

Serum Profile of Cytokines in Iraqi Inflammatory Bowel Disease Patients

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Abstract

Background: Studies indicated that IBD-related tissue damage results from a dynamic interplay between immune and non-immune cells, in which cytokines are crucial mediators.

Aim: To determine serum level of eight cytokines (IL-2, IL-4, IL-8, IL-10, IL-12, IL-17A, IP-10 and IFN- γ) in inflammatory bowel disease (IBD) Iraqi patients.

Patients and Methods: The IBD patients (54 ulcerative colitis; UC and 25 Crohn's disease; CD) attended the Gastrointestinal Teaching Hospital in Baghdad for diagnosis and treatment during the period March-August 2012. Serum level of cytokines was determined by ELISA method.

Results: Four cytokines showed significant variations between UC patients and controls. Levels of IL-8 (2.47 ± 0.35 vs. 0.48 ± 0.19 pg/ml), IL-12 (5.06 ± 0.47 vs. 1.58 ± 0.79 pg/ml) and IP-10 (6.96 ± 1.02 vs. 1.98 ± 0.76 pg/ml) were increased in patients, while level of IL-10 (2.72 ± 0.44 vs. 7.33 ± 2.32 pg/ml) was decreased. In CD patients, levels of IL-8 (3.26 ± 0.56 vs. 0.48 ± 0.19 pg/ml), IL-12 (4.71 ± 0.79 vs. 1.58 ± 0.79 pg/ml) and IP-10 (5.03 ± 1.50 vs. 1.98 ± 0.76 pg/ml) were also significantly increased in patients compared to controls. Comparing UC and CD patients revealed that IL-10 level was significantly decreased in UC patients (2.72 ± 0.44 vs. 5.79 ± 1.10 pg/ml), while IFN- γ was significantly increased (5.99 ± 0.49 vs. 3.78 ± 0.61 pg/ml).

Conclusion: These findings highlight a pathogenic role of these cytokines in UC and CD patients.

Key words: Inflammatory bowel disease, Ulcerative colitis, Crohn's disease, Cytokines.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of inflammatory, idiopathic, conditions affecting the gut. It predominantly consists of two chronic, often relapsing, immunologically mediated gastrointestinal disorders, which are ulcerative colitis (UC) and Crohn's disease (CD). The inflammation in CD may involve any segment of the digestive tract, from mouth to anus, and is associated with discontinuous transmural lesions of the gut wall, whereas in UC the inflammation is confined to the colon and rectum, and lesions are continuous in extent from rectum and upwards to one or several colonic segments and restricted to the mucosa. (1). When the gut is inflamed, there is a breakdown in

intestinal barrier function, abnormal secretion, changes in the patterns of motility and visceral sensation, which contribute to symptoms generation (2). These two clinical entities share multiple similarities; including gastrointestinal inflammation, waxing and waning severity and unknown etiology, but clinical and pathological heterogeneity are also observed (3). However, the pathogenesis and etiology of UC and CD are not well-characterized, but both phenotypes are suggested to be the manifestation of complex and multifactorial processes, in which genetics, environmental influences and immunologic abnormalities may play the most important role to promote an excessive and poorly controlled mucosal inflammatory response directed against components of

the luminal microflora that lead to an IBD-related tissue damage, which results from a dynamic interplay between immune and non-immune cells, in which cytokines are crucial mediators in this interplay. (4,5,6).

Cytokines are low molecular weight proteins produced mainly by immune cells and facilitate communications between cells, stimulate proliferation of antigen-specific effector cells, and mediate local and systemic inflammation (7). They are suggested to play an etiological and pathological role in IBD because cytokines are key signaling molecules of intestinal immune system (8). Different studies revealed that several cytokines; for instance, interleukin-1 (IL-1), IL-6, IL-10, IL-13 and tumor necrosis factor-alpha (TNF- α), may be involved in pathogenesis of IBD (5,6). It is also evident that these mediators play an important role in a local and systemic acute-phase response of inflammatory process in IBD (9). Cytokine profile analyses in IBD patients demonstrated that CD and UC are probably immunologically distinct. Crohn's disease is often described as a prototype of T-helper (Th) 1-mediated diseases because the primary inflammatory mediators are the Th1 cytokines, while UC is usually viewed as a Th2 type condition because of the increased intestinal expression of the Th2-associated cytokines. However, these pathways may not be mutually exclusive as individual cytokines can have diverse and even opposing functions in various clinical and immunological settings (10). Furthermore, it has been demonstrated that CD and UC gut mucosa is heavily infiltrated with another subset of Th cells; termed Th17 cells, which produce a distinct array of cytokines that has been recently targeted in IBD (11).

