOVA), Chi^2, Fisher's exact test, and independent *t*-test, were performed by using the SPSS software version 17.0. A *P*-value less than 0.05 was considered as statistically significant. The serum levels of cytokines were presented as mean ± SD.

Table 1 Demographic and clinical characteristics of patients with irritable bowel syndrome.

Variables	IBS-D *n* (%) 34 (45.9)	IBS-C *n* (%) 29 (39.2)	IBS-M *n* (%) 11 (14.9)
Males, *n* (%)	19 (67.9)	5 (17.9)	4 (14.3)
Females, *n* (%)	15 (32.6)	24 (52.2)	7 (15.2)
Age at diagnosis (years)	34.11 ± 11.58	37.20 ± 12.17	29.09 ± 10.18
Hypertension	1 (2.9)	1 (3.4)	0 (0)
Metabolic syndrome	1 (2.9)	0 (0)	1 (9.1)
Smoking habit	1 (2.9)	0 (0)	1 (9.1)
Narcotics consumption	1 (2.9)	0 (0)	0 (0)
Gastroesophageal reflux disease	0 (0)	0 (0)	1 (9.1)
Gastroesophageal reflux disease + metabolic syndrome	0 (0)	0 (0)	1 (9.1)
Anxiety	5 (14.7)	0 (0)	2 (18.2)
Obsession	0 (0)	0 (0)	1 (9.1)
Depression	0 (0)	0 (0)	1 (9.1)
Other mental diseases	5 (14.7)	0 (0)	4 (36.4)
Sleep disorder	3 (8.82)	—	3 (27.3)
Schizophrenia	—	—	1 (9.1)
Border Line Personality	1 (2.94)	—	—
Antisocial Personality	1 (2.94)	—	—
Somatic diseases	2 (5.9)	1 (3.4)	1 (9.4)

IBS-D: irritable bowel syndrome with diarrhea; IBS-C: irritable bowel syndrome with constipation; IBS-M: irritable bowel syndrome with mixed symptoms.

Results

IL-6, IL-8, and TNF-α in the serum of patients with irritable bowel syndrome and controls

Fig. 1 shows the profile of IL-8, IL-6, and TNF-α in the serum of patients with IBS and healthy controls. The serum levels of IL-6, IL-8, and TNF-α in patients with IBS were 166.40 ± 42.99, 414.73 ± 61.94 and 175.8 ± 47.67 pg/ml, respectively. The serum levels of IL-6, IL-8, and TNF-α in healthy controls were 5.00 ± 0.54, 15.23 ± 0.66 and 175.80 ± 47.67 pg/ml, respectively. There was a significant increase in the serum levels of all cytokines in patients with IBS as compared to controls ($P < 0.001$).

IL-6, IL-8, and TNF-α cytokines in serum of patients with IBS-D

The serum levels of IL-6, IL-8, and TNF-α in patients with IBS-D were 153.84 ± 57.13 pg/ml, 390.06 ± 95.72 pg/ml and 88.35 ± 44.95 pg/ml, respectively. All cytokines showed a significant increase in IBS-D patients as compared to the controls (Fig. 2).

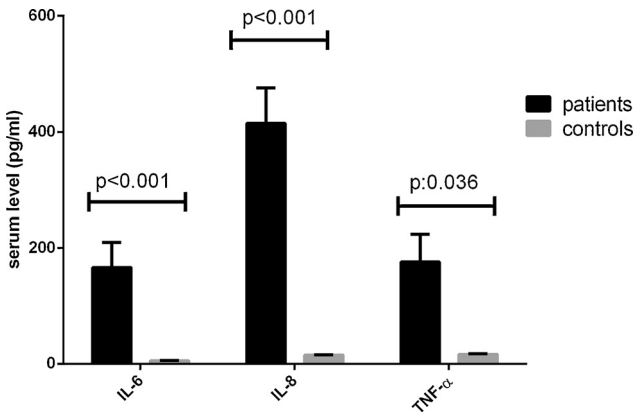

Figure 1 Comparison between serum levels of IL-6, IL-8, and TNF-α in patients with IBS and healthy controls. Statistically significant differences measured by independent *t*-test and values are presented as mean ± SD.

