

Original Article

Serum IL-17 and IL-6 increased accompany with TGF- β and IL-13 respectively in ulcerative colitis patients

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Abstract: Purposes: To explore the serum level of pro- and anti-inflammatory cytokines in the patients of ulcerative colitis and irritable bowel disease. And analyze the correlation between the cytokine's levels and disease's activity of ulcerative colitis patients. Methods: Serum cytokines of ulcerative colitis and irritable bowel syndrome with diarrhea patients including IL-6, IL-10, IL-13, IL-17, TNF- α and TGF- β were analyzed by enzyme linked immunosorbent assay, and ulcerative colitis activity were assessed by Mayo scoring system. The correlation of the serum level of cytokines and ulcerative colitis activity were analyzed by the SPSS 19.0 software. Results: Compared with healthy people, the serum level of IL-6, IL-10, IL-13, IL-17, TNF- α and TGF- β were elevated in ulcerative colitis patients. There is no direct correlation between each cytokines analyzed and the Mayo score. And the level of IL-6 is relevant to IL-13 ($r=0.364$, $P=0.029$), and the level of IL-17 is relevant to TGF- β ($r=0.336$, $P=0.045$). Conclusion: When the pro-inflammatory cytokines increase in the serum of ulcerative colitis, the anti-inflammatory cytokines were increased concomitantly. Some cytokines are positive correlated, such as IL-6 and IL-13, IL-17 and TGF- β , the mechanism of which is complex and needs further investigation.

Keywords: Ulcerative colitis, irritable bowel syndrome, cytokine, mayo score, correlation

Introduction

Ulcerative colitis is a worldwide, chronic, idiopathic, inflammatory disease of the rectal and colonic mucosa. The immune disorder in gut involved the disruption of tight junctions and the mucus film covering the epithelial layer causing increased permeability of the intestinal epithelium, resulting in increased uptake of luminal antigens. Macrophages and dendritic cells (innate immune cells), on recognition of non-pathogenic bacteria (commensal microbiota) through molecular pattern-recognition receptors, change their functional status from tolerogenic to an activated phenotype. Cytokines in UC patients plays an important role in the process of the inflammation of the colon. Activation of NF- κ B pathways stimulates the transcription of pro-inflammatory genes, resulting in increased production of pro-inflammatory cytokines (TNF- α , IL-12, 23, 6, and 1 β). After processing of antigens, macrophages and dendritic cells

present them to naive CD4⁺ T-cells, promoting differentiation into Th2 effector cells, characterised by production of IL-4. Natural-killer T cells are the main source of IL-13, which has been associated with disruption of the epithelial cell barrier [1]. So some scholars argued that, the immune mechanism involves the imbalance of Th1/Th2 and Th17/Treg (regulatory T lymphocytes).

Irritable bowel syndrome (IBS), a chronic and debilitating functional gastrointestinal disorder, with the characteristic of bellyache, abdomen bulge accompanied with the changing of defecating habit and stool character. It is diagnosed on the basis of a characteristic cluster of symptoms in the absence of detectable organic abnormalities. According to the updated ROME III criteria, IBS is a clinical diagnosis and presents as one of the three predominant subtypes: (1) IBS with constipation (IBS-C); (2) IBS with diarrhea (IBS-D); and (3) mixed IBS (IBS-M) [2].

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Table 1. Mean age in each group

Group	N	Age			
		Min	Max	Mean	SD
UC	36	17	62	36.389	1.902
Control	36	19	60	37.667	11.457
IBS-D	27	21	66	42.741	10.737

There is no statistical difference between groups ($F=2.648$, $P=0.076$).

Table 2. Mean score and frequency of each score point

Score point	3	4	5	6	7	8	9	10	12
Case frequency	9	5	4	4	5	2	3	2	2
Mean \pm SD	5.917 \pm 0.444								

Traditionally, IBS has been conceptualized as a condition of visceral hypersensitivity (leading to abdominal discomfort or pain) and gastrointestinal motor disturbances (leading to diarrhea or constipation) [3].

In our study, we evaluated the cytokines of Th1, Th2, Th17 and Treg by ELISA assay. We assuming that, there is a correlation between them and also the disease's activity (Mayo scores).

Materials and methods

Patients and grouping

Participants including criteria: UC (N=36) and IBS-D (N=27) patients in Affiliated Hospital of Guangdong Medical College between 2011 and 2013. Eligible participants were patients with an established diagnosis of UC by conventional clinical, endoscopic and histological criteria.

