Tumor necrosis factor-alpha serum level in assessment of disease activity in inflammatory bowel diseases

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ABSTRACT

Aim To investigate an influence of the concentration of proinflammatory cytokines tumor necrosis factor-alpha (TNF-α) in serum on the activity of inflammatory bowel disease (IBD).

Methods The IBD patients of both genders (n=60) were divided in two equal groups, ulcerative colitis (UC) and Crohn’s disease (CD). Based on the result of activity index each group was subdivided in two subgroups: active and inactive phase of the disease. Age and gender matched apparently healthy individuals (n=30) involved in the control group. Serum TNF-α concentration was determined by enzyme linked immune-adsorbent assay (ELISA).

Results The significant difference (Mann-Whitney Test) in serum TNF-α level was found between healthy controls 28.86 pg/ml (28.74 – 29.19 pg/ml) and CD patients (29.47 pg/ml (29.1 – 29.77 pg/ml) (p < 0.05) and UC patients 29.34 pg/ml (29.14 – 29.71 pg/ml) (p < 0.05) respectively. Serum TNF-α level in patients with CD was higher compared to serum TNF-α level in patients with UC, but the difference was not significant (p > 0.05). There were no significant differences in serum TNF-α concentrations either in CD or UC patients related to the phase of disease activity: active CD 29.53 pg/ml (29.20 – 29.90 pg/ml) vs inactive CD 29.26 pg/ml (29.15 – 29.53 pg/ml); active UC 29.53 pg/ml (29.32 – 29.85 pg/ml) vs inactive UC 29.26 pg/ml (29.10 – 29.63 pg/ml).

Conclusions Since there were no differences in serum TNF-α concentrations related to the disease activity we consider that TNF-α is not an adequate serum biomarker for an assessment of the disease activity in patients with IBD.

Key words: TNF-α, ulcerative colitis, Crohn’s disease
INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic and chronic inflammatory disease of gastrointestinal tract (GIT). Ulcerative colitis (UC) and Crohn’s disease (CD) are two major types of IBD. Ulcerative colitis is characterized by inflammation of mucosa of large bowel, while CD may affect any part of GIT and is characterized by transmural inflammation, fissuring and skip lesion (1). Inflammatory disorder in IBD is resulting from an imbalance between luminal bacterial flora and the immune system due to genetic and environmental factors. The chronic immune response in IBD occurred due to an inappropriate secretion of proinflammatory cytokines in response to initial stimulating events (2). In both UC and CD, activated CD4+ T cells in the lamina propria and peripheral blood secrete inflammatory cytokines (1). CD4+ T cells have two major types: Th1 cells (interferon γ, TNF-alpha) and Th2 cells (IL-4, IL-5, IL-13). Th1 cells appear to induce transmural granulomatous inflammation that resembles CD, and Th2 cells (IL-4, IL-5, IL-13) appear to induce superficial mucosal inflammation resembling UC (1). Tumor necrosis factor-alpha (TNF-α), also known as cachectin, is a pro-inflammatory cytokine largely produced by extravascular effector cells, principally monocytes and macrophages (3). It functions as a multipotent modulator of immune response and further acts as a potent pyrogen. TNF-alpha circulates throughout the body responding to invasive 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 (4). TNF-alpha also inhibits lipoprotein lipase activity and induces a state of anorexia and wasting similar to that observed in chronic neoplastic and infectious diseases (5). It can also increase cell permeability, resulting in impairment of barrier function and edema formation (6). The TNF-α plays a central role in mucosal inflammation, and is elevated in the gastrointestinal tract of some forms of inflammatory colitis (7). Recently, a growing body of information has pointed to a significant importance for TNF-α in the pathogenesis of inflammatory bowel disease (IBD), and anti-TNF agents have proven to be effective treatment of some individuals (1, 8). Previous findings support the hypothesis that proinflammatory cytokines are pivotal in the amplification of intestinal inflammation. TNF-alpha, IL-6 and IL-1β induce the increased secretion of other cytokines, and are themselves released by immune cells in response to cytokine stimulation. As proinflammatory mediators TNF-α, IL-6 and IL-1β have a central role in the complex cytokine network (9). The significance of increased IL-1β secretion in the IBD has been well documented (10). In contrast, the studies of TNF-α in IBD have resulted in conflicting findings. In one study serum TNF-α level was increased (11), while other investigation could not confirm these findings (12).

The aim of this study was to estimate if the serum TNF-α level could be used in the assessment of the disease activity in IBD.