Therefore, the present study aimed to explore the profile of eight cytokines (IL-2, IL-4, IL-8, IL-10, IL-12, IL-17A, IP-10 and IFN- γ) in sera of two groups of Iraqi IBD patients (UC and CD).

PATIENTS AND METHODS

The study was approved by the Medical Ethics Committee of the Iraqi Ministry of Health and was done as 79 Iraqi IBD patients were investigated. The patients attended the Gastrointestinal Tract Unit at Al-kadhamyah Teaching Hospital in Baghdad for diagnosis and treatment during the period March - August 2012. The disease was diagnosed by the consultant medical staff at the hospital by using Lennard-Jones diagnostic criteria (12). The diagnosis was made according to clinical, endoscopic, histological, and radiological findings in patients who were firstly diagnosed, and cases with undetermined colitis were excluded. Exclusion criteria were: age less than 18 years, pregnancy, history of colorectal surgery,

active infections, current malignancy, and autoimmune disorders that could complicate the interpretation of cytokine serum levels. Accordingly, the patients were clinically distributed into two groups: UC, which included 54 patients (34 males and 20 females), and their age mean $\pm$ S.E. was 38.9 ± 1.7 years; and CD, which included 25 cases (15 males and 10 females), and their age was 37.8 ± 2.2 years. In addition, 27 apparently healthy controls of blood donors (15 males and 12 females) matched patients for age (35.9 ± 2.9 years) and ethnicity (Iraqi Arabs) were also enrolled in the study.

Assessment of cytokine serum levels

Five milliliters of venous blood was collected from each participating subject and transferred to a plain tube and left to clot. Then, it was centrifuged at 1000 rpm for 15 minutes to separate serum, which was distributed into aliquots and stored frozen at -20°C until assayed for cytokine levels. The assessment was carried out by using eight ELISA kits (Peprotech, UK). They were designed for a quantitative measurement of human IL-2, IL-4, IL-8, IL-10, IL-12, IL-17A, IP-10 and IFN- γ in serum, by sandwich Elisa technique.

Statistical analysis

Serum level of cytokines was analyzed using the SPSS software (Statistical Package for Social Sciences) version 13. Their data were given as mean $\pm$ standard error (S.E.), and differences between means were assessed by ANOVA (Analysis of Variance), followed by LSD (Least Significant Difference).

RESULTS

Ulcerative colitis patients versus controls

Out of the eight cytokines determined, four showed variations between UC patients and controls. Serum levels of IL-8 (2.47 ± 0.35 vs. 0.48 ± 0.19 pg/ml), IL-12 (5.06 ± 0.47 vs. 1.58 ± 0.79 pg/ml) and IP-10 (6.96 ± 1.02 vs. 1.98 ± 0.76 pg/ml) were significantly ($P \leq 0.001$, 0.01 and 0.001, respectively) increased in patients, while serum level of IL-10 (2.72 ± 0.44 vs. 7.33 ± 2.32 pg/ml) was significantly ($P \leq 0.05$) decreased. Serum level of IL-17A was also increased in patients (3.90 ± 1.53 vs. 2.15 ± 0.92 pg/ml), but the difference was not significant, and IL-2 showed a non-significant decreased level (8.00 ± 0.81 vs. 11.23 ± 5.23 pg/ml) in patients (Table 1).

Crohn's disease patients versus controls

Serum level of IL-8 (3.26 ± 0.56 vs. 0.48 ± 0.19 pg/ml), IL-12 (4.71 ± 0.79 vs. 1.58 ± 0.79 pg/ml) and IP-10 (5.03 ± 1.50 vs. 1.98 ± 0.76 pg/ml) were also significantly ($P \leq 0.001$, 0.01 and 0.01, respectively) increased in CD patients compared to controls, while IL-2 showed a

decreased level in patients (9.74 ± 1.50 vs. 11.23 ± 5.23 pg/ml), but the difference was not significant (Table 2).

Table 1: Mean levels serum of study cytokines of ulcerative colitis patients and controls.

Cytokine	Serum Level (Mean $\pm$ S.E.; pg/ml)		P $\leq$
	Controls (No. = 27)	Ulcerative Colitis (No. = 54)	
IL-2	11.23 ± 5.23	8.00 ± 0.81	N.S.
IL-4	2.67 ± 1.45	2.52 ± 0.38	N.S.
IL-8	0.48 ± 0.19	2.47 ± 0.35	0.001
IL-10	7.33 ± 2.32	2.72 ± 0.44	0.05
IL-12	1.58 ± 0.79	5.06 ± 0.47	0.01
IL-17A	2.15 ± 0.92	3.90 ± 1.53	N.S.
IP-10	1.98 ± 0.76	6.96 ± 1.02	0.001
IFN- γ	4.79 ± 1.88	5.99 ± 0.49	N.S.

N.S.: Not significant

Table 2: Mean levels serum of study cytokines of Crohn's disease patients and controls.

