Tropical Gastroenterology 2012;33(4):251–256

Original Article

Pro-inflammatory and anti-inflammatory cytokine response in diarrhoea-predominant Irritable bowel syndrome patients

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ABSTRACT

Background and aim: Irritable bowel syndrome (IBS) is referred to as a functional bowel disorder which is diagnosed by a number of characteristic symptoms (Rome II criteria) in the absence of detectable structural abnormalities. Low-grade inflammation of the intestine may be one of the reasons for development of diarrhoea-predominant IBS (IBS-D). We undertook this study to estimate the serum levels of pro-inflammatory (IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines in IBS-D patients.

Methods: A total of 108 diarrhoea patients were screened. Out of these only 63 adult IBS-D patients were enrolled. Age and sex matched 62 apparently healthy controls with no GI symptoms were also recruited. Out of 63 IBS-D patients, 37 were males while there were 32 males among the controls. The patients with IBS-D were diagnosed according to the Rome II criteria. Levels of serum IL-6, TNF-α and IL-10 were measured in all subjects using ELISA.

Results: Mean (±SD) age of IBS-D patients (42.6±19.5 years) was comparable (p=0.64) to that of controls (43.5±18.7 years). The mean (±SD) levels of IL-6 in IBS-D patients (32.2±12.01pg/ml) was significantly higher (p<0.001) than in controls (7.48±2.55pg/ml). The levels of TNF-α in IBS-D patients (16.3±5.2 pg/ml) were also significantly higher (p<0.05) than in controls (7.94±2.19 pg/ml). There was no significant difference in the serum levels of IL-10 (p=0.23) between IBS-D patients (5.75±2.1 pg/ml) and controls (5.84±1.9 pg/ml).

Conclusion: Our results indicate that mild inflammation is involved in IBS-D patients as pro-inflammatory cytokines were increased although no difference in anti-inflammatory cytokine was observed.

KEYWORDS: Cytokines, IL-6, TNF- α, IL-10, IBS

Introduction

Irritable bowel syndrome is a common functional bowel disorder characterized by recurrent abdominal pain and altered bowel habits.[1] The condition is common, with a population prevalence of between 5 and 20%.[2-4] Several mechanisms have been proposed to explain the pathophysiology of IBS, including visceral hypersensitivity,[5] altered gut motility,[6] and psychosocial factors.[7] In addition, inflammation and mucosal immune system activation may be important.[8] Studies demonstrate an increased risk for developing IBS after dysenteric illness[9-11] and increased numbers of immunocompetent cells in rectal mucosa of patients with post-infectious IBS up to 1 year,[12] implying that low-grade inflammation may contribute to symptoms. In a recent review it has been suggested that low-grade mucosal inflammation,

particularly mast cell activation, may be a contributory factor in the pathogenesis of IBS.[13] Pro- and anti-inflammatory cytokines are important modulators of the immune response and play a role in intestinal inflammation. The normal gastrointestinal immune response is under tight regulation with the balance between pro- and anti-inflammatory cytokines / mediators defining the immune status of the gut.[14]

IL-6 is a multi-functional cytokine that regulates immune response, acute phase reactions, hematopoiesis and may play a central role in host defense mechanisms.[15] This cytokine exerts many effects ranging from defense to inflammation and tissue damage.[16] It is produced both by macrophages and adipocytes,[17] and by immune cells, fibroblasts and endothelial cells.[18]

Tumor necrosis factor alpha (TNFα) is a polypeptide cytokine produced by monocytes and macrophages which has a crucial role in chronic inflammatory states such as inflammatory bowel disease[17,20] and rheumatoid arthritis.[21] It has been shown that patients with persistent symptoms after an acute infectious gastroenteritis have a five-fold increase in the number of activated macrophages in the rectal lamina propria.[13] TNFα circulates throughout the body responding to stimuli (infectious agents or tissue injury), activating neutrophils, altering the properties of vascular endothelial cells, regulating metabolic activities of other tissues, as well as exhibiting tumoricidal activity by inducing localized blood clotting. Due to its varied actions throughout the immune system, TNF-α may play a role in the pathogenesis of many disease states.[22] Macrophages are the main producers of TNF-α and interestingly are also highly responsive to TNF-α. TNF-α has been shown to play a pivotal role in orchestrating the cytokine cascade in many inflammatory diseases and because of its role as a "master-regulator" of inflammatory cytokine production.[23]