PATIENTS AND METHODS

The study included 60 IBD patients of both genders, 30 ulcerative colitis (UC) and 30 Crohn’s disease (CD) patients. Diagnosis of IBD was determined by a gastroenterologist based on anamnesis, clinical findings, laboratory tests, endoscopy with histopathology and radiological examinations in the respective health institutions. Based on UC activity index (UCAI) defined by Seo et al. (13) and activity index (AI) for CD patients defined by Van Hees et al. (14) studied groups were divided into subgroups of those displaying the active and inactive phases of the disease. Control group (n=30) represented age and gender-matched, apparently healthy individuals. These subjects were recruited from volunteers who had absence of clinical and biochemical detection of inflammatory bowel disease or illness that can affect the observed parameters. All subjects involved in the study went through detailed anamnestic questionnaire, medical history, physical examination and standard laboratory analyses. Standard laboratory analyses include blood erythrocyte, leukocyte and platelets, differential blood count, hemoglobin concentration, hematocrit, erythrocyte sedimentation rate, serum proteins, fibrinogen concentrations and glucose concentrations.
Written informed consents were obtained from all subjects. The study was carried out at the Institute for Clinical Immunology of the University Clinical Center in Sarajevo, with an approval from the Ethics Committee of the School of Medicine of the Sarajevo University. Investigations were carried out in accordance with the Declaration of Helsinki as revised in 2000.

Blood samples for serum TNF-alpha levels measurement were taken from the cubital vein and maintained at room temperature for 30 minutes allowing for spontaneous coagulation. After the coagulation the samples were centrifuged at 3000g for 15 min, separated and frozen at -20°C until the analysis was performed.

Serum TNF-alpha concentration was measured by enzyme-linked immunosorbent assay (ELISA) method (R&D Systems; Quantikine, Inc, USA). The lower limit of detection of this assay was 15.6 pg/mL.

The Shapiro-Wilk test of normality was used to test the distribution of variables. Since all variables were skewed they were presented as median and interquartile ranges. The difference in values of tested parameters was assessed by Kruskal-Wallis test. Afterwards, Mann-Whitney U-test was used to compare differences between two groups. A p value of < 0.05 was considered statistically significant.

**RESULTS**

Serum TNF-alpha level was significantly higher (p < 0.05) both in Crohn’s disease, 29.47 pg/ml (29.15 – 29.77 pg/ml) and in ulcerative colitis patients, 29.34 pg/ml (29.14 – 29.71 pg/ml) than in healthy controls, 28.86 pg/ml (28.74 – 29.19 pg/ml). Serum TNF-alpha level in Crohn’s disease patients was higher compared to serum TNF-alpha level in ulcerative colitis patients, but the difference was not significant (p > 0.05) (Figure 1).

The median and interquartile ranges of TNF-alpha serum level in active CD patients of 29.53 pg/ml (29.20 – 29.90 pg/ml) was higher in comparison with inactive CD patients, 29.26 pg/ml (29.15 – 29.53 pg/ml), but the difference was not statistically significant (p > 0.05). Serum TNF-alpha concentration of active CD patients of 29.53 pg/ml (29.20 – 29.90 pg/ml) showed significant difference (p < 0.05) compared to healthy controls, 28.86 pg/ml (28.74 – 29.19 pg/ml). Comparison of the serum TNF-alpha level of inactive CD patients, 29.26 pg/ml (29.15 – 29.53 pg/ml) with healthy controls showed a significant difference (p < 0.05) (Figure 2).

No significant difference (p < 0.05) was observed between active UC patients, 29.32 pg/ml (29.32 – 29.85 pg/ml) and inactive UC patients, 29.26 pg/ml (29.10 – 29.63 pg/ml). A significant difference (p < 0.05) in serum TNF-alpha level was found when active UC patients, 29.53
pg/ml (29.32 – 29.85 pg/ml), were compared
with healthy controls, 28.86 pg/ml (28.74 –
29.19 pg/ml), as well as inactive UC patients,
29.26 pg/ml (29.10 – 29.63 pg/ml) and healthy
controls (p < 0.05) (Figure 3).

DISCUSSION

The results of previous studies of the TNF-α and
other proinflammatory cytokines (IL-1β, IL-8)
level in plasma, stool and intestinal mucosa in
IBD patients were contradictory (9,12,15). Some
authors (2,11,15,16) reported a significant incre-
ase in serum TNF-α level in IBD patients,
and the others did not find any statistically significant
difference in the serum TNF-α concentration
between patients with ulcerative colitis, Crohn’s
disease and healthy controls (9,12). The results of
our study showed that the serum TNF-α level was
significantly higher in IBD patients compared to
the healthy controls. The observed rise of TNF-α
serum level is in accordance with results of Mae-
da et al. (17) who reported significant difference
of serum TNF-α level in patients with IBD com-
pared to the healthy controls.

In this study there was no statistically significant
difference in serum TNF-alpha level between
two IBD groups although the level was higher in
CD patients than in UC patients. No differences
were found in serum TNF-α level between active
and inactive phase of the disease both in CD pa-
ients and UC patients. However, the comparision
of active CD or UC patients with healthy controls
has shown a statistically significant difference in
serum TNF-α level. Moreover, a significant dif-
ference in serum TNF-alpha level between healthy
controls and in inactive CD patients, as well
as inactive UC patients was found. Our results
are not completely in accordance with results of
Murch et al. (11) who observed a significant rise
of serum TNF-α above control values in CD and
UC patients in the active phase of the disease but
not in the inactive phase.

