Serum transforming growth factor beta 1 levels in multiple myeloma patients

Hasan Şenol COŞKUN[1], Osman İLHAN[2], Muhit ÖZCAN[2], Selime AYAZ[3], Klara DALVA[2], Celalettin ÜSTÜN[2], Mutlu ARAT[2]

[1] Department of Medical Oncology, Süleyman Demirel University School of Medicine, Isparta,
[2] Department of Haematology, Ankara University School of Medicine,
[3] Haematology Laboratory, Yüksek İhtisas Hospital, Ankara, TURKEY

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ABSTRACT

Cytokinesis plays an important role in the etiology of multiple myeloma. The transforming growth factor (TGF) beta 1 levels in 82 sera from 60 patients with multiple myeloma were analyzed by ELISA. Fourty one sample were obtained before treatment from newly diagnosed patients, 22 after treatment from the same patients and 19 from relapsed/refractory patients. Serum median TGF level of newly diagnosed patients was 769.5 ng/mL (126-1853), and the relapsed/refractory patients had similar levels. TGF levels after chemotherapy were not different between patients that reached plateau phase and those who remained refractory. We found a negative correlation between TGF and C-reactive protein and blood urea nitrogen and a positive correlation between TGF and hemoglobin level in newly diagnosed patients. After treatment, it was determined that TGF levels at diagnosis were higher in patients who reached plateau phase than in the refractory patients. Elevated serum TGF concentration at diagnosis in multiple myeloma patients may be a favorable predictor of response.

Key Words: Multiple myeloma, TGF-beta, Survival.

ÖZET

Multipl miyeloma hastalarında serum transforming growth faktör beta 1 seviyesi

Sitokinler multipl miyeloma etyolojisinde önemli bir rol oynar. Altmış hastadan elde edilen 82 örnekte serum transforming growth faktör (TGF) beta 1 seviyesi çalışıldı. Kırkbir örnek yeni tanı konulan hastalardan tedavi öncesi, 22 örnek tedaviden sonra, 19 örnek ise refrakter relaps hastalardan elde edildi. Yeni tanı konulan hastalarda tedavi uygulama öncesi ortanca serum TGF seviyesi 769.5 ng/mL (126-1853) bulundu. Bu değer relaps ve refrakter hastalarla benzerdi. Kemoterapi sonrası TGF seviyeleri plato safhasına ulaşan veya refrakter kalan hastalarda farklı değildi. Yeni tanı hastalarda TGF ile C-reaktif protein ve kan üre nitrojeni negatif korelasyon, hemoglobin ile pozitif korelasyon bulundu. Tanı anında TGF seviyesi kemoterapi ile plato safhasına ulaşan hastalarda refrakter kalan hastalardan daha yüksekti. Multipl miyelomada tanı anında yüksek serum TGF-beta seviyeleri iyi cevabın bir göstergesi olabilir.

Anahtar Kelimeler: Multipl miyeloma, TGF-beta, Sağkalım.

INTRODUCTION

Multiple myeloma (MM) is a disorder of monoclonal malignant plasma cells. It is associated with production of monoclonal immunoglobulin, painful bone destruction, anemia, hypercalcemia, and renal dysfunction[1,2]. MM is also a rare example of a malignancy in which a role for cytokine has been clearly established. Interleukin (IL)-6 has been proposed as an autocrine and/or paracrine growth factor for MM[3,4]. Other cytokines, such as tumor necrosis factor (TNF) and IL-1, are also involved in mediating bone destruction characteristic of melanoma[5,6].

Transforming growth factor (TGF)-beta1, a 25 kDa homodimer peptide, regulates cell growth, differentiation and the expression of extracellular matrix proteins such as collagen, fibronectin, and proteoglycans. This multipotent modulator has also been implicated in the pathogenesis of a variety of diseases including neoplasm, fibrotic disease and immunoglobulin disorders[7-11]. TGF-beta mRNA was observed in 12/12 of tumor samples in MM patients and in 10/10 of human myeloma cell lines[12]. In some reports TGF is recognized as an inhibitory growth factor in myeloma cell growth. High serum levels of TGF in MM patients have been reported[13]. Urashima's study suggests that TGF-beta is produced in MM patients by both MM cells and bone marrow stromal cells in concordance with tumor cell growth[14]. In this report we studied the correlation between serum TGF levels and clinical course in MM patients receiving chemotherapy.

MATERIALS and METHODS

Patients

Sixty MM patients followed in Ankara University Hospital between December 1996 and March 1998 were enrolled into this study. Eighty-two samples were obtained from these patients. Forty-one sample were obtained from newly diagnosed patients, 22 after treatment of newly diagnosed patients, and 19 from relapsed/refractory patients.

Thirty-four (56.7%) patients were male and, 26 (43.3%) female. Median age was 59 years (range 38-76). Female patients were slightly older than male patients, but the difference was not significant [female median 61 years (range 38-76), male median 57 years (range 40-70)]. Seventeen patients (28.3%) had renal involvement, and 23 (38.3%) had hypercalcemia. Forty-nine (81.7%) patients had more than three lytic bone lesions observed by direct X-ray. Patients' characteristics are shown in Table 1.

