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SHORT COMMUNICATION

# Serum Proinflammatory Mediators at Different Periods of Therapy in Patients With Multiple Myeloma

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Multiple myeloma (MM) is a malignant disease characterized by the clonal proliferation of plasma cells within the bone marrow. Several cytokines have been demonstrated to be involved in the control of growth, progression, and dissemination of MM. We determined serum levels of interleukin-1$\beta$ (IL-1$\beta$), soluble interleukin-2 receptor (sIL-2R), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and C-reactive protein (CRP) in 14 newly diagnosed MM patients. The median age of the patients was 63.4 ± 10.8 years and all of the patients were stage III (classified according to the Durie-Salmon classification). The same parameters were measured in 15 healthy controls. In addition, we also examined the effects of vincristine-adriamycin-dexamethasone (VAD) therapy on the same parameters and mediators as well as the relationship among the parameters in the same patient groups. The serum concentrations of TNF-$\alpha$, IL-1$\beta$, sIL-2R, IL-6, IL-8, and CRP (18.6 ± 3.7 pg/mL, 10.1 ± 2.8 pg/mL, 730 ± 220 U/mL, 11.4 ± 3.3 pg/mL, 23.9 ± 8.3 pg/mL, and 49.9 ± 19.5 mg/dL, resp) were significantly higher in newly diagnosed MM patients than in healthy controls ($P < .0001$). All of the parameters were found to be significantly reduced after chemotherapy. In conclusion, we found that after the VAD therapy, the level of these cytokines which are thought to play an important role in the pathogenesis of MM was significantly suppressed. This is the first study demonstrating strong impact of VAD treatment on circulating mediators of sIL-2R and IL-8 levels parameters.

## INTRODUCTION

Multiple myeloma (MM) is a B-cell malignancy characterized by the accumulation of a clonal population of plasma cells in the bone marrow that secretes a monoclonal immunoglobulin protein. Bone marrow stromal cells and myeloma cells produce several proinflammatory cytokines that play an important role in the pathogenesis of multiple myeloma. Of these, Interleukin-6 (IL-6) is known as a growth and survival factor in multiple myeloma via activation of extracellular signal-regulated kinase and phosphatidylinositol 3-kinase signaling cascade [1]. Another proinflammatory cytokine such as Interleukin-1$\beta$ (IL-1$\beta$), a potent osteoclast-activating factor, can increase the expression of adhesion molecules and induce paracrine IL-6 production [2, 3]. These biologic effects of IL-1$\beta$ closely parallel several of the clinical features of human myeloma, such as osteolytic bone lesions, homing of myeloma cells to the bone marrow, and IL-6-induced cell growth [3]. Similar to IL-1$\beta$, tumor necrosis factor-$\alpha$ (TNF-$\alpha$) is also a potent bone-resorbing proinflammatory cytokine that may contribute to the development of the osteolytic bone disease observed in patients with MM [2, 4]. C-reactive protein (CRP) is produced by hepatocytes in response to inflammation and infection and strongly correlated with proinflammatory cytokines particularly IL-6 [5]. On the other hand, soluble interleukin-2 receptor (sIL-2R) can increase during some inflammatory processes and malignant disorders [6, 7]. Levels of serum sIL-2R reflect the total amount of the activated T lymphocytes in tumor-infiltrating lymphocytes of cancer tissues and metastatic organs, because a part of alpha-chain of sIL-2R is released into the bloodstream on the attachment of IL-2 to its specific sIL-2R membrane [7]. Interleukin-8 (IL-8) chemokine for neutrophils and lymphocyte has been demonstrated to be produced by bone marrow stromal cells [8]. Hypothesis suggested that IL-8 may be able to attract circulating malignant plasma cells precursors into an IL-6-rich bone marrow microenvironment [6]. Recently, high-dose therapy (HDT) with

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autologous stem cell transplantation (auto-SCT) has become the standard treatment for many patients with MM. Hence HDT and autologous stem cell rescue are considered to be a standard part of initial therapy for patients with MM [9, 10]. Therefore, the patients accepted as potential transplant candidates are generally treated with dexamethasone-based programs rather than alkylating agents to avoid stem cell toxicity. Chemotherapy consisting of VAD (vincristine-adriamycin-dexamethasone) has commonly been used as the initial chemotherapeutic regimen in MM patients prior to HDT and auto-SCT [10, 11].

The objectives of the present study were to investigate circulating levels of mediators such as IL-1$\beta$, sIL-2R, IL-6, IL-8, TNF-$\alpha$ as well as CRP before treatment and in different time periods after VAD treatment in patients with MM.