Interleukin 10 is a pleiotropic cytokine playing an important role as a regulator of lymphoid and myeloid cell function. Due to its ability to block cytokine synthesis and several accessory cell functions of macrophages, this cytokine is a potent suppressor of the effector functions of macrophages, T-cells and NK cells. In addition, IL-10 participates in regulating proliferation and differentiation of B-cells, mast cells and thymocytes.[24] The immunosuppressive properties of IL-10 suggest a possible clinical use in suppressing transplant graft rejections. IL-10 can furthermore exert strong anti-inflammatory activities.[25]

Therefore, we hypothesize that low-grade inflammation of the intestine may be the reason for development of IBS-D. This study was planned to estimate serum levels of pro-inflammatory cytokines (IL-6, TNF-α) and anti-inflammatory cytokine (IL-10) in adult patients of diarrhoeal type of IBS (IBS-D).

Methods

Patients were diagnosed as per the Rome II criteria.[26] IBS is diagnosed in the presence of abdominal pain or discomfort for at least 12 weeks in the preceding 12 months associated with two of the following: relief after a bowel movement and / or association with a change in frequency of bowel movements; and / or an association with a change in consistency of motions. All IBS subjects were further classified according to the Rome II criteria for bowel habit predominance and only diarrhoea predominant IBS (IBS-D) patients were included for this study. A total of 108 patients suffering from diarrhoea were screened. Out of these only 63 adult IBS-D patients fulfilled the Rome II criteria. Age and sex matched 62 apparently healthy individuals with no GI symptoms were recruited as controls. Biopsies were taken by sigmoidoscopy in order to exclude microscopic colitis in all patients. The study was approved by our Institute ethics committee and informed consent was obtained from all subjects before their enrollment. Patients with other gastrointestinal diseases including inflammatory bowel disease (IBD) confirmed by sigmoidoscopy, and clinically significant systemic diseases were excluded from the study. Pregnant women, individuals diagnosed with lactose intolerance, immunodeficiency, individuals who had undergone any abdominal surgery, with the exception of hernia repair and appendectomy, and those with a psychiatric illness were also excluded.

Cytokine assays

Peripheral venous blood samples were collected from all participants. Five ml blood samples were collected in plain vials. Samples were centrifuged after proper clotting to prevent hemolysis. Serum was separated and stored at -80°C until further analysis. Serum IL-6, TNF-α and IL-10 were measured by a commercially available enzyme-linked immunosorbent assay (Diaclone). IBS-D patients and controls samples were processed in same run for proper comparison. The assays were performed according to the manufacturer's protocols. The minimal

detectable concentration were <2 pg/ml, <3.16 pg/ml, <1.3 pg/ml for IL-6, TNF-α and IL-10, respectively. Inter- and intra-assay assessments of the kit reliability were also conducted.

Statistical analysis

Statistical significance for serum cytokine levels between IBS-D patients and healthy controls was evaluated using an unpaired two-tailed Student's t-test. Values have been reported as means±SD. Any statistical difference was considered significant at $p < 0.05$.

Results

Out of the 108 patients screened, 45 were excluded (21 patients with IBS-A / M, five positive for microcytic colitis ruled out by sigmoidoscopy biopsy, eight positive for celiac diseases confirmed by serum TTG levels, seven patients positive for ova/cyst in stool and four positive for *Clostridium difficile* toxin in stool). Therefore, only 63 IBS-D patients were enrolled according to the Rome II criteria.[26]

Subject characteristics

The demographic data of patients and controls is given in **Table 1**. Mean (±SD) age of IBS-D patients (42.6±19.5 years) was comparable (p=0.64) to controls (43.5±18.7 years). Among the IBS-D patients 37 were males and 26 females while in controls 32 were males and 30 were females. Age and sex distribution among IBS-D patients and controls was found to be comparable. The age range in IBS-D patients was 26-65 years while that in controls was 25-64 years.

Table 1: Demographic characteristics of the study subjects.

Groups	IBS-D (n=63) n (%)	Controls (n=62) n (%)	P value
Males	37 (58.7)	32 (51.6)	0.53
Females	26 (41.3)	30 (48.4)	0.57
Average age (Mean ± SD)	42.6±19.5	43.5±18.7	0.64
Range (Yrs)	(26-65)	(25-64)	

Serum IL-6 profile

Figure 1 shows the scatter diagram of serum IL-6 cytokine profile for IBS-D patients and healthy controls. There was a significant difference ($p<0.001$) between the IL-6 serum levels of IBS-D patients (32.2±12.01 pg/ml) and healthy controls (7.48±2.55 pg/ml).

