Serum cytokine levels in ulcerative colitis: Their relationship to disease activity and circulating acute phase reactants

Ülseratif kolitte serum sitokin düzeyleri: Hastalık aktivitesi ve akut faz reaktantları ile ilişkileri

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SUMMARY: *Our objective was to study the relationship between plasma levels of TNF-α, IL-1β, sIL-2R and disease activity in ulcerative colitis.*

Serum TNF-α, IL-1β and sIL-2R concentrations were measured by enzyme-linked immunosorbent assay (ELISA) in 20 healthy subjects and 44 patients with active ulcerative colitis (UC). Patients were assessed by the ulcerative colitis activity index (UCAI), criteria previously established.

Serum TNF-α levels were found to be 7.33 ± 8.21 pg/mL in the control group, 22.40 ± 22.34 pg/ml in patients with UC. The serum sIL-2R levels obtained were 459.3±168.8 U/ml in the control group and 1375.5±525.1 U/ml in patients with UC. Serum IL-1b levels were 1.02±0.24 pg/ml in the control group and 8.92±5.88 pg/mL in patients with UC. TNF-α, IL-1β and sIL-2R levels were significantly higher in the UC group than those observed in the normal subjects (p<0.0001). There was significant correlation between UCAI with TNF-α (r=0.7351, p<0.05), IL-1β (r=0.7253, p<0.05) and sIL-2R (r=8497, p<0.05) serum levels.

These results suggest that TNF-α, IL-1β and sIL-2R levels are related with activity of these disease and measurement of cytokine serum concentrations may provide a simple way to monitor disease activity in UC.

Key words: Ulcerative colitis, tumour necrosis factor-α, interleukin-1b and soluble interleukin-2 receptor

ÖZET: *Bu çalışmada amacımız ülseratif kolit (ÜK) hastalarında serum TNF-α, IL-1β, sIL-2R düzeyleri ile hastalık aktiviteleri arasındaki ilişkiyi araştırmaktı.*

Çalışmaya 20 sağlıklı kontrol ile 44 aktif ÜK hastası alındı. Hastalarda ELİSA yöntemi ile serum TNF-α, IL-1β, sIL-2R konsantrasyonları ve akut faz reaktanları saptanarak UCAI ile aralarındaki ilşkiler incelendi.

Serum TNF-α düzeyi kontrol grubunda 7.33±8.21 pg/ml ÜK grubunda 22.40±22.34 pg/ml, serum sIL-2R düzeyi kontrol grubunda 459.3±168.8 U/ml, ÜK grubunda 1375.5±525.1 U/ml ve serum IL-1β düzeyi kontrol grubunda 1.02±0.24 pg/ml, ÜK grubunda 8.92±5.88 pg/ml bulundu. TNF-α, IL-1β, sIL-2R ÜK grubunda normal kontrol grubundan anlamlı derecede yüksek bulundu (p<0.0001). UCAI ile TNF-α (r=0.7351, p<0.05), IL-1β (r=0.7253, p<0.05), sIL-2R (r=8497, p<0.05) serum düzeyleri arasında anlamlı korelasyon saptandı.

Bu bulgular ile serum TNF-α, IL-1β, sIL-2R düzeylerinin hastalık aktivitesi ile korelasyon gösterdiği ve serumda bu sitokin düzeylerinin ölçümü ile hastalığın aktivitesinin takibinin mümkün olabileceği sonucuna vardık.

Anahtar sözcükler: Ülseratif kolitis, tümör nekroz faktör-α, interleukin-1b, soluble interleukin-2 reseptörü

The etiology and pathogenesis of idiopathic inflammatory bowel disease (IBD) remain unknown. However, recent progress in knowledge of the regulation of inflammatory and immune responses has focused attention on soluble mediators, particularly on cytokines. Cytokines are proteins produced by activated immune cells that influence the activity, differentiation, or proliferation of other cells (1-5). These proteins may play a key role in the pathogenesis of IBD and serum cytokine concentrations are considered to indicate disease activity. Levels of some cytokines in intestinal inflammation have either been the subject of conflicting reports or have been inadequately investigated.