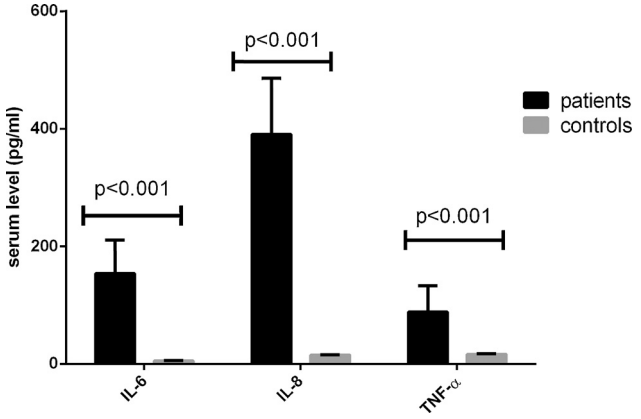

Figure 2 Comparison between serum levels of IL-6, IL-8, and TNF-α in patients with IBS-D and healthy controls. Statistically significant differences measured by independent *t*-test and values are presented as mean ± SD.

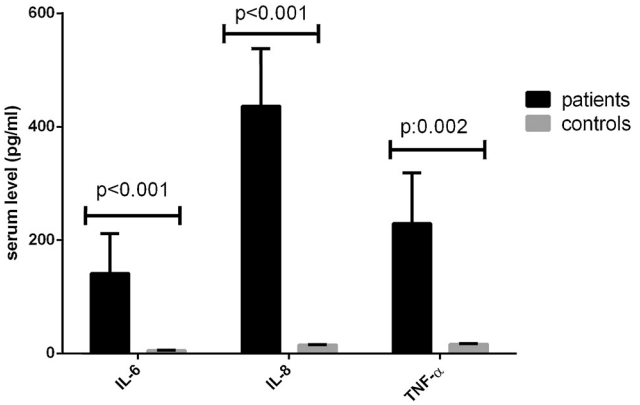

Figure 3 Comparison between serum levels of IL-6, IL-8, and TNF-α in patients with IBS-C and healthy controls. Statistically significant differences measured by independent *t*-test and values are presented as mean ± SD.

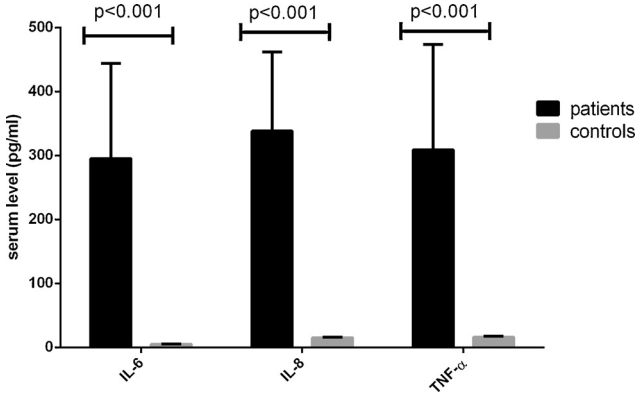

Figure 4 Comparison between serum levels of IL-6, IL-8, and TNF-α in patients with IBS-A and healthy controls. Statistically significant differences measured by independent *t*-test and values are presented as mean ± SD.

IL-6, IL-8, and TNF-α cytokines in serum of patients with IBS-C

The serum levels of IL-6, IL-8, and TNF-α in patients with IBS-C were 141.39 ± 70.27, 436.48 ± 101.56 and 229.11 ± 89.80, respectively. The serum levels of the above-mentioned cytokines in IBS-C patients were significantly higher than in the controls (Fig. 3).

IL-6, IL-8, and TNF-α cytokines in serum of patients with IBS-M

The serum levels of IL-6, IL-8, and TNF-α in patients with IBS-M were 295.34 ± 148.59, 338.45 ± 123.32 and 309.07 ± 164.4 respectively. The serum levels of the above-mentioned cytokines in IBS-M patients were significantly higher than in the controls (Fig. 4).

Comparison of IL-6, IL-8, and TNF-α between irritable bowel syndrome subtypes

The serum levels of IL-6, IL-8, and TNF-α were compared between the IBS subtypes and the difference was not significant (Fig. 5). There was no significant difference between males and females with regard to the level of tested cytokines, not only in all patients with IBS but also in IBS subtypes (data not shown).

