Fecal and Circulating TNF-α Measurement for Assessment of Disease Activity in Inflammatory Bowel Disease

Dr. Ahmet DOBRUCALI, Dr. Murat TUNCER, Dr. Yeşim TUNCALI ERALP, Dr. Kadir BAL, Dr. İbrahim YURDAKUL, Dr. Mehmet ALTIN, Dr. İsmail DİNÇ, Dr. Fatma AŞCIOĞLU

Özet: *İNFLAMATUAR BARSAK HASTALIĞINDA, SERUM VE DIŞKIDA TÜMÖR NEKROZ FAKTÖRÜ ÖLÇÜMÜ HASTALIK AKTİVİTESİNİ GÖSTEREN BİR YÖNTEM OLARAK KULLANILABİLİR Mİ?*

İnflamatuar barsak hastalığı (İBH) olan hastalarda hastalığın aktivitesinin belirlenmesi genelde güçtür. Endoskopik ve radyolojik yöntemler zaman alıcı ve özel ekip gerektiren işlemlerdir. Tümör nekroz faktörü-alfa (TNF-α) inflamasyon ve sepsise cevap olarak mononükleer hücrelerden salgılanan bir sitokindir. Dışkıda TNF-α ölçümü, İBH'da barsaktaki inflamasyonun şiddetini gösteren bir yöntem olarak kullanılabilir. Çalışmamızda İBH ve İBH dışında ishali olan hastalarda ve normal şahıslarda dışkı ve serum TNF-α seviyeleri ölçülerek hastalık aktivitesi ile ilişkisi incelendi. Aktif dönemdeki İBH'lı hastaların (13 hasta, 7 ülserli kolit, 6 Crohn) dışkı TNF-α düzeyleri İBH dışında ishali olan hastaların (7 hasta) ve normal şahısların dışkı TNF-α düzeylerine göre anlamlı ölçüde yüksek bulundu, (sırasıyla 294±229 pg/g, 3,4±1,3 pg/g ve 2,8±1,9 pg/g), (p<0.001). Tedavi sonrasında, İBH olan hastalarda saptanmış olan bu yüksek değerlerin kontrol grubu ile benzer seviyeye düştüğü gözlendi (2,2±1,5 pg/g). Serum TNF-α düzeylerinde ise hastalığın aktivitesi ile ilişki saptanmadı (aktif İBH'da 2,1±2 pg/ml, tedavi edilmiş İBH'da 2,7±1,9 pg/ml, İBH dışında ishali olanlarda 2,3±1,8 pg/ml ve normal şahıslarda 2,5±2,3 pg/ml) (p> 0.05).

Çalışmamızın sonuçları, İBH'da dışkıda TNF-α ölçümünün hastalık aktivitesinin tayininde ve tedaviye cevabın izlenmesinde kullanılabilecek bir yöntem olabileceğini düşündürmektedir.

Anahtar kelimeler: İnflamatuar barsak hastaları, tümör nekroz faktörü.

Summary: *Measurement of disease activity in patients with inflammatory bowel disease (IBD) is difficult. The best available methods such as endoscopy and radiology are time consuming and spesific personnel requiring. Tumor necrosis factor alpha (TNF-α) is a cytokine released by mononuclear cells in response to inflammation and sepsis. It may be possible to use TNF-α concentration in stool as a marker of disease activity in IBD. In our study, we measured TNF-α concentrations in stool and blood samples from normal persons (n= 9), patients with active IBD (n= 13, 7 with crohn's disease and 6 with ulcerative colitis) and patients with diarrhea which caused by other reasons such as infection and gluten sensitivity (n= 7). The concentration of TNF-α found in the stools of the patients with active IBD were significantly greater than those detected in the stools of normal perons and patients with diarrhea, (294±229 pg/g, 2,8±1,9 pg/g and 3,4±1,3 pg/g respectively), (p<0,001). After the treatment, high concentrations of stool TNF-α were decreased to similar levels measured as in the controls (2,2±1,5 pg/g). There was no correlation between the serum concentrations of TNF-α and disease activity, (2,1±2 pg/ml in active IBD, 2,7±1,9 pg/ml in treated IBD, 2,3±1,8 pg/ml in patients with diarrhea and 2,5±2,3 pg/ml in controls).*

The results of our study suggest that measurement of stoo TNF-α concentration may provide a simple way to monitor disease activity in inflammatory bowel disease.

Key words: Inflammatory bowel disease, tumor necrosis factor.