Cytokine	Serum Level (Mean $\pm$ S.E.; pg/ml)		P $\leq$
	Controls (No. = 27)	Crohn's Disease (No. = 25)	
IL-2	11.23 ± 5.23	9.74 ± 1.50	N.S.
IL-4	2.67 ± 1.45	2.99 ± 0.84	N.S.
IL-8	0.48 ± 0.19	3.26 ± 0.56	0.001
IL-10	7.33 ± 2.32	5.79 ± 1.1	N.S.
IL-12	1.58 ± 0.79	4.71 ± 0.79	0.01
IL-17A	2.15 ± 0.92	2.33 ± 0.36	N.S.
IP-10	1.98 ± 0.76	5.03 ± 1.50	0.01
IFN- γ	4.79 ± 1.88	3.78 ± 0.61	N.S.

N.S.: Not significant

Ulcerative colitis versus Crohn's disease

Comparing UC and CD patients revealed that IL-10 serum level was significantly decreased in UC patients (2.72 ± 0.44 vs. 5.79 ± 1.10 pg/ml; $P \leq 0.01$), while IFN- γ was significantly increased (5.99 ± 0.49 vs. 3.78 ± 0.61 pg/ml; $P \leq 0.01$).

DISCUSSION

Although the exact etiology remains unknown, it is thought that IBD results from a dysregulated and aberrant immune response to intestinal flora in the context of a genetic predisposition, in which pro- and anti-inflammatory cytokines are critical mediators of IBD pathogenesis (5,6), and results of present study favor such generalization. Cytokines are key regulators of the intestinal immune system and are known to participate in the disruption of normal state of controlled inflammation (13). Early innate-type cytokines, primarily secreted by the intestinal epithelium, as well as activated antigen presenting cells; including dendritic

cells and macrophages, have been suggested to actively regulating the inflammatory response observed in UC and CD patients; moreover IBD has generally been characterized by aberrant overreactive and autoreactive cells and an imbalance between Th1, Th2, Th17 and Treg cell populations and their respective cytokines (14). In the present results, UC and CD patients shared a significant increased serum level of IL-8, IL-12 and IP-10 compared to controls, although with different level of significance, while UC patients were also observed with a significant decreased level of IL-10.

The first of these cytokines is IL-8, which is a potent chemoattractant and activator of neutrophils. It is produced by a wide variety of cell types, including macrophages, neutrophils, endothelial cells, fibroblasts, chondrocytes and osteoclasts (15). In agreement with the present results, Nielsen *et al.* (16) found that IL-8 was significantly increased in active UC patients and was positively correlated with the disease activity. It has also been reported that increased local production of IL-8 in active stage of UC is probably caused by both an increased number and enhanced activity of macrophages (17). It has also been suggested that the increased IL-8 production in the inflamed mucosa may play a role in stimulating the influx of neutrophils into areas of active inflammation and that neutrophils activated by IL-8 may also induce mucosal injury by the release of lysosomal enzymes, leukotriene B and toxic free radicals (18). Colonic lamina propria of patients with IBD has also been shown to produce IL-8, but its concentration showed no difference between inflamed mucosa of patients with CD or UC (19); an observation that is confirmed in the present study, although it was based on measuring IL-8 level in sera of patients. Thus, IL-8 might be involved in inflammation and pathogenesis of the two IBD entities. Mucosal IL-8 protein and IL-8 mRNA concentrations have also been correlated with the degree of inflammation, and IL-8 mRNA was strongly expressed by intestinal inflammatory cells but not by intestinal epithelial cells suggesting that virtually all IL-8 is produced by interstitial inflammatory cells (20). Therefore, an imbalance in the intestinal immune system with a shift towards pro-inflammatory mediators has been considered as a characteristic feature of IBD, and among them, IL-8 has been suggested to play an important role in disease activity, and a dysregulation of IL-8 has been implicated in the pathogenesis of IBD (21). The tissue level of IL-8 was found to be higher in active UC compared to normal colonic tissue, and its serum level was also related to endoscopic and histological severity of UC (22). Based on these findings and the present study results, IL-8 seems to be a reliable biomarker, closely related to a disease activity, but its

pathogenic role in initiation and maintenance of colitis needs to be further studied.