Participants' exclusion criteria: UC patients with tumor and UC patients with immune disease.

Grouping: Control group (N=36), mainly compose of healthy people who derived from Physical Centre of Affiliated hospital of Guangdong Medical College.

This study was approved by the Ethics Committee of the Affiliated Hospital of Guangdong Medical College; patients and healthy volunteers were recruited after obtaining informed consent.

Mayo scoring

Disease's activity of UC were assessed Mayo scoring system described previously [4]. And the scoring criteria are listed below:

Stool frequency: 0=Normal number of stools for this for this patient; 1=1-2 stools more than normal; 2=3-4 stools more than normal; 3=5 or more stools more than normal.

Rectal bleeding: 0=No blood seen; 1=Streaks of blood with stool less than half the time; 2=Obvious blood with stool most of the time; 3=Blood alone passed.

Findings of flexible proctosigmoidoscopy: 0=Normal or inactive disease; 1=Mild disease (erythema, decreased vascular pattern, mild friability); 2=Moderate disease (marked erythema, absent vascular pattern, friability, erosions); 3=Severe disease (spontaneous bleeding, ulceration).

Physician's global assessment: 0=Normal; 1=Mild disease; 2=Moderate disease; 3=Severe disease.

Sample preparation

Three milliliter of venous blood were obtained from participants. Blood sample were collected in serum separation tube. After separation, the serum was stored in -80°C until testing by enzyme linked immunosorbent assay (ELISA) at one time.

ELISA analysis

Serum level of IL-6, IL-10, IL-13, IL-17, TNF- α and TGF- β were determined by ELISA kit purchased from Shanghai Bogu Biotechnology Co., Ltd. (Shanghai, China). Serum samples from 36 patients with UC and 27 patients with IBS-D were evaluated. The control group consisted of 36 healthy individuals with no family history of IBD and non-immune mediated disorders. According to the detecting step instructions, the concentration of cytokines in serum was calculated by the standard curve.

Statistical analysis

All the data were expressed as Mean \pm Standard Deviation (Mean \pm SD). SPSS 19.0 soft-

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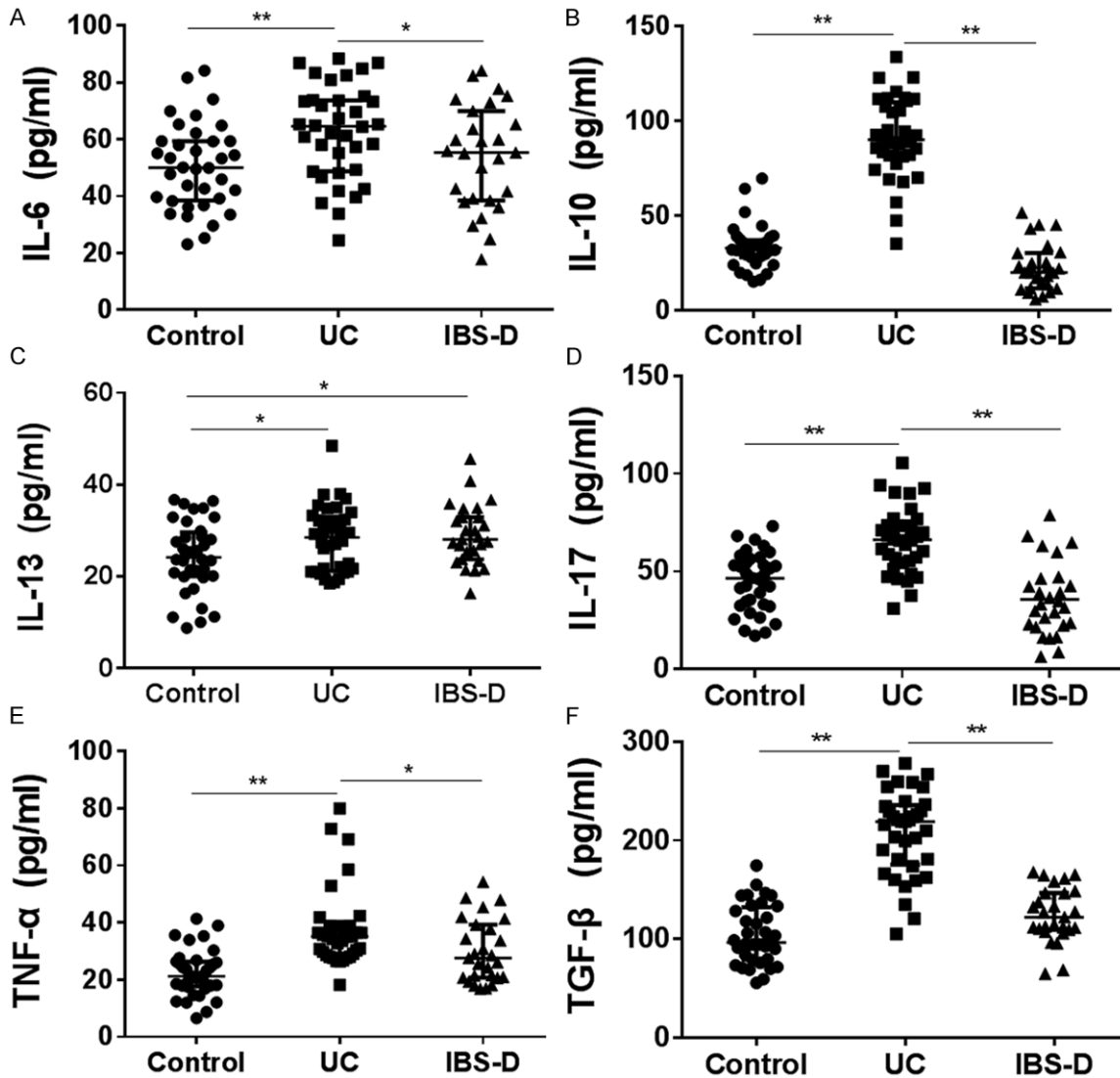