Serum TNF-α profile

Figure 2 shows the cytokine profile of IBS-D patients and healthy controls. There was a significant difference ($p<0.05$) in serum levels of TNF-α between IBS-D patients and healthy controls (16.3±5.2 vs. 7.94±2.19 pg/ml).

Serum IL-10 profiles

Figure 3 shows the IL-10 cytokine profile observed in IBS-D patients and healthy controls. No significant difference (p=0.23) was observed between the serum levels of IL-10 (5.75±2.1 pg/ml) in IBS-D patients and the healthy controls (5.84±1.9 pg/ml).

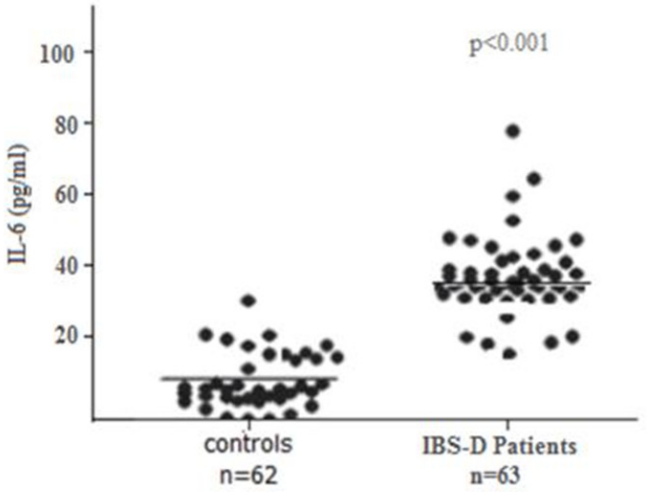

Figure 1: Serum IL-6 cytokine profiles of diarrhoea-predominant IBS (IBS-D) patients and healthy controls

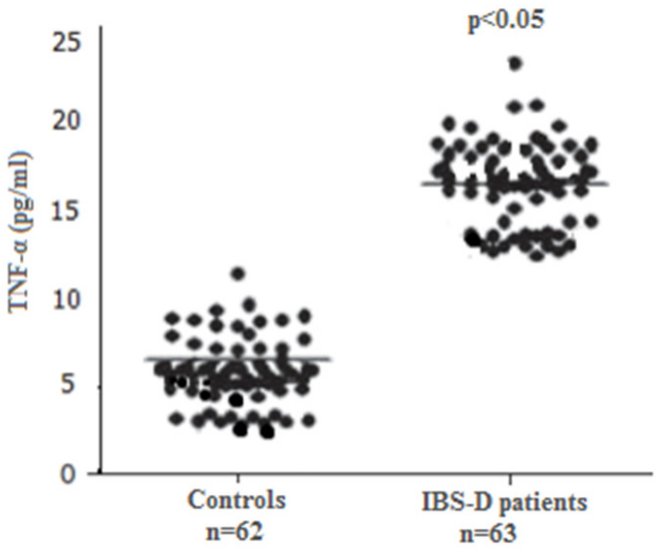

Figure 2: Serum TNF-α cytokine profiles of diarrhoea-predominant IBS (IBS-D) patients and healthy controls

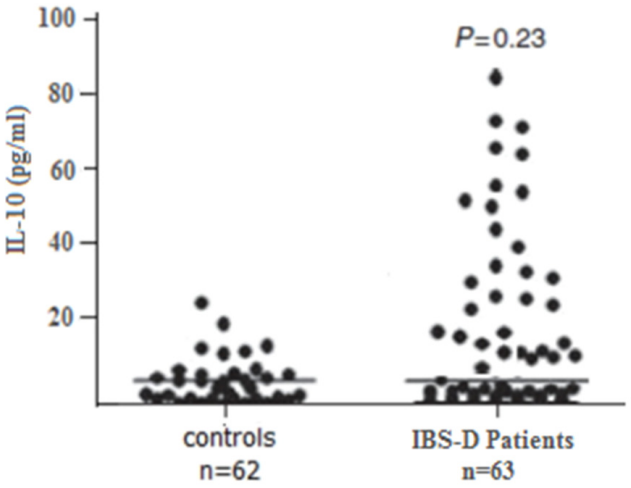

Figure 3: Serum IL-10 cytokine profiles of diarrhoea-predominant IBS (IBS-D) patients and healthy controls

Correlation between pro-inflammatory cytokine levels and severity of symptoms

No correlation was noted between pro-inflammatory cytokine levels and severity of symptoms. Values of correlation were as follows: IL-6 vs. stool frequency (r=0.42, p=0.14); IL-6 vs. Bristol stool scores (r=0.32, p=0.17); IL-6 vs. abdominal pain (r=0.45, p=0.15); IL-6 vs. bloating scores (r=0.35, p=0.42); TNF-α vs. stool frequency (r=0.32, p=0.21); TNF-α vs. Bristol stool scores (r=0.35, p=0.19); TNF-α vs. abdominal pain (r=0.38, p=0.18); TNF-α vs. bloating scores (r=0.39, p=0.22).

