RESEARCH ARTICLE

Editorial Process: Submission:09/08/2021 Acceptance:09/18/2022

Serum Levels of Interleukin-1β and Disease Progression in Multiple Myeloma Patients: A Case and Control Study

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Abstract

Objective: Multiple myeloma (MM) is known as an incurable heterogeneous plasma cell malignancy that presents with a variety of clinical manifestations. Inflammation plays an important role in this disease. Cytokines and Chemokines cause the progression of the disease. One of them is interleukin-1β (IL-1β), which may be involved in the pathogenesis of MM. Other markers such as calcium, albumin, creatinine, globulins, and total protein are also used to diagnose and prognosis patients. The main purpose of this study was to evaluate the serum level of IL-1β and various forms of calcium (total calcium, ionized calcium, and corrected calcium), albumin, creatinine, globulin, and total protein on stage-I of MM patients and healthy controls. **Methods:** Serum samples from 30 stage-I MM patients and 30 healthy subjects as controls were examined in this study. The protein concentrations of serum IL-1β was assessed by enzyme-linked immunosorbent assay (ELISA), total calcium, albumin, creatinine, total protein, and globulin Measured by auto analyzer BT3000, an electrolyte analyzer was used to measure ionized calcium (Ca++) and a special equation was used to calculate the corrected calcium. **Result:** The mean level of IL-1β was significantly elevated in stage-I MM. The mean levels of IL-1β were 7.04±1.15 ng/ml in stage-I MM and 3.12± 0.90 ng/ml in controls ($p<0.001$). The mean levels of total calcium (total Ca) were 9.45±0.56 mg/dl in stage-I MM and 9.09±0.43mg/dl in controls ($p=0.008$). The mean levels of ionized calcium (Ca++) was 4.65±0.28mg/dl in stage-I MM and 4.75±0.33mg/dl in controls ($p=0.2$). The mean ratio of serum ionized calcium to total calcium (Ca++/ total Ca) was 0.49±0.054 in stage-I MM and 0.52±0.047 in controls ($p=0.02$). The mean ratio of serum ionized calcium to corrected calcium (Ca++/corrected Ca) was 0.42±0.033 in stage-I MM and the Mean ratio of serum ionized calcium to calcium total (Ca++/ total Ca) was 0.52±0.047 in controls, Comparison of the mean of the two groups shows a significant difference ($p<0.001$). The mean level of albumin was 1.72±0.35 g/dl in stage-I MM and4.32±0.41g/dl in controls ($p<0.001$). The mean level of total protein was 12.65±0.81g/dl in stage-I MM and 7.07±0.4 g/dl in controls ($p<0.001$). The mean level of globulin was 11.00±0.96 mg/dl in stage-I MM and 2.85±0.77 mg/dl in controls ($p<0.001$). The mean level of creatinine was 1.15±0.25 mg/dl in stage-I MM and 0.96±0.15 mg/dl in controls ($p=0.001$). **Conclusion:** The results of the study indicate the possible involvement of IL-1β at stage-I MM and it can indicate the role of chemokines in the disease process, especially in the early stages. Changes in the chemical profiles mentioned can help in the diagnosis and prognosis of the disease.

Keywords: Multiple myeloma- interleukin- 1β- total calcium, ionized calcium - corrected calcium- albumin- creatinine

Asian Pac J Cancer Prev, **23 (9)**, 2937-2942

Introduction

Multiple myeloma (MM) is a systemic malignancy of B cells in the blood caused by uncontrolled clonal proliferation of plasma cells in the bone marrow (BM) (Gerecke et al., 2016). The second most common malignancy is in the blood after non-Hodgkin's lymphoma and in most cases it is incurable. The disease is preceded by a pre-malignant condition monoclonal gammopathy of unknown significance (MGUS) (Nikesitch and Ling, 2016). The BM environment in people with MM shows high levels of interleukin and interferon-γ-induced cytokines (Cao et al., 2010). Cytokines are involved in the inflammatory and anti-inflammatory processes. Many of these factors present in the serum or bone marrow of people with MM have pro-inflammatory activity, such as interleukin-1 (IL-1). IL-1 is a major mediator of the host response to a variety of infections, inflammatory and immune challenges. The primary source of IL-1 is also blood monocytes and tissue macrophages. IL-1 contains two cytokines, IL-1α and IL-1β. (Cao et al., 2010; Landskron et al., 2014). IL-1β is expressed in almost all plasma cells of MM patients, whereas it is not present in normal plasma cells (Gabay et al., 2010; Akdis et al., 2011). IL-1β stimulates stromal cells in the BM environment to produce large amounts of IL-6, which in