There is no simple and reliable method to determine disease activity in patients with inflammatory bowel disease (IBD). Acute phase reactants such as CRP and erythrocyte sedimantation rate (ESR) do not always correlate with disease activity and pathologic findings. Therefore, it's mandatory to evaluate the clinical status, together with the laboratory datas in order to assess the severity of the disease. Colonoscopy, contrast barium enema and indium labeled granulocyte scintigraphy are com-

I. Ü. Cerrahpaşa Tıp Fakültesi İç Hastalıkları, Gastroenteroloji, Merkez Araştırma Laboratuvarı, Anabilim Dalı.

Table I: Abbreviations: CRP: C reactive protein, Sed.: Sedimantation TNF-α; Tumor necrosis factor.

Patients		No	Stool TNF-α (pg/g)	Serum TNF-α (pg/ml)	Sed. mm/h	CRP mg/ml
Small bowel Crohn's disease	Active	3	20,6 ± 23	2,7 ± 2,2	55,3 ± 6,6	11 ± 3
	Inactive		2,13 ± 0,1	3,3 ± 2,3	19,3 ± 6,8	5,6 ± 1,4
Small and large bowel Crohn's disease	Active	3	506 ± 438	2,11 ± 1,8	61,6 ± 1,9	17,3 ± 5
	Inactive		2,76 ± 1	2,61 ± 1,9	17 ± 2,6	3 ± 1
Ulcerative colitis	Active	7	219 ± 200	1,7 ± 2,3	55,4 ± 11	14,5 ± 5
	Inactive		1,8 ± 2,3	2,13 ± 8	15,7 ± 4	4,3 ± 2
All inflammatory bowel disease	Active	13	294 ± 229	2,1 ± 2	56,8 ± 10	14,3 ± 4
	Inactive		2,2 ± 1,5	2,7 ± 1,9	16,9 ± 4,6	4 ± 1,7
Diarrhoea	Active	7	3,4 ± 1,3	2,3 ± 1,8	36,7 ± 15	7,1 ± 3,5
	Inactive		1,8 ± 0,9	1,4 ± 1,8	12,4 ± 3,6	2,5 ± 1,1
Healthy controls		9	2,8 ± 1,9	2,5 ± 2,3	9,4 ± 4	1,8 ± 1,1

plex and time consuming diagnostic methods and require trained personnel.

It has been known that there is an increase in the concentration of inflammatory cells within the inflamed colonic mucosa of patients with ulcerative colitis (1,2). Moreover raised circulatory levels of TNF-α have been found in patients with active ulcerative colitis (UC) and Crohn's disease and it has been suggested that, the cytokine could be secreted by the inflamatory cells in the involved colonic mucosa(3).

This study aims to determine the correlation between serum and fecal TNF-α levels and disease activity in patients with inflammatory bowel disease.

MATERIALS and METHOD

29 patients were included in this study; 20 had diarrhea (7 patients had ulcerative colitis, 6 had Crohn's disease and the remaining 7 had different clinical disorders which appeared with diarrhea) and 9 normal individuals were taken as controls. In the IBD group, the male to female ratio was 6:7, the mean age was 33.3±15. Diagnosis, activation and recovery crytreia of Crohn's disease and UC had been determined on the basis of history, physical examination, radiologic, histologic and microbiologic findings. In the non-IBD group, the male to female ratio was 4:3, the mean age was 34±18 and the cause of diarrhea was found out to be enteropathogenic E. coli in 2, shigella in 2, salmonella in 1, amoebic colitis in 1 and non-tropical sprue in the remaining 1 patient. The control group constituted of 5 male and 4 female patients, the mean age was 43±20.

Serum and stool samples for ESR, CRP and TNF-α were taken twice from the IBD group and once from the non-IBD group. For serum TNF-α measurement, venous blood samples were centrifuged at 2000 rpm, supernatants were collected and stored at -70°C. For fecal TNF-α determinations, 30 gr stool samples were obtained in steril containers from each patient. After preparation with the same amount of isotonic fluid buffered in sterile phosphate solution, the stool samples were certrifuged with 15000 rpm for 30 minutes and supernatants were stored at -70°C. TNF-α was measured by ELISA (TNF-α ELISA kit, Cistron Biotechnology). TNF-α levels were expressed as pg/ml in serum and as pg/gr in stool. Mann-Whitney U-test was used for statistical analysis.