In this study, we investigated serum TNF-α, IL-1b and sIL-2R levels in active ulcerative colitis (UC) and also searched for a correlation between cytokine levels and disease activity.

MATERIALS AND METHODS

Forty four patients with ulcerative colitis (24 women, 20 men, mean age 39.18) and 20 healthy age matched volunteers (12 men, 8 women, mean age 30.8) were studied. The diagnosis of UC was established according to clinical, endoscopic, pathological and radiological criteria. The ulcerative colitis activity index (UCAI) was used to

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Table 1. The characteristic of the patients with UC

	Control	*UC*
	n:20	*n:44*
Age (year)	32±7	39±18
ALB (g/dL)	4.1±0.3	3.1±0.7*
Leukocyte (mm3)	5820±1531	8894±4467*
CRP (mg/dL)	7.2±2.5	137.2±55.3*
ESR (mm/h)	13.8±5.1	71.0±34.6 *
Activity Index	-	47.4±16.6

*p<0.05; compared with levels in control and patients with UC.

Table 2. Serum TNF-α, sIL-2R and IL-1β levels in patients with UC.

		TNF-α (pg/ml)	sIL-2R (U/ml)	IL-1β (pg/ml)
	n	*mean±SD*	*mean±SD*	*mean±SD*
Control	20	7.33±8.21	459.3 ±168.8	1.02±0.24
UC	44	22.40±22.34*	1375.5±525.1*	8.92±5.88*

*p<0.0001 when compared with control

assess disease activity (2). The maximum index of UCAI is 100% , we defined active disease as a UCAI of 20%.

UCAI was determined by clinical symptoms (stool quality, frequency of diarrhoea, melaena, abdominal pain, general status, body weight loss, fever), laboratory data (CRP, total protein, albumine/globuline, potassium), and complications (joint and skin manifestations and others). Each parameter was scored (range 0-3) and then maximum score was calculated (range 3-10). UCAI was formulated as follows: UCAI=100 x score/max.

In 20 patients with UC, the entire colon was involved, in 12 patients left sided colitis and the remaining 12 patients had distal sigmoiditis. At the time of study no patients had extra-intestinal manifestations or extra-intestinal infection. Patients with viral, alcoholic, metabolic, autoimmune liver disease, primary biliary cirrhosis, human immunodefiency virus (HIV) infection, overt infectious disease (septicemia, pneumonia, urinary tract infection), cancer, leukaemia, autoimmune disease and renal insufficiency were excluded from both groups. The patients were taking no medications. No patients had undergone previous surgery.

Blood collection: blood samples were taken from all patients at the same time (8 am). Venous blood was collected into sterile vacuum blood collection tubes and then allowed to clot at room temperature for 30 minutes. Grossly hemolyzed or lipemic specimens were not used. Serum was stored at-70°C until it was used. Cytokine analysis was performed within 6 months of serum storage. Prior to assay, frozen sera was slowly brought to room temperature and gently mixed by hand. We were careful to avoid microbial contamination and did not use any suspect specimens.

Cytokine assays: TNF-α, IL-1ß and sIL-2R levels were measured using commercially available enzyme like immunosorbent assay (ELISA) kits with specific of monoclonal antibodies human IL-1ß (Advanced Magnetic Inc. Cambridge, MA, USA), TNF-α and sIL-2R (T-cell Sciences, Cambridge, MA, USA). The steps used in the assays followed manufacturer's guidelines. All samples were assayed in duplicate and variation between duplicate samples was less than 15%. Cytokine concentrations were calculated by means of duplicate samples. Minimum detectable concentrations of TNF-α and IL-1ß were 1pg/ml and detection limit of sIL-2R was 50 U/ml.