Discussion

Irritable bowel syndrome is a stress-related disorder associated with disturbed brain-gut communication, gastrointestinal homeostasis and based on recent evidence, low-grade inflammation and an altered microbiota. Our study examines the cytokine profiles of IBS-D patients and healthy individuals. The presence of elevated serum IL-6 and TNF-α in diarrhea-predominant IBS patients as compared to controls signifies ongoing mild inflammation in IBS-D. Our findings are consistent with a recent study by Scully et al.[27] Their analysis of pro-inflammatory cytokines in female IBS patients with extra-intestinal co-morbidities showed increased levels of IL-6, IL-8, and TNF-α. McKerman et al[28] also demonstrated elevated cytokine levels in patients with the irritable bowel syndrome, indicating some immune dysregulation in these patients. Pro-inflammatory cytokines IL-6 and TNF-α have been shown to control the release of corticotrophin-releasing hormone, the main hypothalamic regulatory peptide of the hypothalamic-pituitary-adrenal (HPA) axis.[29,-31] Thus an increase in these cytokines as seen in our IBS patients may be involved in exaggerated activation of the HPA axis. The significant increase in IL-6 and TNF-α levels in our IBS-D patients tend to corroborate with the above hypothesis. Similar findings of increased serum IL-6 and TNF-α in IBS patients have been reported in other studies.[29,32,33] This may be due to enhanced cellular immune response with increased pro-inflammatory cytokine production in diarrhea-predominant IBS. It has further been seen that IL-6 and TNF- α cytokine gene polymorphisms could change an individual's susceptibility to IBS and these might have pathophysiological role.[34] However, no differences were observed in TNF-α and IL-6 levels between patient groups with strong and mild/moderate pain intensity.[35] It has also been observed by Dinan et al[36] that the IL-6 cytokine profile in IBS is driven by responses mediated by muscarinic receptors.

IL-10 is a potent suppressor of macrophage, T cell and NK cell effector functions. In addition, IL-10 participates in regulating proliferation and differentiation of B-cells.[24] We did not find any significant difference in the IL-10 levels between our patients and controls. The ongoing mild inflammation discussed above might be the reason for such observations for IL-10. These findings are similar to those reported by other studies.[27,29] IL-10 has also been proposed to have a putative role in enteric infections and other causes of intestinal inflammation. High producer TNF-α and low producer IL-10 genotypes have been reported to be significantly more prevalent in IBS patient than in healthy individuals and also in diarrhea-predominant IBS as compared to other IBS subtypes.[37] The lower prevalence of the high producer genotype in IBS suggests that high production of IL-10 may have some protective role or, conversely, that individuals predisposed to produce lower amounts of this cytokine might be more likely to develop IBS.[38] Studies have documented the onset of IBS following bacteriologically confirmed gastroenteritis, while others have provided evidence of low-grade mucosal inflammation[12,39] and immune activation[40,41] in IBS. Mast cells, which are increased in number and activated in the jejunum and colon in IBS, may play a critical role in mediating the neural response to these inflammatory triggers. The results by Dinan et al[42] show that mast cells increased significantly in the IBS-diarrhea group compared to IBS-constipation and control groups. Despite the expected heterogeneity of the disorder, differences in mucosal chemokine signaling were evident in this cross-sectional study of IBS patients at the level of both gene expression and protein secretion.[43] It has also been

reported that specific environment events such as acute gastrointestinal infection can trigger the manifestations of IBS. Moreover, in animal studies the role of transient mucosal inflammation[44] and the role of the severity of inflammation in terms of the manifestation of specific abnormalities of function have been identified.[45] Thus it seems reasonable to speculate that inflammatory process can play an important role in the manifestations of IBS-D.

This study demonstrates that patients with IBS-D can have increased levels of pro-inflammatory cytokines IL-6 and TNF-α with no difference in anti-inflammatory cytokine levels of IL-10. Our data provides evidence to support the view that mild inflammation may be involved in diarrheal type of irritable bowel syndrome.

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