Diagnosis and Treatment

Diagnostic criteria included more than 10% of plasma cells in bone marrow aspiration, lytic bone lesion and monoclonal protein in serum or urine. Complete blood count, chemistry analysis, erythrocyte sedimentation rate, bone survey, protein electrophoresis, C-reactive protein (CRP) and beta 2 microglo-

Table 1. Patient characteristics

Patient number	60
Median age (range)	59 (38-76)
Sex	
Female	34 (56.7%)
Male	26 (43.3%)
M-protein	
IgG	39 (65%)
IgA	8 (13.3%)
Light chain	13 (21.7%)
Light chain type	
Lambda	9 (15%)
Kappa	51 (85%)
Stage (durie salmon)	
Stage 1	10 (16.7%)
Stage 2	2 (3.3%)
Stage 3	48 (80%)
Lytic lesion of bone	49 (81.7%)
Renal involvement	17 (28.3%)
Hypercalcemia	23 (38.3%)

bulin (B2M) were studied routinely. Staging was done by Durie-Salmon system. Newly diagnosed patients were treated by melphalan 10 mg/m^2, day, days 1-4 oral and prednisolon 2 mg/kg/day, days 1-4 oral. Vincristine 0.4 mg continuous intravenous (IV) infusion days 1-4, doxorubicin 9 mg/m^2 continuous IV infusion days 1-4, and dexamethasone 40 mg peroral days 1-4, 9-12, 17-20 every three or four weeks were administered to 19 relapsed/refractory patients.

Analysis of TGF-beta

TGF concentration was measured using a TGF-beta ELISA kit (R&D System). Serum was collected before and after chemotherapy and then stored at -70°C until analysis. Sera were activated before analysis with 2.5 N acetic acid/10 M urea (250 mL) and 2.7 N NaOH/1 M HEPES (250 mL). Reference range for TGF-beta was between 34.7 and 63.9 ng/mL with a median of 48.6 ng/mL. Sensitivity of system was 7.1 pg/mL.

Statistics

Comparisons between groups were performed by the Mann-Whitney U test or chi-square test when appropriate. Comparisons of related parameters such as before and after treatment were performed using Wilcoxon test. Correlations between two parameters were estimated by the Spearman rank correlation analysis. Results were considered statistically significant when p values were less than 0.05.

RESULTS

We analyzed 82 sera from 60 patients. Forty-one patients were newly diagnosed and 19 were in the relapsed/refractory group. Twenty-two of 41 newly diagnosed patients were evaluated after treatment. We achieved a plateau phase in 14 patients and eight patients remained refractory.

TGF concentration was measured in newly diagnosed and relapsed/refractory patients as median 708 ng/mL (range 173-1409) and 722 ng/mL (range 126-1853), respectively. Whole results of TGF-beta1 concentration were higher than reference range for using monoclonal antibody (reference range 34.7-63.9 ng/mL; median 48.6).

Concentration of B2M was higher in relapsed/refractory patients than in newly diagnosed patients but CRP concentration was not (Table 2).

Concentration of TGF after treatment was slightly higher in patients who reached a plateau phase in comparison with those who remained refractory, but the difference was not statistically not significant. TGF after treatment was increased in all of the 22 available patients (Table 3). Median pretreatment TGF was 708 ng/mL and median post-treatment TGF was 954 ng/mL in patients who achieved plateau phase and 843 ng/mL in patients who remained refractory. There was no statistically significant difference. B2M con-

Table 2. Serum concentration of TGF-beta1, CRP and B2M in newly diagnosed and relapsed/refractory patients (median and range)

	Untreated patients (n= 41)	Relapsed/ refractory patients (n= 19)	All patients (n= 60)	Reference range
B2M (ng/mL)*	1610 (640-9800)	2800 (1000-20000)	2250 (476-20000)	1010-1710
CRP (mg/L)	11.5 (0.9-188)	18 (0.9-120)	10.9 (0.9-188)	< 5
TGF-beta1 (ng/mL)	708 (173-1409)	722 (126-1853)	769.5 (126-1853)	34.7-63.9

* p= 0.02. B2M: Beta 2 microglobulin, CRP: C-reactive protein, TGF: Transforming growth factor-beta1.

Table 3. Pre-and post-treatment serum concentration in newly diagnosed MM patients (median and range)

		After treatment (n= 22)	
	Before treatment (n= 41)	Plateau phase (n=14)	Refractory (n= 8)
B2M (ng/mL)	1610* (640-9800)	1512** (476-3160)	4200*,** (1721-7980)
CRP (mg/L)	11.5 (0.9-188)	4*** (0.9-243)	11.3*** (3.5-56)
TGF-beta 1 (ng/mL)	708 (173-1409)	954 (154-1378)	843 (188-1170)

* p= 0.0076, ** p= 0.0036, *** p= 0.02. B2M: Beta 2 microglobulin, CRP: C-reactive protein, TGF: Transforming growth factor-beta1.

centration was decreased in plateau phase patients and increased in refractory patients (1512 ng/mL versus 4200 ng/mL respectively p< 0.05). There were no significant differences in CRP concentrations after treatment in refractory patients. CRP concentration was decreased in patients in plateau phase.