RESULTS

Fecal TNF-α levels in patients with active IBD were significantly greater than from controls

(294±229 pg/gr and 2.8±1.9 pg/gr respectively, (p<0.001). There was no difference in circulatory TNF-α levels between the two groups (2,1±2 pg/ml and 2,5±2,3 pg/ml respectively, p>0.05). Following treatment in all patients with IBD, fecal TNF-α levels decreased significantly to 2.2±1.5 pg/gr (p<0.001). Serum TNF-α levels did not display a meaningful difference before and after treatment; estimated values were 2.1 ± 2 pg/ml and 2.7±1.9 pg/ml respectively (p>0.05) (Table I).

The mean fecal and circulatory TNF-α levels in patients with active Crohn's disease were 264±244 pg/gr and 2.4±1.8 pg/ml, while the same parameters in patients with ulcerative colitis were measured as, 219±200 pg/gr and 1.7±2.3 pg/ml respectively, (p>0.05). Fecal TNF-α levels in patients with active Crohn's disease were significantly greater with both small and large bowel involvement, compared with only small bowel involvement; the mean estimated values were 506±438 pg/gr and 20.6±23 pg/gr respectively, (p<0.001). Circulatory TNF-α levels were not statistically different in either group (2.1±1,8 pg/ml and 2.7±2,2 pg/ml respectively, p>0.05).

The mean serum and fecal TNF-α concentrations in the-non-IBD group were not statistically different compared with controls. Fecal levels were estimated as 3.4±1.3 pg/gr and 2.8±1.9 pg/gr, while circulatory concentrations were measured as, 2.3±1.8 pg/ml and 2.5±2.3 pg/ml, respectively (p>0,05) (Table I).

Serum CRP and ESR values were significantly higher in both IBD and non-IBD groups, compared with controls (p<0.01). Nevertheless, IBD group displayed considerably greater levels than the non-IBD group (p<0.01). However, following treatment, the CRP and ESR values were not statistically different in either group (Table I).

DISCUSSION

TNF-α a cytokine secreted by the activated macrophages in response to inflammation and sepsis, has important functions in the inflammatory process (2,4,5). Although it is shown to be produced by the Kuppfer cells in the liver, cerebral astrocytes and microglial cells, TNF-α is mainly secreted by the activated macrophages and monocytes(6,7). It induces a strong cathabolic effect by increasing lipolysis and glycolysis(8). Moreover, by displaying an autocrine manner, it can stimulate the differentiation of other macrophages into epitheloid cells(9). In experiments conducted with animal models, chronic exposure to TNF-α has been shown to cause anorexia, weakness, weight loss, a discrepancy in nitrogen balance, deterioration in hemopoetic capacity, decrease in the life span of red blood cells and also to serve as a mediator in endogenous pyrogenic activity (5,10). Furthermore, it has been suggested that the TNF-α has a deleterious effect on growth, by interfering with the collagen metabolism (11,12). Further discoveries on TNF-α, has led to an increase in subsequent trials. In animal models, it has been shown that TNF-α creates a cytopathic effect in the colonic mucosa (10), probably by giving way to multifocal microinfarcts, mediated by platelet activating factor (PAF) and interleukines(13). In mice, systemic exposure to TNF-α has caused intestinal hemorrhage and necrosis. On the basis of this data, it has been suggested that the main pathological disorder might be the deterioration of the vascular structure, caused by an aggrevation in the procoagulant activity, neutrophilic adhesion and stimulation of mediator secretion, as related above(14). Experiments have shown that, TNF-α is produced by activated macrophages and T cells in the intestinal mucosa. This observation reminds that the increased circulatory and fecal TNF-α levels in active IBD originates from the activated inflammatory cells(9). However, in rats with experimentally induced colitis, no change was reported in systemic TNF-α concentrations. This observation suggests that other mediators of inflammation, having similar biologic activities, such as LTB-4 and interleukines could have a role in the inflammatory porcess(5). In another study, the concurrence of local and systemic findings together with an augmentation in the TNF-α receptor quantity in IBD has been observed, which confirms the role of TNF-α as a mediator in the inflammatory response(15).

