Original Article

Serum levels of bone sialoprotein, osteopontin, and β2-microglobulin in stage I of multiple myeloma

Aria Maaroufi, Mohammad-Hasan Khadem-Ansari, Hamid-Reza Khalkhali[1], Yousef Rasmi

Departments of Biochemistry and [1]Biostatistics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

For correspondence: Dr. Mohammad-Hasan Khadem-Ansari, Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. E-mail: mhansari1@gmail.com

ABSTRACT

Context: The fluctuations of proteins in multiple myeloma (MM) are well-known markers for checking the status of the patients.

Aims: The objective of this study was to examine three proteins that have an important role in disease progression.

Subjects and Methods: The study was performed with two groups: 30 MM stage I patients' (14 females/16 males; aged 60.83 ± 12.38 years) as case group and 40 healthy individuals (18 females/22 males; aged 57.65 ± 6.43 years) as control group. Both groups have been matched in gender and age. Bone sialoprotein (BSP), osteopontin (OPN), and β2-microglobulin (β2M) were measured with an enzyme-linked immunosorbent assay.

Results: Serum BSP levels of MM-I patients was significantly higher than that of healthy controls (29.24 ± 5.57 vs. 20.89 ± 3.67, $P = 0.001$). OPN levels of MM-I patients were significantly lower than that of healthy individuals (12.03 ± 3.45 vs. 19.35 ± 4.67, $P = 0.001$). β2M levels of patients and controls were similar (1.49 ± 0.67 vs. 1.29 ± 0.55, $P = 0.193$).

Conclusions: The results suggested that myeloma cells may affect the production of BSP and OPN, which possibly contributes to osteoclastic bone resorption in MM-I patients. Their levels may be a useful biomarker for assessing bone destruction in MM-I patients and distinguishing MM-I from healthy individuals.

KEY WORDS: Bone sialoprotein, multiple myeloma stage I, osteopontin, β2-microglobulin

INTRODUCTION

Multiple myeloma (MM) is a malignant disorder characterized by the proliferation of a single clone of plasma cells derived from B cells in the bone marrow. Frequently, there is invasion of the adjacent bone, which destroys skeletal structures and results in bone fractures and pain. Occasionally, plasma cells infiltrate multiple organs and produce a variety of symptoms.[1]

MM accounts for >1% of all cancer diagnosis. A major aspect of MM is osteolytic bone disease, present in about 75% of patients at diagnosis. In addition, to increase bone resorption, a significant decrease in bone formation by osteoblasts is observed in MM, further disrupting the normal coupling of bone formation and resorption. As a result, bone lesions do not normally heal even if MM goes into remission after high-dose chemotherapy. Thus, investigators have recently explored the possibility of monitoring the myeloma bone disease through markers on bone resorption and formation in an effort to improve the assessment of disease progression.[2]

Human bone sialoprotein (BSP), a 33 kDa glycoprotein, is a major noncollagenous extracellular protein of mineralized tissues such as bone, dentin, cementum, and calcified cartilage.[3] BSP has an apparent molecular weight of 60–80 kDa due to extensive posttranslational modifications including N- and O-linked glycosylation, serine and threonine phosphorylation, tyrosine sulfation, and sialylation. BSP is produced by osteoblasts, osteoclasts, osteocytes, and hypertrophic chondrocytes during bone morphogenesis.[4] The high glutamic acid content of BSP (22%) suggests it is the focal point for mineralization of hydroxyapatite during bone formation.[5] The activity of BSP in bone

Cite this article as: Maaroufi A, Khadem-Ansari MH, Khalkhali HR, Rasmi Y. Serum levels of bone sialoprotein, osteopontin, and β2-microglobulin in stage I of multiple myeloma. J Can Res Ther 2020;16:98-101.

Submitted: 22-Jun-2016
Revised: 30-Jun-2018
Accepted: 10-Nov-2018
Published: 29-Jul-2019

Access this article online
Website: www.cancerjournal.net
DOI: 10.4103/jcrt.JCRT_666_16
Quick Response Code:

homeostasis may be dependent on additional regulatory factors in the bone microenvironment. For example, Xu *et al*.[6] and colleagues report that BSP–collagen implants placed into surgically created rat calvarial defects stimulate osteoblast differentiation and bone repair.[6]

Osteopontin (OPN) is a secreted phosphoglycoprotein, originally isolated from the bone extracellular matrix.[7] OPN is now suggested to be involved in normal tissue remodeling processes such as bone resorption, angiogenesis, wound healing, and tissue injury as well as certain diseases such as vascular restenosis, atherosclerosis, renal diseases, and tumorgenesis.[8]

β2-microglobulin (β2M) is a low-molecular-weight protein synthesized by nucleated cells and originally isolated from human urine. Its structure is similar to the constant region of immunoglobulin molecules.[9] β2M has been proposed as a prognostic factor in MM, but β2M levels are reported to correlate with other prognostic indicators such as stage and other proteins levels.[10]

In the current study, we aimed to determine the levels of BSP, OPN, and β2M in MM-I patients.