## MATERIALS AND METHODS

This study involved a total of 14 newly diagnosed MM patients who were admitted to the Department of Hematology, Turgut Ozal Medical Center, Inonu University, Malatya, Turkey. They were 6 males (42%) and 8 females (58%). The mean age was 63.4 ± 10.8 (range 42–71) years. The patients were in stage III of MM according to the Durie-Salmon classification. The study was carried out in compliance with the guidelines prescribed by the Institutional Health Committee at the Medical Faculty, Inonu University. All of the patients received VAD and bisphosphonates (pamidronate) treatment following diagnosis. Venous blood samples were taken before initiation of VAD therapy (0 day) and on the 3rd, 7th, 10th, and 28th days after the first course of VAD. The VAD regimen consisted of vincristine (0.4 mg/day for 4 days IV), doxorubicin (9 mg/$m_2$/day for 4 days IV), and dexamethasone (40 mg/day per orally on days 1–4, 9–12, and 17–20). The treatment cycles were repeated at 4-week intervals. None of the patients or healthy controls was receiving any topical or systemic medication on admission. Following centrifugation of the blood samples at 2000 x*g* for 10 minutes, sera were separated and kept at −70°C until the analysis of cytokines.

### *Cytokine analysis*

The analyses were performed using the Immulite chemiluminescent enzyme immunometric assay (Diagnostic Products, Los Angeles, Calif). The technique was based on a solid-phase (bead) two-site chemiluminescent enzyme immunometric assay. For each cytokine calibration, a master curve was constructed according to remarks of the National Institute for Biological Standards and manufacturer's instructions. TNF-$\alpha$ assay has a calibration range of up to 1000 pg/mL. The assay is standardized in terms of the National Institute for Biological Standards and Control reference preparation number 87/650. The detection limit of the assay was approximately 1.7 pg/mL. IL-1$\beta$ assay has a calibration range up to 1000 pg/mL. The assay is standardized in terms of the international standard for IL-1$\beta$, 86/680. The detection limit of the assay was approximately 1.5 pg/mL. sIL-2R assay has a calibration range up to 7200 U/mL. The detection limit of the assay is approximately 10 U/mL. IL-6 assay has a calibration range up to 2000 pg/mL. The assay is standardized in terms of the international standard for IL-6, 89/548. The detection limit of the assay was approximately 1 pg/mL. IL-8 assay has a calibration range up to 7500 pg/mL. The assay is standardized in terms of the National Institute for Biological Standards and Controls reference preparation number 89/520. The detection limit of the assay was approximately 2 pg/mL. The coefficient of variance of intra-assay and interassay was generally in the range of 4.3%–9.7%.

### *Statistical analysis*

Mean and standard deviations were calculated for each parameter. Variable normality analysis was performed. Variables in Table 1 showed normal distribution and comparison of means calculated by parametric unpaired *t* test. Whereas parameters in Table 2 did not show normal distribution and repeated measures, ANOVA test was done. Pearson's linear correlation test was used for assessment of correlation between parameters. The minimum level of significance was defined at $P < .05$. All the above-mentioned analysis was performed using the Standard Package for Social Sciences (SPSS) version 9.0 for Windows (SPSS, Chicago, Ill).

## RESULTS

The levels of cytokines and CRP were determined in newly diagnosed patients prior to and after VAD therapy. The results were expressed as means and standard deviation (SD). Summaries of the concentrations of the measured parameters prior to treatment are provided in Table 1. Pretreatment cytokine levels and CRP were found to be increased at diagnosis but significantly decreased 3, 7, and 10 days after VAD treatment (Table 2). The decrease in cytokine levels temporarily the occurred since the studied values started to increase on the 28th day after VAD therapy. Average levels of TNF-$\alpha$, IL-1$\beta$, sIL-2R, IL-6, IL-8, and CRP differed according to the days after conducting VAD treatment. All cytokines except IL-1$\beta$ were found to be positively correlated with CRP. Repeated measures ANOVA test for measured cytokines and CRP within and between different periods can be seen in Table 2.

## DISCUSSION

The significant point in this study is that serum levels of IL-1$\beta$, sIL-2R, IL-6, IL-8, TNF-$\alpha$, and CRP in newly diagnosed stage III MM patients were significantly higher compared to normal controls and were significantly lower after chemotherapy. IL-6 is an important multifunctional

Table 1. Comparison of serum levels of cytokines and CRP (mean ± SD) in patients with MM.

| Parameters | Control | Patients | *P* value |
|---|---|---|---|
| TNF-$\alpha$ (pg/mL) | 6.3 ± 1.3 | 18.6 ± 3.7 | $P < .0001$ |
| IL-1$\beta$ (pg/mL) | 4.3 ± 0.2 | 10.1 ± 2.8 | $P < .0001$ |
| sIL-2R (U/mL) | 597 ± 80 | 730 ± 220 | $P = .021$ |
| IL-6 (pg/mL) | 4.4 ± 0.2 | 11.4 ± 3.2 | $P < .0001$ |
| IL-8 (pg/mL) | 6.4 ± 1.6 | 23.9 ± 8.3 | $P < .0001$ |
| CRP (mg/dL) | 4.1 ± 1.5 | 49.9 ± 19.4 | $P < .0001$ |

Table 2. Serum cytokines and CRP levels (mean ± SD) in different periods after VAD treatment.

