RABMS Journal of Research in Applied and Basic Medical Sciences

Urmia University of Medical Sciences

Serum levels of IL-6, TNF-α, and YKL-40 in patients with stage I multiple myeloma

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Abstract

***Background & Aims*:** Multiple myeloma (MM) is a type of plasma cell malformation accounting for 10% of the blood cancers with unknown ethiopathology. MM causes changes in inflammatory proteins. The aim of this study was to evaluate the serum levels of IL-6, TNF-α, and YKL-40 in patients with stage I MM.

Materials & Methods: This case-control study was performed on 28 patients with stage I MM including 14 males and 14 females with mean age of 62.11 ± 6.71 years and 40 individuals as controls including 20 males and 20 females with mean age of 60.25 ± 4.81 years matched in both age and sex. Serum concentrations of inflammatory factors including IL-6, TNF-α and YKL-40 were measured using the sandwich Enzyme Linked Immunosorbent Assay (ELISA) method and the data were analyzed using SPSS software.

Results: The serum levels of IL-6 in patients with stage I MM (7.42±5.89 pg/ml) were significantly higher compared to the control group (1.67±1.08 pg/ml); (p< 0.0001). Also, the serum levels of YKL-40 in the patients (45.86 ± 9.14 ng/ml) were significantly higher compared to the control group (35.87 ± 11.2 ng/ml); (p <0.0001). Also, the levels of TNF-α (8.02±1.75 pg/ml) were slightly high compared to the control group (7.77 ± 1.91 pg/ml); (p=0.419).

Conclusion: According to the results in MM, inflammatory proteins such as YKL-40 andIL-6 are the major growth factor of the myeloma cells and help their survival.

***Key words*:** Multiple myeloma, IL-6, TNF-α, YKL-40

Received 10 February 2020; accepted for publication 28 April 2020

Introduction

Multiple myeloma (MM) is a plasma cell disorder that includes about 10% of the blood cancers(1). MM usually results from a benign pre-cancerous Monoclonal Gammopathy of Undetermined Significance (MGUS) disorder. In MGUS, the number of plasma cells in the bone marrow is lower than 10%. Approximately, 10% of the patients with MGUS enter into myeloma phase, in which the number of the plasma cells in the bone marrow is more than 10% (1, 2). The mean age of patients at the time of diagnosis is 68 years and 99% of the patients are more than 40 years old. Black-skinned

[DOI: 10.13183/rabms.6.3.160]

people are more susceptible to this disease compared to white-skinned people, and white-skinned people are more susceptible to this compared to yellow-skinned people. Moreover, males are more susceptible compared to females (3). Some of the symptoms and complications of this disease are bone pains, especially vertebrae bones, osteolytic lesions (80%), bone fractures, kidney failure, amyloidosis caused by immunoglobulin deposition in tissue of kidney, frequent infections, hyperproteinemia, anemia, hypercalcemia, and reduction in the alkaline phosphatase and osteocalcin values (4-7). Among these symptoms, bone fractures and lesions are considered as the most important complications of MM (7, 8). The disease is geographically distributed unequally, so that the highest prevalence is seen in northern Europe, Australia, and New Zealand, while the lowest prevalence is found in Japan and Greece. MM generally includes approximately 1% of malignancies and 10% of the blood cancers in white-skinned people and 20% in African Americans (9). Other studies indicated that MM prevalence is 2 to 4 times more in people whose relatives suffer from this disease compared to others. Family history of other hematological malignancies might also have a relationship with the increased prevalence of the MM (10). International staging system divides MM into three stages based on the serum values of β2-microglobulin (β2M) and albumin. In stage I, the β2M value is lower than 3.5 mg/l and the albumin value is ≥3.5 g/dl. In stage II, the value of β2M is between 3.5 - 5.5 mg/L and the albumin value is lower than 3.5 g/dl. In stage III, the serum value of β2M is higher than 5.5 mg/l (11).

In most cases, the diagnosis of MM is based on the presence of a monoclonal M-protein or free-light chains in the blood and also the presence of at least 10% plasma cells in the bone marrow(12).

IL-6 as a potential growth factor for myeloma/plasmacytoma cells involves in their production. Studies conducted on the myeloma cells which were separated from the bone marrow of the patients, suggested that IL-6 is an autocrine and paracrine growth factor for human myeloma cells produced from them (13, 14), IL-6 has anti-apoptotic effects on the myeloma cells, thus, it increases the survival and proliferation rates of the myeloma cells (13).

TNF-α leads to IL-6 production from stromal cells of the bone marrow that are regarded as the main growth factors for tumor cells and show pro-angiogenic properties in the body (15-17). The bone marrow cells extracted from the patients, produce greater amounts of TNF-α, compared to the control group, depending on the disease stage, osteolytic bone disease, and the number of plasma cells in the bone marrow (18, 19). These findings suggested the key role of TNF-α in the growth, survival, and development of malignant cell colonies.