In IBD patients TNF-α level was measured not
only in serum but also in stool (15), intestinal
specimens (18) and isolated lamina propria mo-
nonuclear cells (2). Braegger et al. (15) reported
that children with IBD have a significant increase
in stool TNF-α concentration compared to healthy
children. They also found significantly higher
concentrations of TNF-α in the stool of children
in the active phase both of Crohn’s disease and
ulcerative colitis compared to the control group,
which is in accordance with our results related to
serum TNF-α. The stool TNF-α concentration in
inactive phase of the disease, due to surgery or
treatment with steroids, fell down to the control
level. These results suggest that measurements
of stool TNF-α concentration may provide a sim-
ple way to monitor the disease activity in IBD.

Reinecker et al. (2) studied the secretion patterns
of proinflammatory cytokines (TNF-α, IL-6 and
IL-1β) from isolated lamina propria mononucle-
ar cells (LPMNC) isolated from colonic biopsi-
es from CD and UC patients. They showed that
the spontaneous secretion of TNF-α by isolated
LPMNC was very low in normal mucosa, as well
as noninvolved IBD mucosa. On the contrary,
the spontaneous secretion of TNF-α by LPMNC
obtained from involved CD mucosa or UC mu-
cosa was elevated in comparison with the control
group. These authors suggested that determinati-
on of proinflammatory cytokine secretion by iso-
lated LPMNC from colonoscopic biopsies may
be a sensitive method for monitoring severity of
mucosal inflammation in IBD patients.

Although our findings suggest that serum TNF-α
level may not be associated with the disease ac-
tivity in IBD patients, additional studies may be
necessary. The reason we have not found a signi-
ficant difference in serum TNF-α related to the
disease activity may be due to the limit size of our
It is also possible that IBD patients might be exquisitely sensitive to low TNF-α levels or that we missed elevation in TNF-α during our sampling, because of diurnal variation in serum TNF-α level. The serum TNF-α level in our patients could be also influenced by prednisone that is a potent inhibitor of TNF-α production. It is possible that TNF-α may be unstable during storage because some authors report that TNF-α is unlikely to degrade during frozen storage.

In conclusion, due to absence of significant differences in serum TNF-α level among IBD patients with active and inactive phase of the disease, serum TNF-α level cannot be recommended for assessment of the disease activity in IBD.

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REFERENCES
Serumska koncentracija faktora nekroze tumora alfa u procjeni aktivnosti inflamatornih bolesti crijeva

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SAŽETAK

Cilj Ispitati utjecaj koncentracije proupalnog citokina, faktora nekroze tumora alfa (TNF-α) u serumu na aktivnost inflamatornih bolesti crijeva (IBC).

Metode U studiju je bilo uključeno 60 pacijenata, oba spola, koji su podijeljeni u dvije jednakobrojne grupe, s ulceroznim kolitisom (UK) i Crohnovom bolešću (CB). Svaka je grupa, na osnovu aktivnosti bolesti, bila podijeljena u dvije podgrupe, aktivna i inaktivna faza. Aktivnost bolesti utvrđena je na osnovu indeksa aktivnosti bolesti. Kontrolnu grupu (n=30) činili su zdravi ispitanici, odgovarajuće dobi i spola. Koncentracija TNF-α u serumu određivana je enzimskim imuno-vezujućim testom (ELISA).

Rezultati Utvrđena je značajna razlika (Mann-Whitney Test) u koncentraciji TNF-α u serumu zdravih ispitanika, 28.86 pg/ml (28,74 – 29,19 pg/ml) i bolesnika s Crohnovom bolešću (CB), 29,47 pg/ml (29,15 – 29,77 pg/ml) (p < 0,05) i ulceroznim kolitisom 29,34 pg/ml (29,14 – 29,71 pg/ml) (p < 0,05). Koncentracija TNF-α u serumu bolesnika CB-a bila je veća u odnosu na koncentraciju TNF-α u serumu bolesnika UK-a, ali utvrđena razlika nije bila statistički značajna (p > 0,05). Ni kod bolesnika CB-a, ni kod bolesnika UC-a, nije utvrđena statistički značajna razlika u koncentraciji TNF-α u serumu u odnosu na aktivnost bolesti: aktivni CB 29,53 pg/ml (29,20 – 29,90 pg/ml) vs. inaktivni CB 29,26 pg/ml (29,15 – 29,53 pg/ml); aktivni UK 29,53 pg/ml (29,32 – 29,85 pg/ml) vs. inaktivni UK 29,26 pg/ml (29,10 – 29,63 pg/ml).

Zaključak Budući da se koncentracija TNF-α u serumu nije značajno mijenjala u ovisnosti od aktivnosti bolesti, TNF-α nije adekvatan serumski biomarker u procjeni aktivnosti bolesti kod bolesnika s IBC-om.

Ključne riječi: TNF-α, ulcerozni kolitis, Crohnova bolešć.