| Periods | TNF-$\alpha$ (pg/mL) | IL-1$\beta$ (pg/mL) | sIL-2R (U/mL) | IL-6 (pg/mL) | IL-8 (pg/mL) | CRP (mg/dL) |
|---|---|---|---|---|---|---|
| Before treatment (I) | 18.6 ± 3.7 | 10.1 ± 2.8 | 730 ± 220 | 11.4 ± 3.3 | 23.9 ± 8.3 | 49.9 ± 19.5 |
| 3 days after treatment (II) | 13.6 ± 4.3 | 6.5 ± 1.1 | 670 ± 340 | 7.1 ± 1.4 | 16.2 ± 5.9 | 25.8 ± 8.3 |
| 7 days after treatment (III) | 11.9 ± 3.9 | 5.9 ± 0.6 | 540 ± 125 | 5.2 ± 0.8 | 13.5 ± 4.2 | 3.1 ± 1.2 |
| 10 days after treatment (IV) | 8.1 ± 1.1 | 5.9 ± 0.6 | 436 ± 28 | 4.6 ± 0.5 | 5.2 ± 0.7 | 1.8 ± 2.5 |
| 28 days after treatment (V) | 11.8 ± 2.5 | 8.8 ± 2.7 | 779 ± 97 | 7.5 ± 1.2 | 8.6 ± 1.7 | 23.9 ± 10.9 |
| Repeated measures ANOVA $P > .05$ | II-V | I-V, III-IV | I-II, I-V, II-III, II-V | II-V, III-IV | None | II-V |
| Repeated measures ANOVA $P < .05$ | Rest | Rest | Rest | Rest | All | Rest |

proinflammatory cytokine involved in tumor growth and metastasis [12]. IL-6 is also the major growth and survival factor for MM and has been shown to protect MM cells from apoptosis induced by a variety of agents [13, 14]. Serum and bone marrow IL-6 levels were found to be elevated in myeloma patients, and levels correlate with disease activity and disease status [6]. CRP serum levels reflect IL-6 *in vivo* and it may be regarded as a powerful prognostic factor in patients with MM [5, 15]. TNF-$\alpha$ increases in all types of cellular and humoral immune response and shows a synergistic effect with IL-6 [4, 16]. IL-1$\beta$ and TNF-$\alpha$ are also potent bone-resorbing cytokines that may contribute to the development of the osteolytic bone disease observed in patients with MM [2, 4]. Increased proinflammatory cytokines (IL-1$\beta$, IL-6, TNF-$\alpha$) and CRP serum levels have been reported in different series of MM patients [1, 2, 3, 4, 6, 17]. Similar to the previous findings from literature, our results showed that these proinflammatory cytokines were higher in the MM patients than control groups. Bisphosphonates seem to have an anti-inflammatory activity caused by the inhibition of the release of inflammatory mediators from activated macrophages, such as IL-6, IL-1$\beta$, TNF-$\alpha$, and CRP [18, 19, 20, 21]. Several studies have demonstrated bisphosphonates to be powerful inhibitors of bone resorption [19, 20, 21]. According to our results, the combination of VAD/bisphosphonate chemotherapy produced a significant reduction of the circulating serum levels of the cytokines.

There are sufficient data for serum IL-8 and sIL-2R levels of MM patients before and after treatment in the literature. IL-8 plays a crucial role in inflammatory and tumor-associated angiogenesis, as well as in tumor progression [8, 22]. Bone marrow stromal cells (BMSC) from myeloma patients also aberrantly secrete the chemokine IL-8 [8, 23]. It is possible that this aberrant production of IL-8 by the BMSC of patients with myeloma does not have a direct effect on myeloma growth and survival *per se* but may instead function to promote angiogenesis [8, 23, 24]. Nevertheless, according to literature reviews although some mediators were studied in MM, sIL-2R, and IL-8 in particular, have not yet been investigated in MM in relation to chemotherapy. High levels of sIL-2R have been measured in many inflammatory and malignant diseases [6, 7]. Although the specific role of sIL-2R in the immune reponse has not yet been fully described, elevated serum sIL-2R levels are correlated with MM activity and MM stage [25, 26]. Experimental data from a study conducted by Vacca et al report that serum and urinary values of sIL-2R were significantly increased in MM patients compared with normal controls [26]. Our results showed that circulating serum levels of the cytokines sIL-2R and IL-8 were increased in MM patients compared to controls and were positively correlated with the other cytokines. Circulating serum levels of the cytokines sIL-2R and IL-8 decreased significantly following chemotherapy. Our study is the first one demonstrating reduction of circulating serum mediators, sIL-2R and IL-8 levels, after chemotherapy.

In summary, our data also supported involvement of several mediators in the pathogenesis of MM. Decreased circulating serum mediators of sIL-2R and IL-8 levels may also give a preliminary idea of the assessment of therapy response. Our results also suggest that after a time interval (10–28 days), cytokine levels reincrease. This could mean that cytokines suppression was temporary and one

VAD therapy course of recommended dose and period is likely inadequate. Repeated VAD/bisphosphonate courses may provide better cytokines suppression. So, more efforts should be directed not only toward understanding the significance of the variability of these cytokines in patients with MM, but also their potential role in therapeutic approaches to MM. Further investigations are warranted in larger patient groups to support our present findings.

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