Plasma YKL-40 increases in the patients suffering from acute myeloid leukemia (AML) (20), MM (21, 22), and Hodgkin's lymphoma (23). A high correlation has been found between YKL-40 and IL-6 among the patients suffering from myeloma and Hodgkin's lymphoma (22, 23). Myeloma patients with high plasma YKL-40 experience severe bone destruction including increased bone resorption and early development of bone diseases related to myeloma (21). YKL-40 expression in myeloma increased in cells around the myeloma in the bone marrow microenvironment. This protein is considered as a predictive marker in MM (24).

MM is one of the blood malignancies with no definitive treatment; thus there is an increasing need to establish new accurate biomarkers for early diagnosis of the disease. Measuring the YKL-40 protein as a new marker, which is produced by inflammatory cells and solid tumor cells, contributes to diagnosis of this disease in the early stages.

Materials and Methods

This case-control study was conducted on 28 MM patients (62.11 ± 6.71 years) and 40 healthy subjects (60.25 ± 4.81 years). The study groups were selected from individuals who were referred to Laboratory of Imam Khomeini Hospital in Urmia, Iran during the years 2016-2017. Clinical tests including CBC, bone marrow, and radiologic examinations were carried out in the patients suspicious for MM, after wards, these

[DOI: 10.13183/rabms.6.3.160]

patients were referred to the laboratory for using electrophoresis of serum proteins and immunotyping via capillary electrophoresis.

All patients with high peaks in gamma (higher than 30%) or beta zone together with the kappa or lambda chains in terms of the IgG, IgM, and IgA immunoglobulins were categorized in stage I of the MM. The staging was performed based on the international staging system (ISS) and the values of serum β2M and albumin. Moreover, all patients with β2M values lower than 3.5 mg/l, measured by luminescent immunoassay (Diasorin, Liaison, Italy), were categorized in the stage I. This study was approved by the Ethics Committee of Urmia University of Medical Sciences to the code number IR.umsu.rec.1395.216.

Five milliliters of blood were taken from the patients and the control group. Serum samples were stored in the freezer at -40°C.

Serum levels of IL-6 (IBL International Germany), TNF-α (IBL International Germany), and YKL-40 (Bioassay Technology Laboratory, China) were measured by Enzyme Linked Immunosorbent Assay (ELISA).

Statistical Analysis:

Data were analyzed by using SPSS software version 22. The mean difference in two groups was determined by independent samples t-test for variables distributed normally and Mann Whitney U was determined for variables distributed non-normally. Results were presented as mean ± SD. The significance level was considered to be $p < 0.05$. In addition, the correlation among the variables distributed normally was examined using the Pearson test and the correlation among the variables distributed non-normally was examined using Spearman test.

Results

In the current case-control study, the serum concentrations of IL-6, TNF-α, and YKL-40 were examined and compared in 28 patients with MM and 40 healthy control subjects. The demographic characteristics and serum concentrations of IL-6, TNF-α, and YKL-40 were evaluated between the groups. Furthermore, the correlation between the mentioned variables was evaluated. The mean age of patients with stage I MM was 62.11 ± 6.71 years, and the mean age of the subjects in the control group was 60.25 ± 4.81 years. The age range in the patient group was 49-74 years, while it was 53-70 years in the control group. A significant difference was not found between two groups in this regard (p=0.078).

The mean serum values of IL-6 in stage I MM were 7.42 ± 5.89 pg/ml, while they were 1.67 ± 1.08 pg/ml in the control group. ($p < 0.0001$). The mean values of TNF-alpha were 8.02±1.75 pg/ml and 7.77 ± 1.91 pg/ml in stage I multiple myeloma patients and control group subjects, respectively but the difference between two groups was not statistically significant ($p = 0.419$). The mean values of YKL-40 in stage I MM patients and control group subjects were 45.86 ± 9.14 and 35.87 ± 11.2 ng/ml, respectively. A significant difference was found between groups ($p < 0.0001$) (Table 1).

Table 1. Comparison of variables in stage I multiple myeloma patients and control group

Variable	Control group N=40	Patients group N=28	P-value
Age (year)*	60.25 ± 4.81	62.11 ± 6.71	0.078
Gender (male/female)**	20/20	14/14	1.000
IL-6 (pg/ml)***	1.67 ± 1.08	7.42 ± 5.89	0.0001
TNF-α (pg/ml)***	7.77 ± 1.91	8.02 ± 1.75	0.419
YKL-40 (ng/ml)***	35.87 ± 11.2	45.86 ± 9.14	0.0001

*comparing by Independent samples t-test

** comparing by Chi-square test

*** comparing by U Mann-Whitney test

[DOI: 10.13183/rabms.6.3.160]

The correlation between IL-6, TNF-α, and YKL-40 was examined in the patients and the results are presented in Table 2.