Statistical analysis: All results are given in mean ±SD. Differences among the various groups were analysed using the Mann-Withney U test. Correlation analysis was done by Spearman's rank correlation test. The analysis was performed using computer software with Microstast Ver4.0 (Ecosoft Inc.); $p<0.05$ was considered significant.

RESULTS

The characteristics of patient groups are in table 1. Serum TNF-α levels were found to be 7.33 ±8.21 pg/mL in the control group, 22.40±22.34 pg/ml in patients with UC. Serum sIL-2R level obtained were 459.3±168.8 U/ml in the control group, 1375.5 ±525.1 U/ml in patients with UC. Serum IL-1ß levels were 1.02 ± 0.24 pg/ml in the control group and 8.92 ±5.88 pg/mL in patients with UC. In the disease group, the TNF-α, IL-1ß and sIL-2R levels were significantly higher than those observed in normal subjects ($p<0.0001$). Analysis of the relationship between individual cytokines with biochemical and histological parameters of UC disease was as follows; there was significant correlation of UCAI with TNF-α ($r=0.7351$, $p<0.05$), (Figure 1), IL-1ß ($r=0.7253$ $p<0.05$),

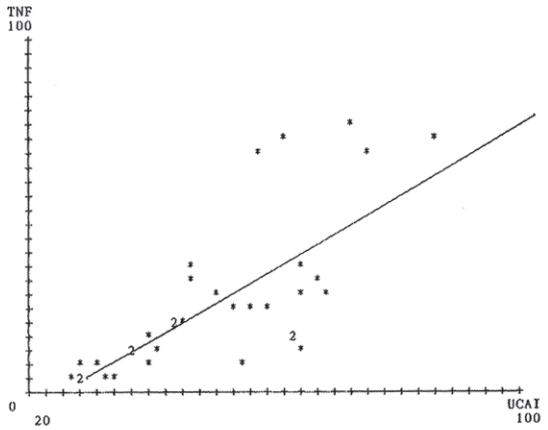

Figure 1. Correlation between serum TNF-α level and UCAT

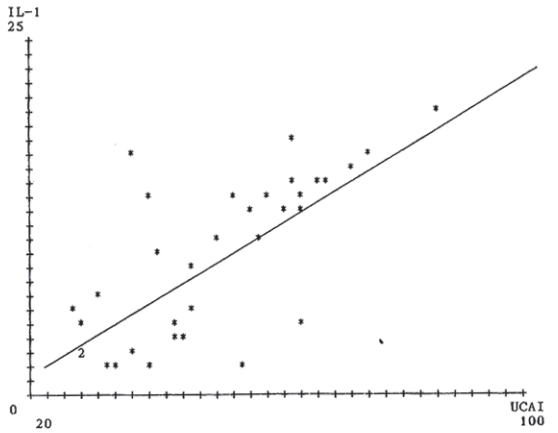

Figure 2. Correlation between serum IL-1β level and UCAT

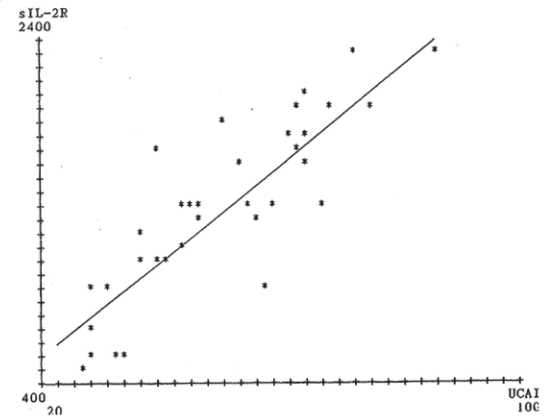

Figure 3. Correlation between serum sIL –2R level and UCAT

(Figure 2), sIL-2R (r=8497, p<0.05), (Figure 3) serum levels. Furthermore, in patients with UC, TNF-α, IL-1ß and sIL-2R levels were positively correlated with CRP blood levels (r=0.6427, r=0.8350, r=0.5001 respectively, p<0.05) and TNF-α and sIL-2R levels were negatively correlated with serum albumin levels (r=-0.5347, r=-0.4935, p<0.05).