We found a negative correlation between TGF and CRP and blood urea nitrogen in newly diagnosed patients. There was a positive correlation between TGF-beta1 concentration and hemoglobin. Correlation analysis is summarized in Table 4.

Concentration of BZM at diagnosis in patients who reached plateau phase after treatment was lower than in refractory patients (median 1517 ng/mL vs. 2730 ng/mL, p= 0.04). CRP and B2M concentration at diagnosis were statistically significantly higher in patients who remained refractory (Table 5).

Table 4. Correlations of TGF concentration with other parameters in newly diagnosed patients

	r	p
B2M	-.213	0.317
CRP	-.380	0.019
Hemoglobin	+.493	0.002
Blood urea nitrogen	-.455	0.007
Creatine	-.246	0.148
Monoclonal protein	-.153	0.464
Erythrocyte sedimentation rate	-.165	0.330
Plasma cell rate in bone marrow	-.258	0.138

B2M: Beta 2 microglobulin, CRP: C reactive protein.

Patients with renal involvement had lower TGF and higher CRP and B2M concentration in comparison to patients without renal involvement. We did not find any significant difference in serum levels of TGF, CRP and B2M according to presence of lytic bone lesion.

DISCUSSION

Transforming grawth factor is a multifunctional regulator of cellular activity belonging to a larger family of polypeptides which regulate cell growth, cell differentiation and cell function[7-9,15]. The mRNA of TGF-beta 1 has been detected in MM patients[12]. In addition the TGF-beta 1 may increase hematologic disorders such as aplasia characterized by increased plasma cells in bone marrow. Some studies show that TGF is a growth factor in plasma cells in MM patients. In contrast, some studies have reported that TGF-beta1 was an inhibitory factor[14]. An ex vivo study shows that TGF-beta1 inhibits the growth of OPM-2 myeloma cells, which express glucocorticoid receptors. This inhibitory effect was blocked by anti-TGF-beta antibody[16]. The studies show that TGF from MM cells inhibits proliferation and is associ-

Table 5. TGF, CRP and B2M concentration at diagnosis in 22 patients with available post-treatment data

	Plateau phase patients (n= 14)	Refractory patients (n=8)	p
B2M (ng/mL)	1517 (680-2550)	2730 (1200-4200)	0.05
CRP (mg/L)	4.9 (0.9-25.5)	24.3 (1.9-188)	0.05
TGF-beta1 (ng/mL)	970 (268-1206)	564 (126-939)	0.04

B2M: Beta 2 microglobulin, CRP: C reactive protein, TGF: Transforming growth factor-beta1.

ated with suppression of IL-2 induced T cell proliferation[17,18]. Kyrtsonis et al. reported that serum TGF level was related to the degree of immunoparesis in patients with multiple myeloma[19].

Kroning et al. reported that the serum concentrations of TGF-beta1 in 23 MM patients were higher than normal range[13]. In this study median TGF was 9.5 ± 7.4 ng/mL (reference range 3.5 ± 1.6), and 10.8 ± 3.5 ng/mL in peripheral blood and bone marrow respectively. There was no significant difference between peripheral blood and bone marrow. Jiang et al. compared plasma, urinary and intraplatelet TGF concentration in 27 MM patients and 22 healthy people. They found that intraplatelet and urinary TGF concentration was significantly higher in MM patients than in the control group. Plasma concentration of TGF was not different between MM patients and control group. The concentration of TGF was higher in patients with lytic bone lesion than in those without[20].

IL-6 is the main cytokine in MM patients. The other cytokines may play a role in growth of myeloma cells and especially developing lytic bone lesion[21-25]. TGF-beta1 has been demonstrated in MM patients. The main effect of this cytokine on the immunological system is inhibitory effect. A high level of TGF-beta1 has been reported[13,20]. Some studies have show that TGF-beta1 was an inhibitory factor in MM patients, but result of some studies do not agree. TGF may play a role in myeloma, and serum levels may be associated with good clinical course in MM patients.

In conclusion our results show a strong relationship between TGF-beta1 levels and clinical course in patients with MM. Elevated serum TGF concentration at diagnosis in MM may be a favorable predictor of response. TGF may play a role in myeloma and serum levels may be associated with good clinical course in MM patients. The determination of TGF-beta1 and levels of other cytokines may be useful in the monitoring of MM.

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Address for Correspondence:

Hasan Şenol COŞKUN, MD

Department of Medical Oncology
Süleyman Demirel University
School of Medicine
Isparta, TURKEY

e-mail: *hscoskun@erciyes.edu.tr*