Table 2. The correlation between the variables in multiple myeloma patients

Variables having correlation	Correlation Co.	P-value
TNF-α -IL-6*	-0.037	0.850
TNF-α -YKL-40*	0.353	0.066
IL-6-YKL-40**	0.141	0.473

* Pearson correlation coefficient

**Spearman correlation coefficient

Discussion

MM is the second most common cancer among hematologic neoplasms. In recent years, many studies examined the diagnosis and treatment of this disease(25). Rapid progression of the disease and lack of proper diagnosis have led MM to be considered as one of the major cancers with the high rate of mortalities (from diagnosis to death). Therefore, it is essential to design comprehensive studies on the effective factors for appropriate treatment and follow-up (26).

The results of this study indicated that in patients with MM, the serum levels of IL-6 and YKL-40 increased significantly compared to the control group, while the TNF-α level showed a slight and non-significant increase.

Different clinical studies have examined the association of increased the YKL-40 with low survival in several cancers such as breast, colorectal, ovarian, prostate, and blood cancers, and suggested YKL-40 as a prognostic biomarker for cancer (27, 28). Overall, the present results were in line with results of the other studies, which introduced the serum levels of YKL-40 as an effective biomarker in MM prognosis (21, 24, 29). However, it is essential to find the relationship between increased YKL-40 and survival rate in MM.

Pre-inflammatory mediators play an important role in the pathogenesis of MM. IL-6 has anti-apoptotic effects on the myeloma cells, thereby it can increase the survival and proliferation rate of these cells. Also, the TNF-α plays a key role in bone resorption and is likely to improve osteolytic disease in patients with MM. Kuku et al. examined the levels of serum IL-6, TNF-α, and CRP in patients with stage III MM. They showed that IL-6 and TNF-α (3 times) and CRP (10 times) levels increased significantly compared to the control group before chemotherapy. In this study, a significant increase in IL-6 levels was consistent with the results of our study. However, in our study, the TNF-α levels had no significant increase which seems to be related to the stage of the disease or the number of samples. Kuku et al. showed that after chemotherapy with bisphosphonate and VAD (vincristine-adriamycin-dexamethasone), a significant reduction was observed in the studied cytokines. These results suggested that evaluation of cytokines was useful not only in the understanding the pathogenesis of MM, but also in the assessment of the effects of therapeutic methods (13, 30, 31).

Terpos et al. had conducted a study in 2003 on the effects of pamidronate and ibandronate on the reduction of bone mineral density, IL-6, and β2-microglobulin in patients with MM. The result of this study showed that bone resorption in MM patients was associated with an increase in IL-6 and other acute-phase proteins which were stimulated by IL6(32). Then, it was suggested that there should be a relationship between IL-6 and bone resorption. Similarly, Kaminska et al. reported significantly higher levels of IL-6 and TNF-α in patients with MM as stimulants for the production of angiogenesis growth factors by plasma cells. In their study, there was also a positive correlation between IL-6 and TNF-α, which was inconsistent with findings of the present study. This could be probably due to differences in the study of the different stages of the disease or the number of samples (33).

Lee et al. studied the mechanism of biological action of TNF-α in human myeloma cells and indicated that

[DOI: 10.13183/rabms.6.3.160]

TNF-α as a pre-inflammatory cytokine involved in various processes such as cell growth, cell death, and differentiation in the development of MM. In this study increased amounts of TNF-α and IL-6 have been shown to be associated with poor prognosis. The results also showed a 1.5-fold increase in bone loss in patients with high levels of TNF-α compared to those with low levels (34).

Studies about YKL40 and MM

Johansen et al. declared that TNF-α decreased the production of YKL-40 in cultured single-layer chondrocytes. In the present study, there was no significant increase in TNF-α values, therefore, it seems that it is likely to have a weaker inhibitory effect on YKL-40 production in these patients. However, IL-6 has shown to stimulate the secretion of YKL-40 in newly-isolated human chondrocytes, and the significant increase in YKL-40 in our study might be related to the significant increase in the IL-6 levels. Consistently, a correlation between YKL-40 and IL-6 concentrations in synovial fluid and serum of patients with rheumatoid arthritis has been shown. Thus, the association between YKL-40 and IL-6 may be another pathological process in MM (35).

Conclusion

According to the results, in MM, in addition to IL-6, which is considered as the main growth factor of the myeloma cells, several other cytokines such as TNF-α also support the life of the myeloma cells. All in all, in the present study we used a multifactorial approach, like IL-6, TNF-α and YKL-40 that could provide another accurate tool for differential diagnosis of MM patients.

Acknowledgment The authors would like to thank the financial support of Vice Chancellor for research and technology.

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[DOI: 10.13183/rabms.6.3.160]

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[DOI: 10.13183/rabms.6.3.160]