DISCUSSION

In spite of numerous experimental and clinical studies, the etiology and pathogenesis of the IBD remains unknown. Whether the trigger for the development of IBD, is bacterial, viral, dietary or environmental, there is general agreement that immune mechanisms are of probable importance (1-5). Both UC and Crohn's disease (CD) are characterized by an influx of inflammatory and immune cells into the diseased mucosa and local production of soluble mediators of inflammation occurs. (1, 7). These mediators include cytokines, arachidonic acid metabolites, reactive oxygen intermediates and growth factors that have important regulatory and effector activities relevant to intestinal inflammation (8). Cytokines have autocrine, paracrine and endocrine activities that mediate the local and systemic manifestation of intestinal inflammation. These molecules regulate and amplify the immune response, induce tissue injury and mediate complications of inflammatory response. These also have a critical role in suppressing inflammation and mediating repair and healing (1, 2, 8, 9). IL-1ß and TNF-α are secreted by activated macrophages. IL-1 stimulates the production of IL-2 and sIL-2R on T lymphocytes. In vivo and in vitro exposure to cytokines, particularly TNF-α and IL-1, can produce many of the universal features of intestinal inflammation, including activation of immune, mesenchymal, endothelial and epithelial cells, diarrhea; recruitment of circulating inflammatory cells, tissue damage (villous atrophy, crypt hyperplasia and ulceration) and fibrosis (10-14). Most cytokines, produced by activated macrophages (IL-1, IL-6, and the IL-8 family) are reproducibly increased in actively inflammed tissue of UC and CD, infectious colitis and experimental colitis (15-17). IL-1ß production was significantly increased in active UC and CD; in addition, there was significant correlation between IL-1ß and disease activity (2, 18, 19). Elevated serum, stool and lamina propria cell levels of TNF-α have been described in children with CD and also compared with diarrheal controls: stool TNF-α concentration was significantly increased in children with active

CD and UC (20, 21). Inflammatory bowel disease is associated with evidence of T cell activation; expression of sIL-2R may be an early marker of such activation (22). IL-2R expression is increased on lamina propria mononuclear cells in patients with CD and is accompanied by increased serum levels of sIL-2R (3, 8, 23). Tissue and serum levels of sIL-2R are somewhat lower in UC than in patients with CD, but remain higher than healthy controls. Studies of serum concentration of most cytokines have yielded conflicting results; most investigators agree that serum levels of IL-2R and IL-6 are increased in active CD but are less regularly increased in UC (19). However, several studies have reported normal tissue level of TNF-mRNA and protein in adults with IBD and experimental colitis (17).

In this study, we obtained increased circulating levels of TNF-α, IL-1ß and sIL-2R in patients with active UC. Cytokine concentrations have the potential to serve as objective markers of inflammatory activity. Optimal methods of using tissue, serum and stool concentrations of cytokine as indicators of disease activity are still being evaluated, but several appear promising. Tissue concentration of some cytokines correlate with disease activity because they are dramatically increased with active inflammation and barely detectable in inactive UC and normal controls. Tissue levels of IL-1, sIL-2R, IL-6 and IL-8 correlate with endoscopic and histological evidence of inflammation in UC but are somewhat less reliable in CD because of increased production of these cytokines in inactive tissue in some studies (1). We found significant correlation between disease activity and TNF-α, IL-1ß, sIL-2R levels in patients with active UC.

Our study shows that, TNF-α, IL-1ß, and sIL-2R may play an important role in the pathogenesis of IBD and these cytokine levels are related with disease activity and correlated acute phase responses. We conclude that measurement of serum TNF-α, IL-1ß and sIL-2R concentrations may provide a simple way to monitor disease activity in UC.

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