There are contradictory results in human models. In a study by Maeda and collegues, serum TNF-α levels in 33 patients with IBD were measured. Increased serum TNF-α levels were obtained in 4 of the 5 patients with Crohn's disease and 9 out of 28 patients with ulcerative col-

itis. Furthermore, it was shown that in all patients with active Crohn and in 8 of 11 patients with active ulcerative colitis, serum TNF-α levels increased significantly, emphasizing the possible role of TNF-a in the pathogenesis of IBD (16). In another study conducted on 45 patients, consisting of 18 with ulcerative colitis and 27 with Crohn's disease, there was no significant difference in serum TNF-a levels, among patient and control groups(17). In a similar trial with 20 active Crohn and 10 active ulcerative colitis patients, increased serum TNF-a levels were obtained in 21 of all patients. However, compared with controls, the increase was not significant. Particularly, circulatory TNF-a concentrations in patients with ulcerative colitis did not corralate with the increased levels in those with active Crohn's disease. Mucosal TNF-a levels in corresponding biopsy specimens showed a slight increase in only 3 patients. Finally, it was concluded that, TNF-a is not an important mediator in the pathogenesis of IBD. However, in this study fecal TNF-a levels were not determined (18).

In a study by Murch and coll., although in low concentrations, serum TNF-a levels in patients with active ulcerative colitis and Crohn patients with colonic involvement were estimated to be significantly higher than controls. No difference was noted in the non-IBD group. In the same trial, serum TNF-a levels were reported to be higher in Crohn patients with both small and large intestinal involvement, than those with only small intestinal disease. In addition, the ESR and CRP values were in corralation with the severity of inflammation in both IBD and non-IBD groups. These investigators have claimed that, the increase in serum TNF-a levels in IBD probably originates from the activated macrophages in the colonic mucosa and a possible explanation for the low levels is the shortness of circulatory half-life and dilution in the body fluids(6). It has been shown that, the inflammatory cell concentration in the colonic mucosa is significantly higher than controls; 500-1200/1000000 and 60-580/1000000, respectively. It has also been stated that, serum TNF-a levels do not corralate with the secretory cell concentration(9). As mentioned above, this condition may have arised form short circulatory half life and dilution. Nevertheless, the results of this survey seem to support the idea that TNF-a is an important mediator of intestinal inflammation.

Taking into consideration that serum TNF-a concentrations yield contradictory results, it has been proposed that fecal TNF-a measurement might give a reliable results on disease activity. It is stated that two mechanisms might be responsible for fecal TNF-a secretion. It is possibly secreted by the activated mononuclear cells passed into the stool from the ulcerative lining or the locally produced TNF-a passes into the lumen from the lamina propria by epithelial loss. In addition, colonic bacteria and enzymes would cause further degradation of TNF-a in the lumen; which could bring out a reasonable explanation for the increased fecal TNF-a levels in Crohn patients with both small and large bowel involvement(19). Furthermore, studies have proven that, histiocytes and macrophages in the small and large intestine have different morphological and functional features(10). In patients with colonic IBD, the normal colonic cells are replaced totally by a different kind of macrophage group, which can be recovered completely by treatment(21). The variance in macrophage cell lines may also be responsible for the increased fecal TNF-a levels in Crohn's disease with generalized bowel involvement.

In our study, a significant increase in fecal TNF-a levels was obtained in patients with active inflammatory bowel disease, than those of the non-IBD group and controls ($p<0.001$). (Table I). Serum TNF-a levels did not show disparity in either group, when compared with controls. Following treatment, fecal TNF-a concentrations returned to baseline levels. Serum TNF-a levels did not display a significant difference before and after treatment in IBD or non-IBD groups. No difference in fecal and circulatory TNF-a concentrations was found among patients with active ulcerative colitis and crohn's disease. Although fecal TNF-a levels corralated well with colonic involvement in Crohn patients, corresponding serum TNF-a levels were not statistically different in either group. In two Crohn's disease patients with only small bowel involvement, fecal TNF-a concentrations were estimated as 6 and 8 pg/gr, which were not quite different than values

obtained from patients in the non-IBD group. Fecal TNF-a levels also showed an increase in the non-IBD group; however this ratio is quite low with respect to the IBD group(max. 6 pg/gr). Therefore, it can be concluded that, low fecal TNF-a levels do not exclude active disease in Crohn patients with only small bowel involvement. In those patients, ESR, CRP and ileal fluid TNF-a (during colonoscopy) measurements may provide information about the severity of disease. In this study ESR and CRP levels were shown to increase in all patients with diarrhea, with the IBD group displaying a significantly prominent increase than the non-IBD group (Table I). Normal circulatory TNF-a levels showing discordance with the incrased fecal TNF-a levels in patients with active disease, suggests that serum TNF-a measurement is not a reliable method in assesment of disease activity.

The results of our study imply that, in patients with ulcerative colitis and Crohn's disease with colonic involvement, fecal TNF-a measurement is a sensitive and even more specific method in assessing the severity of the inflammatory response, compared with ESR and CRP values. Nevertheless, besides being an expensive and time consuming method and the requirement of trained personnel restricts the availability of this method in clinical practise.