Figure 1. Serum cytokine level in each group. A: IL-6; B: IL-10; C: IL-13; D: IL-17; E: TNF- α ; F: TGF- β ; * $P < 0.05$; ** $P < 0.01$.

ware was used for one-way ANOVA. The correlation analyses were performed by using Pearson Correlation analysis. A P -value of less than 0.05 was considered significant.

Results

Descriptive statistics of the participants

The mean age of each group are list in **Table 1**. And there is no statistically difference between the 3 groups ($F=2.648, P=0.076$). It suggested that the age of each group were well matched. The minimum and maximum of Mayo score were 3 and 12 respectively. The mean score and the case frequency in each point of mayor score are list in **Table 2**.

Both Pro- and anti-inflammatory cytokines are increased in the serum of ulcerative colitis patients

IL-6, IL-10, IL-13, IL-17, TNF- α and TGF- β were increased in the serum of UC patients (**Figure 1**). The details are stated below:

(1) IL-6: UC group was higher than Control and IBS-D group ($P=0.577, P=0.381$).

The IBS-D group showed no statistically significant difference with Control group.

(2) IL-10: UC group was higher than IBS-D group and control group (both $P < 0.001$). The IBS-D group showed no statistically significant difference with Control group.

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Table 3. Correlation analysis of cytokines and Mayo score for UC patients

N=36		IL-6	IL-10	IL-13	IL-17	TNF- α	TGF- β	Mayo score
IL-6	Pearson Correlation	1	0.100	0.364*	0.000	-0.153	0.051	0.026
	P value	-	0.563	0.029	0.998	0.372	0.769	0.881
IL-10	Pearson Correlation	0.100	1	-0.008	0.075	0.007	-0.057	-0.063
	P value	0.563	-	0.963	0.664	0.967	0.740	0.717
IL-13	Pearson Correlation	0.364*	-0.008	1	0.061	-0.038	0.126	0.206
	P value	0.029	0.963	-	0.723	0.827	0.464	0.228
IL-17	Pearson Correlation	0.000	0.075	0.061	1	-0.162	0.336*	-0.064
	P value	0.998	0.664	0.723	-	0.345	0.045	0.710
TNF- α	Pearson Correlation	-0.153	0.007	-0.038	-0.162	1	-0.068	0.137
	P value	0.372	0.967	0.827	0.345	-	0.694	0.426
TGF- β	Pearson Correlation	0.051	-0.057	0.126	0.336*	-0.068	1	0.061
	P value	0.769	0.740	0.464	0.045	0.694	-	0.725
Mayo score	Pearson Correlation	0.026	-0.063	0.206	-0.064	0.137	0.061	1
	P value	0.881	0.717	0.228	0.710	0.426	0.725	-

*. Correlation is significant at the 0.05 level (2-tailed).