Discussion

Irritable bowel syndrome is a common disorder that occurs at any age and in any gender. Its incidence is more common in women in their second and third decades of life. This functional GI disorder has a significant impact on the quality of life of the patients [17,18]. The main findings of our comparison study on the immune markers of the serum between patients with IBS and healthy controls were:

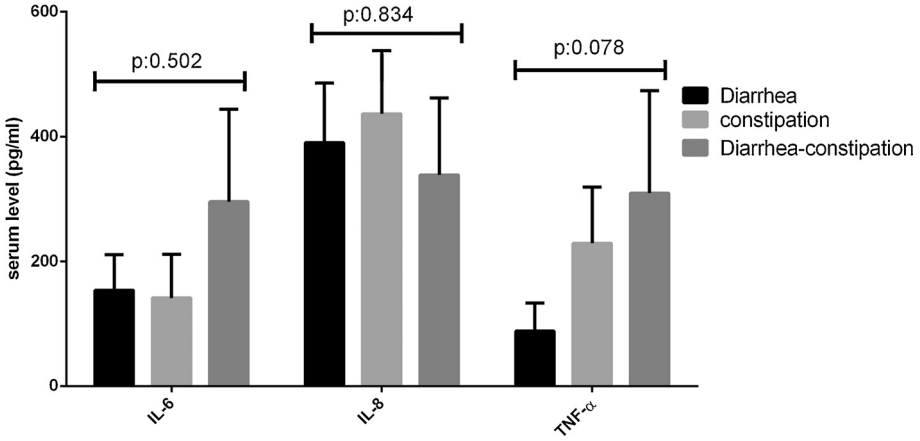

Figure 5 Comparison of IL-6, IL-8, and TNF-α between IBS subtypes. Statistically significant differences measured by one-way ANOVA test and values are presented as mean ± SD.

- patients with IBS showed significantly higher serum levels of IL-6, IL-8, and TNF-α, as compared to healthy controls;
- the serum levels of cytokines were significantly higher in each IBS subtype, including IBS-D, IBS-C, and IBS-M as compared to healthy controls;
- there was no significant difference in the serum levels of cytokines between the IBS subtypes.

Here we detected an increase in the serum levels of proinflammatory cytokines including IL-6, IL-8, and TNF-α, in patients with IBS when compared to healthy controls. Our findings are supported by other previous studies, where increased levels of IL-6, IL-8, and TNF-α in the serum/plasma were reported [19—24].

Darkoh et al. [19] observed that the levels of inflammatory mediators such as TNF-α and IL-1β in the serum and stool samples of patients with IBS were significantly higher than in healthy individuals. Dinan et al. [20] measured the plasma levels of IL-10, IL-8, IL-6, IL-6 receptor, TNF-α and cortisol in 76 patients with IBS and compared them with 75 healthy controls. They reported a significant elevation of IL-6, IL-8, and IL-6 receptor, but not TNF-α in all patients as compared to the controls. Scully et al. [21] showed that plasma levels of IL-6 and IL-8, but not TNF-α, IL-13, IL-10, IL-12p70, IL-1β, and interferon gamma (IFN-γ) were significantly higher in 21 IBS patients, without comorbidity than in the 54 healthy controls. In another study on the cholinergic-mediated proinflammatory response in IBS, a significant increase in serum levels of IL-6 and IL-8 in 37 patients with IBS was confirmed when compared to the 37 controls [22]. Rana et al. [23] indicated that the serum levels of TNF-α and IL-6 in 63 patients with D-IBS were significantly higher than in the 62 healthy controls. Bashashati et al. [24] investigated the relationship between mucosal expression or serum/plasma levels of TNF-α/IL-10 and IBS in a systematic review and meta-analysis. They showed a significant increase in the serum/plasma levels of TNF-α in IBS subtypes when compared with controls only in female patients with IBS. However, serum/plasma levels of IL-10 in IBS subtypes did not show any difference. Interestingly, their results showed a significant decrease in the serum/plasma levels of IL-10 in male patients with IBS comparing with controls. On the other hand, our findings differed from other reports, wherein an insignificant difference in serum/plasma levels of IL-6, IL-8, and TNF-α was observed between patients with IBS and the healthy controls [25—27].