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turn stimulates the survival of myeloma cells(Donovan et al., 1998). Myeloma cells produce IL-1β, which acts as a bone-absorbing activity in MM. (Carter et al., 1990; Donovan et al., 1998; Xiong et al., 2006), Because IL-1β has prominent osteoclast activity and can increase the expression of adhesion molecules, the expression of these cytokines may be important in the pathogenesis of MGUS to active myeloma (Costes et al., 1998). In addition, we examined several laboratory factors associated with MM Including calcium (total calcium, ionized calcium, and corrected calcium), albumin, total protein, globulin, and creatinine. In MM, increased osteoclastogenesis by suppressing osteoblastic activity is the main mechanism of the disease (Terpos et al., 2017). Receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) plays the most important role in bone regeneration and imbalance in this system is one of the factors responsible for osteolysis seen in MM (Giuliani et al., 2004). As a result, in myeloma patients, the main cause of hypercalcemia is extensive bone destruction due to the tumor. (Stewart, 2005).

Till now there are different staging methods have been introduced and some became applicable in clinic or laboratories, for instance Mohammad et al., (2019) introduced staging based on Beta2 microblubolin, percentage of bone marrow plasmacells, serum free light chaing (FLC), serum protein electrophoresis, and serum and urine immunofixation, by this approche the biochemical staging of the MM introduced three stages Mohammad et al., (2019), by the way some researches have been established the predictive or prognostic factors (Bekadja et al., 2022) based on clinical or biochemical factors.

There are three types of calcium in serum: it binds mainly to the albumin protein, another form of complex formation with citrate, lactate, or phosphate anions, and the other form is ionized (Ca ++). Ca++ is biologically active. Diagnosis of hypercalcemia is based on the concentration of Ca++, approximately half of serum calcium is bound to albumin, and an increase or decrease in albumin affects the measurement of calcium (Mateu-de Antonio, 2016b). Since the amount of albumin in patients with MM is low and affects the total calcium, so we used the corrected calcium equation. In patients with MM, the ratio of Ca++/ total Ca and Ca++/corrected Ca are used to better assess calcium(Smith et al., 1984).

The specific cause of decreased serum albumin levels in MM patients with poor prognosis is unknown. Several studies have shown that low serum albumin levels are associated with increased serum concentrations of interleukin-6 (IL-6), a myeloma cell growth factor that reflects disease severity and cell proliferation. an abnormal immunoglobulin-like monoclonal protein and immunoglobulin light chains (kappa or lambda) produced in MM, increases the levels of total serum protein and globulins. In addition, serum total protein and globulin concentrations were evaluated as a diagnostic clue for monoclonal gammopathy (Dash and Mohanty, 2012). Kidney failure is a common feature of MM and is characterized by an increase in serum creatinine. Depending on the definition of renal failure, this complication occurs in 20-40% of newly diagnosed people with MM (Kyle et al., 2003; Camp et al., 2008; Jemal et al., 2009; Waxman et al., 2010).

In this study, we hypothesized that IL-1β may be increased in stage-I of MM patients and contribute to disease progression. To investigate this issue, we conducted a case-control study to assess the serum level of IL-1β in stage-I of MM patients and healthy controls. Also in this study, factors such as calcium, albumin, creatinine, total protein, and globulin were evaluated for early diagnosis of the disease.

Materials and Methods

Patient and control

The study population includes 30 diagnosed stage-I MM patients (21 males and 9 females; with mean age 62.9±6.1 years) and 30 healthy controls (21 males and 9 females; with a mean age of 64.6±6.5 years). Patients with advanced stages were excluded from the study. Also, none of the patients had received chemotherapy or radiotherapy before sampling. Healthy controls were examined for the absence of inflammatory diseases and other underlying conditions that could affect the outcome of the study. Eventually, the samples that confirmed the lack of evidence of impairment entered the study.

Common tests and medical procedures to confirm stage-I MM disease

The laboratory tests used to classify patients in stage-I MM, are listed in Table 1(Kohsari et al., 2020).

The International Staging System (ISS) uses data collected from individuals with MM to classify patients. It is based on the measurement of serum albumin and β2-M. This system has recently been revised to include serum lactate dehydrogenase (LDH) and high-risk gene abnormalities defined by the FISH test(Greipp et al., 2005).

Stage I: β2-M<3.5 mg/dl and albumin≥3.5 g/dl and absence of high-risk DNA abnormalities and normal LDH (Greipp et al., 2005).