In addition to IL-8, the present CD and UC demonstrated a significant increased serum level of IL-12, and the increase was almost three-fold than in controls. Further studies share such presentation, and IL-12 showed an increased serum or intestinal level in IBD, especially CD patients (23,24). Furthermore, transcripts of IL-12 subunits have been detected in gastric and intestinal mucosa of patients with CD. In addition, it has been shown that lamina propria mononuclear cells isolated from intestinal mucosal areas of CD patients, but not UC, released *in vitro* functionally active IL-12 (25). IL-12 is a key cytokine that drives the inflammatory response mediated by Th1 cells. As such, it underlies both normal host responses to a variety of intracellular bacterial, fungal and protozoal pathogens, and abnormal inflammatory responses that accompany many autoimmune diseases, such as UC and CD (26). For CD, it has been characterized by increased production of IL-12 by antigen-presenting cells in intestinal tissue and IFN- γ and TNF- α by intestinal lymphocytes and macrophages (27). These inflammatory cytokines induce and sustain the granulomatous inflammation and bowel-wall thickening that are hallmarks of CD. Therefore, IL-12 has been suggested to be a target for anti-IL-12 antibody therapy in CD patients, and such target has been considered as an effective treatment for the intestinal inflammation in animal models of CD (28). Experimentally, it was found that using such therapy not just targeting IL-12, but also reducing the levels of downstream effectors such as IFN- γ and TNF- α . The reduced secretion of IL-12 after treatment with anti-IL-12 was associated with a decreased number of IL-12-producing macrophages and consequently reduced secretion of IFN- γ , which eliminated the enhancing effect of IFN- γ exerted on the secretion of IL-12 by macrophages (29). A further study has also shown that depletion of macrophages in mouse prevented the development of colitis, which otherwise occurred owing to unregulated production of IL-12 and IL-23 by macrophages (30). In addition, expression of IL-12-related molecules in human intestinal microvascular endothelial cells has been demonstrated to be regulated by TLR3 (Toll-like receptor) that is associated with intestinal infections (31). Mucosal levels of IL-12 have also been demonstrated to be a predictor of recurrence and of need for surgery in perianal CD patients (32).

These findings clearly highlight the role of IL-12 in initiation and pathogenesis of CD, and the present Iraqi CD patients has a such role. However, the UC patients of present study also demonstrated a significant

increased serum level of IL-12, and the pathogenic mechanism involved in CD might also be involved in UC patients; especially if we consider that IL-12 expression has been found to be up-regulated in both active UC and CD biopsies, and it was positively correlated with the activity index score (23).

The third cytokine that showed a significant increased serum level in UC and CD patients was IP-10, which is a key mediator of the interferon response that preferentially attracts activated Th1 lymphocytes to sites of inflammation (33). Studies agree that IP-10 plays two roles in pathophysiology of IBD, especially UC. First, it is a chemokine that differentiates naïve T cells into Th1 cells and recruits them into inflamed colon. Second, it is a negative regulator for intestinal epithelial proliferation and regeneration (34). Therefore, its role in IBD might be expected because chemokines and their receptors play a dominant role in orchestrating the activity of monocytes, macrophages and T cells, and in particular, it has been suggested that IP-10 is involved in human CD pathogenesis (35,36). In addition, as IP-10 is a ligand for the CXCR3 receptor, the activation of which can result in the recruitment of T cells and the perpetuation of mucosal inflammation (37). These findings were further confirmed by Hosomi *et al.* (38) who demonstrated that IP-10 (CXCL10) mRNA levels in colonic mucosa of inflamed IBD were higher than in controls. They also showed abundant CXCR3-positive immature plasma cells in the inflamed colonic mucosa of UC. Increased numbers of immature plasma cells may migrate towards inflammatory sites of UC via the CXCR3 axis, and may participate in UC pathogenesis. Based on these findings, IP-10 has been a target for therapies in IBD, and blockade of IP-10 has been found to attenuate colitis in mice with newly established murine colitis through blocking cellular trafficking and protecting intestinal epithelial cells, and the suggestion was that IP-10 plays a key role in the development of IBD, as well as in chronic experimental colitis (39), and these results were further confirmed, although different IP-10 inhibitors were employed (40).

The increased serum level of IL-8, IL-12 and IP-10 was paralleled by a decreased serum level of IL-10 in UC and CD, but the difference was significant in only UC patients. These findings suggest that IL-10 was down-regulated in the present IBD patients, and consequently the presented results might be expected, because IL-10 is an important immuno-regulatory cytokine produced by many cell populations especially Treg cells, and its main biological function is the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, NK cells, APCs,

mast cells and granulocytes (41). Recent investigations are also in favor of this, and Begue *et al.* (42) demonstrated that impaired IL-10 signaling is considered as a characterization of IBD, especially in early onset patients. Glocker *et al.* (43) and Shah *et al.* (44) further confirmed that IL-10 and IL-10 receptor (IL10R) deficiency are present in IBD patients, and demonstrated that such deficiency cause severe early-onset enterocolitis, and a loss of IL-10 function can cause a severe intractable enterocolitis in infants and young children.

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CONCLUSION

Up-regulation (IL-8, IL-12 and IP-10) and down-regulation (IL-10) of cytokines are suggested to have a role in pathogenesis of UC and CD of Iraqi IBD patients.

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