There are controversial reports regarding the levels of proinflammatory cytokines secreted by cultured peripheral blood mononuclear cells (PBMCs) of patients with IBS. Liebregts et al. [28] have shown significant increase in the LPS-induced IL-6, TNF-α and IL-1β levels in cultured PBMCs of 55 patients with IBS as compared with 36 healthy controls. On the contrary, Elsenbruch et al. [29] reported that LPS-stimulated whole blood cells cultured from 15 female patients with IBS had lower levels of TNF-α and similar levels of IL-6 as compared to 15 healthy women.

Inconsistencies are revealed between studies on the levels of cytokines in the colonic mucosa of patients with IBS, too. Chang et al. [30] showed a significant decrease in mRNA expression levels of mucosal cytokines including IL-2 and IL-6 in patients with IBS, as compared to the controls. Macsharry et al. [9] reported a significant reduction in the secretion of IL-8, CXCL9 and MCP-1, but not TNF-α, IL-6, and IL-1β in the ex vivo biopsy cultures of patients with IBS as compared to the controls.

The lack of correlation between levels of cytokines in serum/plasma and mucus of colonic tissue in patients with IBS suggests that the source and/or clearance of cytokines in the serum may not be the same as in the gut. Additionally, controversies between studies might be related to the heterogeneity of subject data, because the diagnosis of IBS is entirely symptom-based. The other reason for the dissimilarity of findings may be because of the small sample sizes and variation in laboratory assays or kits that were utilized.

Many genetic studies performed so far seek to determine a possible link between cytokine gene polymorphisms and IBS. However controversy between the results prevents a clear conclusion, which might be due to small sample size, differences in ethnicity or differences in the classification of IBS and low statistical power of the studies. Bashashati et al. [31] conducted a systematic review and meta-analysis to define the correlation between cytokine gene polymorphisms and IBS. They detected an association between high producer of proinflammatory cytokine TNF-α (−308 G/A) and IBS in the Asian population, but not in the overall meta-analysis. The variability of results obtained from different studies might be due to the genetic variations among different populations and the influence of other known/unknown polymorphisms on the disease. Additionally, the strengths of epigenetic modifications are changeable by environmental factors, such as stress. Gastrointestinal infections or stress are important factors involved in IBS [32] and may also have influence on the cytokine production. There are few studies that have evaluated the contribution of diet in IBS. Hayes et al. [33] discussed the role of diet to the pathophysiology and symptoms of IBS. They suggested that administration of probiotics, limitation in uptake of foods containing gluten and restriction in eating fermentable foods have great potential for assessing the effect of diet on the pathogenesis of IBS, which might be defined better by carrying out the fundamental studies in the future. Therefore another explanation for the differences between the results of studies on the levels of cytokines might be the diet.

Our study has some limitations. Our cytokine findings are limited to the serum and it is possible that there are differences in the mucosal level of cytokines in the colonic tissues. Even as the cytokine levels were measured in the serum, there was no measurement of the cytokine mRNA expression levels in the peripheral blood mononuclear cells and colonic tissue. Lack of study on the cytokine profile of T-helper 2 and T-regulator was another limitation. Although the phenotypes of our patients were carefully determined and our sample size was comparable to other similar studies, the complexity of IBS and heterogeneity of patients with IBS may have resulted in the lack of difference between cytokine profiles in the IBS subtypes. In the present study, no significant differences in the serum levels of cytokines between IBS subtypes might be related to the lack of statistical power. Our research protocol did not include effects of diet in patients with IBS. We did not also include the effect of high producer IL-8, IL-6 and TNF-α gene polymorphisms on the levels of cytokines. The lack of information regarding possible GI infections and stress was the other limitations in our protocol.

In conclusion, we have demonstrated higher serum levels of IL-6, IL-8, and TNF-α in patients with IBS than in the controls, suggesting an important role of cytokines as immune mediators in the pathogenesis of IBS. Cytokines in the serum may be readily accessible and valuable biomarkers for the diagnosis of IBS. To investigate the role of cytokines on IBS subtypes, further investigations with larger sample sizes are desired.

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Authors' contributions

All authors performed the studies, drafted the manuscript and data analysis. All authors read and approved the final draft of manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

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