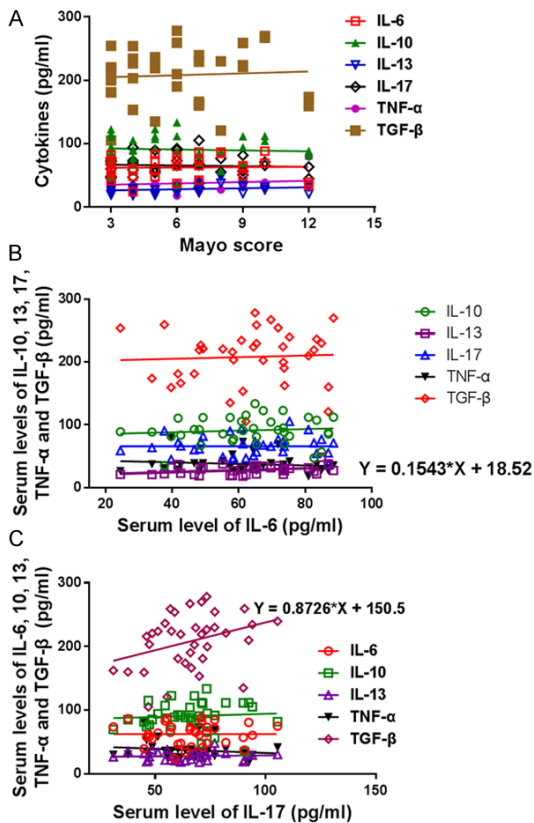


Figure 2. The correlation between cytokines and Mayo score. A: There is no correlation between cytokines and Mayo scores; B: The level of IL-6 is relevant to the level of IL-13 ($r=0.364$, $P=0.029$); C: The level of IL-17 is relevant to the level of TGF- β ($r=0.336$, $P=0.045$).

(3) IL-13: UC and IBS-D group was higher than Control group ($P=0.019$, $P=0.010$). There was

no significant difference between UC and IBS-D group.

(4) IL-17: UC group was higher than IBS-D and Control group ($P < 0.001$). The IBS-D group showed no statistically significant difference with Control group.

(5) TNF- α : UC group was higher than IBS-D group and Control group ($P < 0.001$, $P=0.011$). The IBS-D group showed no statistically significant difference with Control group.

(6) TGF- β : UC group was higher than IBS-D group and Control group ($P < 0.001$). The IBS-D group showed no statistically significant difference with Control group.

Correlation analysis

The results of Pearson Correlation analysis show that, there was not significant correlation between cytokines level and Mayo score in UC group (Table 3; Figure 2A). But there 2 set of cytokines were correlated with each other, IL-6/IL-13 and IL-17/TGF- β ($P=0.029$, $P=0.045$, Table 1), and the equation of linear regression were “ $Y=0.1543*X+18.52$ ” and “ $Y=0.8726*X+150.5$ ” respectively (Figure 2B, 2C). However, there was no any cytokines correlated in IBS-D group (Table 4).

Discussion

Traditionally, Th2 response was considered to be the predominant immune response in UC

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Table 4. Correlation analysis of cytokines for IBS-D patients

		IL-6	IL-10	IL-13	IL-17	TNF- α	TGF- β
IL-6	Pearson Correlation	1	-0.035	0.051	-0.017	-0.082	0.209
	P value	-	0.861	0.799	0.931	0.686	0.297
IL-10	Pearson Correlation	-0.035	1	-0.202	0.205	-0.266	0.192
	P value	0.861	-	0.313	0.305	0.180	0.337
IL-13	Pearson Correlation	0.051	-0.202	1	0.235	-0.257	0.120
	P value	0.799	0.313	-	0.239	0.195	0.550
IL-17	Pearson Correlation	-0.017	0.205	0.235	1	-0.314	0.326
	P value	0.931	0.305	0.239	-	0.110	0.097
TNF- α	Pearson Correlation	-0.082	-0.266	-0.257	-0.314	1	-0.156
	P value	0.686	0.180	0.195	0.110	-	0.436
TGF- β	Pearson Correlation	0.209	0.192	0.120	0.326	-0.156	1
	P value	0.297	0.337	0.550	0.097	0.436	-

while Th1 response is mainly observed in CD. For both the disorder, pro-inflammatory cytokines include TNF- α , IL-17 and et al. At present, the symbol cytokines IL-4 and IL-13 of Th2 response have been well investigated in UC. In our study, both the Th1 and Th2 cytokines were evaluated at one time, and we expected to find some relation between of them, as well as the Mayo score.

The results show that, compared with the control and IBS-D group, IL-6, IL-10, IL-17, TNF- α , TGF- β in UC group were increased. As anti-inflammatory cytokines, IL-10 and TGF- β in the UC group have a higher level than the other two groups ($P < 0.01$), which is coincided with Liberek and colleagues' finding [5]. We speculated the mechanism of negative feedback plays a role on this regulation.