Blood sampling

Blood samples were obtained through overnight fasting. Blood samples were taken using a Sigma refrigerated centrifuge at 4°C for 10 minutes at 3000 rpm. After centrifugation, it was transferred to the cryotubes and stored at -70°C, and assayed at the end of the study, to avoid inter-assay variability.

Statistical methods

Data analysis was performed by SPSS software (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, USA). All measured parameters are expressed as mean ± SD. Before the analysis, the data were checked for normality with the Kolmogorov-Smirnov test. The parametric Independent-Samples T-test was used to perform statistical comparisons between the stage-I of MM patients and controls. Correlation between the measured parameters was calculated by Pearson's correlation. P-values <0.05 were considered as statistically significant.

Measurement of IL-1β

The serum level of IL-1β was measured by a quantitative sandwich enzyme immunoassay method (LDN Labor Diagnostika Nord GmbH & Co. KG.). The IL-1β-ELISA is a solid phase enzyme amplified sensitivity immunoassay performed on the microtiter plate. The assay uses a monoclonal antibody (mAbs) directed against distinct epitopes of IL-1β. Calibrators and samples react with the capture monoclonal antibody (mAbs1) coated on microtiter well and with a monoclonal antibody.

(mAbs 2) labeled with horseradish peroxidase (HPR). After an incubation period allowing the formation of sandwich: coated Mab1-human IL-1β-Mab2-HRP, the microtiter plate is washed to remove the unbound enzyme-labeled antibody. The bound enzyme-labeled antibody is measured through a chromogenic reaction. The reaction is stopped with the addition of stop solution and then the microtiter plate is then read at the 450 nm and 650 nm wavelength. The amount of substrate turnover is determined by colorimetry measuring the absorbance which is proportional to the IL-1β concentration.

Measurement of Calcium, total protein, albumin, globulin, and creatinine

Serum samples were defrosted and centrifuged for testing, according to the protocol of Pars Azmun kit (Tehran, Iran), total calcium, total protein, albumin, globulin, and creatinine were measured automatically by BT3000 auto analyzer and An electrolyte analyzer PL1000B was used to measure Ca++. we used this equation in our study to measure corrected calcium. Equation of corrected calcium: (serum calcium +0.8) (4-serum albumin) (Mateu-de Antonio, 2016a).

Results

Comparison of clinical features and mean serum levels of the cases mentioned between the patient group and the control group is reported in Table 2. The mean serum level of IL-1β in stage-I MM patients was 7.04±1.15 ng/ml and 3.12± 0.90 ng/ml in controls (P<0.001) (Figure 1). There was also a significant difference between the mean level of IL-1β among MM patients and controls. There was no significant difference in serum levels of IL-1β concentration of either gender.

The mean serum level of albumin was 1.72±0.35 g/dl in stage-I MM and 4.32±0.41g/dl in controls, there was also a significant difference between the mean level of albumin among MM patients and controls (P<0.001). The mean serum level of total protein was 12.62±0.81g/dl in stage-I MM and 7.07±0.4 g/dl in controls, There was also a significant difference between the mean level of total protein among MM patients and controls (P<0.001). The mean serum level of creatinine was 1.15±0.25 mg/dl in stage-I MM and 0.96±0.15 mg/dl in controls, There was a significant difference between the two groups (p=0.001). The mean serum level of globulin was 11.00±0.96 mg/dl in stage-I MM and 2.85±0.77 mg/dl in controls (P<0.001). There was a significant difference between the two groups. The mean ratio of Ca++/total Ca was 0.49±0.054 in stage-I MM and 0.52±0.047 in controls, There was a significant difference between the two groups (P=0.02). We compared the mean ratio of Ca++/total Ca in healthy individuals with Ca++/ Corrected Ca in patients. Considering that the mean was 0.42±0.033 in the control group and in the patient group was 0.52±0.047, a significant difference was observed between the two groups (P<0.001). The result of the study shows that there is a significant difference in the mean serum level of IL - 1β, albumin, total protein, globulin, creatinine, the ratio of ionized calcium to total calcium, and comparison of the Ca++/ Corrected Ca inpatient group with Ca++/total Ca in the control group.