REFERENCES

1. Kormbluth A, Salomon P, Sachar DB, Jewell DP. Crohn Disease and Ulcertaive colitis. In: Gastrorintestinal Diseases Ed: Sleisenger and Fordtran. W.B. Saunders Company, London, 1993 pp. 1270-1327.
2. Oppenheim JJ, Franchis WR, Faltynek C. Cytokines. In: Basic and Clinical Immunology Ed: Daniel PS, Abba IT. Lange Medical Book Company, California, 1991, pp. 78-100.
3. Mursh SH, Lamkin VA, Savage MO, Walker Smith JA, MacDonald TT. Serum concentrations for tumor necrosis factor-α in childhood chronic inflammatory bowel disease. Gut, 1991; 32: 913-17.
4. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumour necrosis factor. Lancet, 1989; 1:1122-5.
5. Mack DR, Lau AS, Sherman PM. Systemic tumor necrosis factor-alpha production in experimental colitis. Dig. Dis. and Scien. 1992; (37), 11; 1738-45.
6. Murch SH, Lamkin VA, Savage MD, Walker JA, Mac Donald TT. Serum concentrations of tumour necrosis factor a in childhood chronic inflammatory bowel disease. Gut, 1991; 32: 913-17.
7. Beutler B. The presence of cachectin/tumor necrosis factor in human disease states. Am J Med. 1988; 85: 278-80.
8. Fong Y, Lowry SF, Cerami A. Cachectin/TNF: a macrophage protein that induces cachexia and shock. J Paerenter Enter Nutr. 1988; 12: 72-7 (Abst).
9. MacDonald TT, Hutchings P, Choy MY, Murch S, Cooke A. Tumour necrosis factor-alpha and interferon-gamma production measured at the single cell level in normal and inflamed human intestine. Clin Exp Immunol. 1990; 81: 301-305.
10. Tracye KJ, Wei H, Manogue KR. Cachectine (tumor necrosis factor) induces cachexia, anemia and inflammation. J Exp Med. 1988; 167: 1211-27 (Abstr).
11. Bertolini DR, Nedwin GE, Bringham IS, Smith DD, Mundy GR. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumor necrosis factors, Nature, 1986; 319: 516-8 (Abstr).
12. Buck M, Rippe R, Chojker M, Brenner DA. Inhibition of collagen gene expression by tumor necrosis factor: molecular mechanisms. Hepatology 1989; 20: 617.
13. Wakefield AJ, Dhillon AP, rowles PM et al. Pathogenesis of Crohn's disease, multifocal gastrointestinal infarction. Lancet, 1989, ii: 1057-62.
14. Sun XM, Hsueh WI. Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet activating factor. J Clin Invest, 1988; 81(5): 1328-31.
15. Bautler BA, Milsark IW, Cerami A. Cachectin/tumor necrosis factor, production, distribution and metabolic fate in vivo. J Immunol, 1985; 135: 3972-3977.
16. Maeda M, Watanabe N, Neda H, Yamauchi N et al. Serum tumor necrosis factor activity in inflammatory bowel disease. Immun Pharm Immun Toxic. 1992; 14(3): 451-61.
17. Hyams JS, Treem WR, Evangeline E, Wyzga N and Moore RE. Tumor necrosis factor-a is not elevated in children with inflammatory bowel diseases. J of Ped Gatst and Nutr. 1991; 12: 233-236.
18. Nielsen OH, Bnynkov J, Bendtzen KL. Circulating and mucosal concentrations of tumour necrosis factor and inhibitors in chronic inflammatory bowel disease. Dan Med 1998; 40(2): 247-91 (Abstr).
19. Braegger CP, Nicholls S, Murch SH, Stehenes S, MacDonald TT. Tumor necrosis factor alpha in stool as a marker of intestinal inflammation. Lancet 1992; 339: 89-91.
20. Selby WS, Poulter LW, Hobbs S et al. Heterogenity of HLA-DR positive histiocytes in human intestinal lamina propria: a combined histochemical and immunohistological analysis. J Clin Pathol. 1983; 36: 379-84.
21. Choy MY, Walker JA, Williams CB, McDonald TT. differential expression of CD25 (interleokin receptor) on lamina propria T cells and macrophages in the intestinal lesions in Crohn's disease and ulcerative colitis. Gut 1990; 31: 1365-70.