IL-6 is derived from a variety of immune cells, and with multi-bioactivity. In inflammatory local of lamina propria, IL-6 can antagonize apoptosis of T cells and to maintain the survival of them [6, 7], it is considered to be the "central factor" of IBD. Some scholars believe that, the serum level of IL-6 can predict the risk of recurrence after corticoid induced remission, it can be regarded as a marker of IBD [8].

It is reported that, TNF- α , IL-6 and other cytokines are expressed increasingly in the intestinal lamina propria mononuclear cells (LPMC), which are associated with the mucosal inflammatory level observed by endoscopy [9]. And there were large-scale studies found that the increased serum IL-6 levels in patients with CD. The results mentioned above were coincided with our study.

IL-10 is considered to be an important anti-inflammatory cytokine, mainly secreted by Th2 cells, and monocytes, macrophages. T cells and B cells also secrete IL-10, it inhibits the peripheral mononuclear cells and lamina propria mononuclear cells to secrete IL-1 β , TNF- α , IFN- γ , and thus exert an anti-inflammatory effect [10]. In our study, the serum level of IL-10 of UC patients was elevated in response of the increase of the pro-inflammatory cytokines. It seems

to be positively correlated with serum levels of pro-inflammatory cytokines, but actually the correlation was not significant (**Table 3; Figure 2B, 2C**). Contradictory, a study has reported that, compare with healthy people, serum IL-10 levels of CD patients elevated but did not observed in UC patients [11]. But another study found that, serum IL-10 increased both in active UC and CD [12], which is coincident with our data. Furthermore, compared with the control group, expression of IL-10 mRNA expression in UC patients also increased [13]. Increased expression of IL-10 in patients with UC may contact with tumor [14].

Th17 related cytokines such as IL-17 had been identified to involve in the colonic mucosal inflammation in UC patients [15, 16]. For IBS patients, the cytokines of Th17 are seldom investigated, except for infection such as IBS-D. There was reported that, IL-17 were increased in the colonic mucosa of IBS mice infected with the *Trichinella Spiralis* [17]. Another study found that, the cytokines including IL-4, 12, 17 in peripheral blood of IBS patients showed no difference between IBS with histological abnormal and other IBS without histological abnormal [18].

Th2 cytokines IL-13 usually correlated with IL-4, there were many studies have performed to reveal the relation between them. The expression of IL-4 mRNA and IL-13 in colonic biopsies of UC patients were increased, in particular, express more pronounced in active ulcerative colitis. The expression is positive correlated with the severity assessed by endoscopy and the histopathologic score.

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IL-13 is mainly secreted by invariant NKT (iNKT) cells, regulates the function of monocyte-macrophage cells and B cells, particularly the cytotoxic monocyte-macrophage cells; and inhibits the secretion of inflammatory cytokines on the intestinal mucosal barrier and is benefit for the barrier. In gut, IL-13 is usually with bi-properties. The right amount of IL-13 secretion can increase intestinal motility and enhanced epithelial secretory function, promote the secretion of IL-10 to inhibit the activity of Th17, limit the inflammatory damage of the colon and maintain the homeostasis of intestinal tract [19-21]. Over expression of IL-13 in mouse colon will cause damage in mucosa, because the excessive IL-13 activate the apoptosis molecules of epithelial cell, and destroy the tight junctions of epithelial cells, resulting in damage of epithelial barrier. This process involves cytokines of TNF family such as TWEAK and TNF- α . Therefore, the activity of those cytokines mention above increased in UC patients [22]. Another study showed that, with the increase of inflammatory infiltration of UC, IL-13 levels decline gradually [23], and negatively correlated with the development of UC. In our study, serum level of IL-13 in UC group is higher than Control group, but there is no statistical difference compared to the IBS-D group (**Figure 1C**). The reason is possibly because of infection leading Th1/Th2 shifting, Th1 response enhanced [24], in the intestinal mucosa of patients with IBS-D. In our study, IL-6 and IL-13, TGF- β and IL-17 levels are proportional. It was believed that, the secretion of IL-17 are induced by IL-6 and TGF- β [25], while it is inhibited by IL-13 [26], so when the serum levels of IL-6 and TGF- β were up-regulated, in order to down regulate the excess of IL-17, IL-13 were up-regulated.

Acknowledgements

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Disclosure of conflict of interest

None.

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