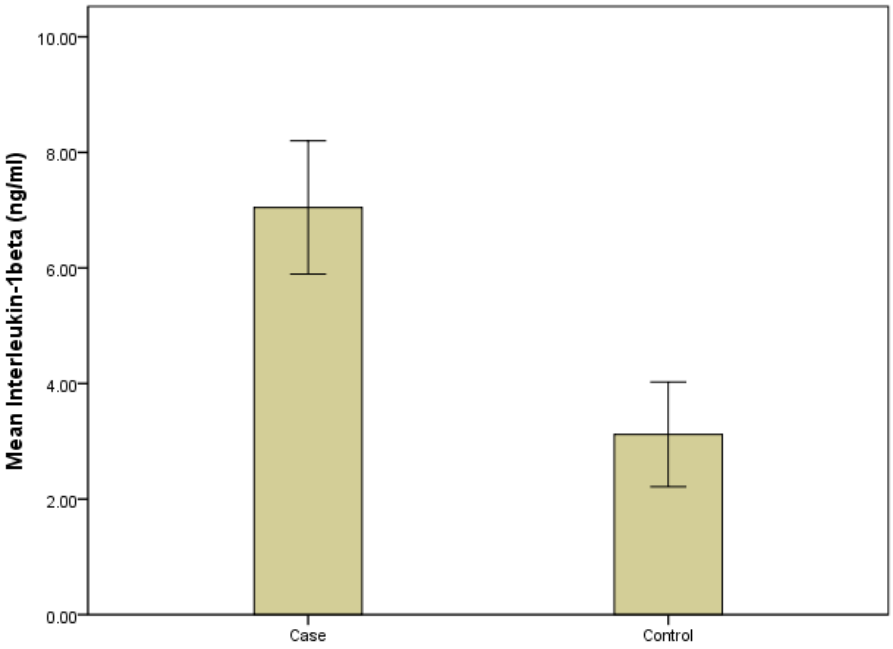

Figure 1. Distribution of Serum Level of IL-1β between MM (multiple myeloma patients) and Controls. A significant difference is observed (P< 0.001).

Table 1. Diagnostic Tests and Criteria for MM Patients

Diagnostic test	Aim	Results
Complete blood count	Measurement of blood cells	Low level may signal anemia, increased risk of infection
Chemistry profile (albumin,calcium,lactate dehydrogenase,creatinine)	Evaluation of kidney function, liver and bone	Abnormal levels may indicate a change in organ status
Beta2-microglobulin (β2-M.)	Determine the level of a protein that indicates the presence of MM and kidney function	Higher-level indicate more extensive disease
Antibody levels	Determine the level of antibodies that are overproduced by myeloma cells	High levels indicate the presence of myeloma
Serum protein electrophoresis	Detect the presence and level of protein made by myeloma cells-monoclonal protein	High levels indicate the presence of myeloma
Bone marrow biopsy	Determine the number and percentage of normal plasma cells in the bone marrow	A higher percentage of myeloma cells indicate more extensive disease

Discussion

MM is the second most common blood cancer. It is cancer in which plasma cells accumulate in the bone marrow, causing severe pain, anemia, and kidney failure. Despite many advances in the diagnosis of this disease and the emergence of new treatments, it is considered an incurable disease (Cho et al., 2018). The exact cause of the disease has not been known until now(Angtuaco et al., 2004). The interaction between MM cells and the BM microenvironment will provide the framework for the development of novel therapies to further improve the outcome of patients. In our previous studies, we focused on the role of interleukin-8 (IL-8) and soluble interleukin-6 receptor (sIL-6R) (Kohsari et al., 2020). No studies have been performed on serum IL-1β levels in MM on stage-I patients.

In this study, we aimed to investigate changes in serum levels of IL-1β in stage-I MM patients. IL-1β promotes the absorption of inflammatory cells and the expression of adhesion molecules on endothelial cells and the secretion of chemokines by stromal cells. (Gabay et al., 2010). IL-1β is expressed in almost all MM plasma cells, stimulates stromal cells to produce large amounts of IL-6, and promotes myeloma cell survival (Donovan et al., 1998; Gabay et al., 2010; Akdis et al., 2011). IL-1β has osteoclastic activity and causes osteolytic lesions. The osteolytic lesion is seen in advanced cases of the disease, so IL-1β is involved in the pathogenesis of MGUS to active myeloma (Cozzolino et al., 1989). Our results show that increased serum levels of IL-1β in patients compared to healthy controls. In a study by Kawano et al., (1989)., they found that bone resorption in myeloma was neutralized by the antiIL-1β antibody. Other previous studies have revealed the similar role of IL-1β. Breast cancer bone metastases are incurable. Endogenous production of IL-1β by tumor cells has been shown to cause bone metastasis (Tulotta et al., 2019). The study by Landvik et al., (2009) Showed that IL-1β is a major mediator in initiating the inflammatory response in lung diseases, including chronic obstructive pulmonary disease and lung cancer. A study by Zhong et al., (2016) found a role for IL-1B in skin cancer, The role of IL-1β in skin cancer has also been seen Mutations to increase NLRP1 function are responsible for the secretion of IL-1β by keratinocytes, which causes inflammation of the skin and hyperplasia, thus leading to skin cancer. Large amounts of IL-1β have also been detected in the murine adenomatous polyposis coli (APC) colon cancer model (Dmitrieva-Posocco et al., 2019). According to the available evidence, more studies on this factor are needed to improve the course of MM. In myeloma patients, the main cause of hypercalcemia is extensive bone destruction caused by the tumor. This is primarily due to increased osteoclastic bone resorption due to strong cytokines that are expressed or secreted locally by myeloma cells. This

Table 2. Mean Serum Levels of the Cases Mentioned between the Patient Group and the Control Group

	Case (Mean±SD)	Control (Mean±SD)	*P-value*
Total Calcium (Ca) (mg/dl)	9.45±0.56	9.09±0.43	0.8
Ionized calcium (Ca++) (mg/dl)	4.65±0.28	4.75±0.33	0.2
Total protein (g/dl)	12.65±0.81	7.07±0.40	<0.001
Albumin (g/dl)	1.72±0.35	4.32±0.41	<0.001
Globulin (mg/dl)	11.00±0.94	2.85±0.77	<0.001
Creatinine (mg/dl)	1.15±0.25	0.96±0.15	0.001
Corrected Ca (mg/dl)	11.30±0.69	-	-
Ca++/ Corrected Ca	0.42±0.033	-	-
Ca++/ total Ca	0.49±0.054	0.52±0.47	0.02
IL-1β (ng/ml)	7.04±1.15	3.12±0.90	<0.001

bone resorption in turn leads to an influx of calcium into the extracellular fluid (Stewart, 2005). on study targeting RANKL as a potential new treatment strategy in myeloma bone disease and myeloma-associated hypercalcemia (Oyajobi, 2007). In our study, we also discussed the importance of assessing changes in serum calcium levels and comparing stage-I MM patients with healthy controls showed a significant difference. Zhong et al., (2016) show that patients with MM with hypercalcemia had a much lower survival. Serum albumin as a prognosis in patients with MM can indicate the severity of the disease (Hussain et al., 2019). In our study, we also examined changes in serum albumin levels and due to the results, albumin levels in stage-I MM patients had decreased. Chen and Magalhaes, (1990) concluded that hypoalbuminemia in patients with MM is primarily related to tumor cell growth and that serum albumin levels may serve as a simple indicator of disease activity. Monoclonal protein increases the level of total serum protein and globulin, thus providing a clue for the diagnosis of MM. In our study, we also showed an increase in total serum protein and globulin in patients compared to the healthy control group. van Hoeven et al., (2011) found that an increase in total serum protein and serum globulin are signs of MM. Dimopoulos et al., (2008) found that renal dysfunction is a common clinical feature of MM during a presentation or during the disease Which may increase serum creatinine levels and decrease glomerular filtration. In the present study, we examined the changes in serum creatinine levels in stage-I MM patients and the control group. The mean serum creatinine level in patients was higher than in the control group. In conclusion, the results of the study suggest that IL-1β may play an important role in the pathogenesis and progression of stage I of MM. this factor can be used to choose better therapeutic strategies. Measurement of calcium, albumin, total protein, globulin, and creatinine can help in the prognosis and diagnosis of the disease.

Author Contribution Statement

During the current Study Shirin Teimouri Nobari1 prepared the primitive proposal and finalized it, done all the steps of the study and prepared the primary manuscript. Rasmi Yousef1, worked as conselor in the study process and data preparing and analyzing and finalizing the main manuscript, and Mohammad Hasan Khadem Ansari suprevized all the project and prepared the samples and facilities and atmospheare of the research and final reviewing the manuscript.

Acknowledgments

This work derived from a Master of Science thesis in biochemistry and we thank the Urmia University of Medical Sciences (department of biochemistry) for kindly providing and supporting this project.

Funding Statement

This research has been financially supported by the Research and Technology Deputy of Urmia Medical University (No.:10207) and the research methods ethically have been confirmed by medical research ethical committee No. IR.UMSU.REC.1399.075.

Data Evalability

The whole data and data files related to the current study will be evailabe for interested persons by emailing to corresponding author.

Conflicts of Interests

The authors declare no conflicts of interests.

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