SUBJECTS AND METHODS

The study includes 30 MM stage I patients (14 females/16 males; aged 60.83 ± 12.38 years) diagnosed by the Department of Oncology, Faculty of Medicine, Urmia University of Medical Sciences (UMSU), Urmia, Iran, and 40 sex- and age-matched (18 females/22 males; aged 57.65 ± 6.43 years) healthy individuals as controls.

In all individuals, complete histories were taken at the time of sample collection. Before study entry, none of the patients had been treated with chemotherapy.

Patients with known coronary or peripheral vascular disease, ecstatic coronary arteries, nonischemic dilated cardiomyopathy, renal and hepatic dysfunction, evidence of ongoing infection or inflammation, hematological disorders, known malignancy, and diabetes mellitus were excluded from the study. Written informed consent was obtained from all participants before the collection of blood samples. The study was approved by the Local Ethics Committee and performed in accordance with the UMSU protocols. Patients were staged using Durie-Salmon staging system.[11] In the control group, healthy people do not have any disease and drug consumption. In patients' group, the status of bone marrow, number of plasma cells and amount of urinary proteins were checked. Ten milliliters of whole-blood samples was collected from the basilic vein into tubes. The serum samples were stored at −40°C until the time of examination. At the time of the test, the serum samples were defreezed and centrifuged at 800 g for 20 min at room temperature. Blood proteins' of MM-I patients were checked using capillary zone electrophoresis and it showed a special pattern in blood proteins. In these patients in gamma fraction is a peak in comparing with normal people that can use in diagnosing of MM [Figures 1 and 2]. BSP, OPN, and β2M levels were measured by enzyme-linked immunoassay sorbent assay based on the biotin double-antibody sandwich technology (Bioassay Technology Laboratory, China).

The data were analyzed using SPSS statistical software: Version 22 (IBM Co., Chicago, USA). To check the normality of the distribution, Kolmogorov–Smirnov test was performed. In case of a normal distribution, the *t*-test was used. All values were expressed as means ± standard deviation. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

In MM Stage I and control groups, the mean differences in levels of BSP and OPN were statistically significant. Changes of β2M level were statistically not significant.

There was a significant increase in the BSP levels of MM-I patients versus controls (29.24 ± 5.57 vs. 20.89 ± 3.67; $P = 0.001$) [Figure 1]. A significant decrease in OPN level of patients versus control individuals (12.03 ± 3.45 vs. 19.35 ± 4.67, $P = 0.001$) was observed [Figure 2]. The differences in the β2M level were statistically insignificant between patients' and control groups (1.49 ± 0.67 vs. 1.29 ± 0.55.; $P = 0.193$) [Figure 3].

DISCUSSION

Progressive MM is associated with both suppression of bone formation and stimulation of bone resorption as shown by histomorphometric studies.[12] This uncoupling of bone turnover is thought to be responsible for the development of osteolytic lesions. OPN and BSP are two bone matrix proteins with significant roles in normal bone metabolism. Pathological studies suggest a routine relevancy between levels of BSP, OPN,

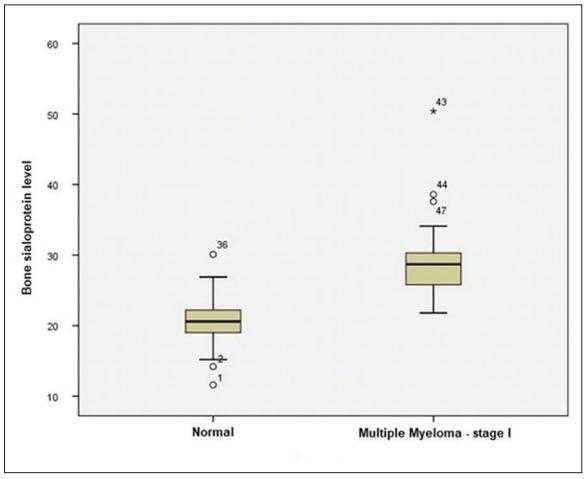

Figure 1: Changes of bone sialoprotein level in the multiple myeloma-I patients

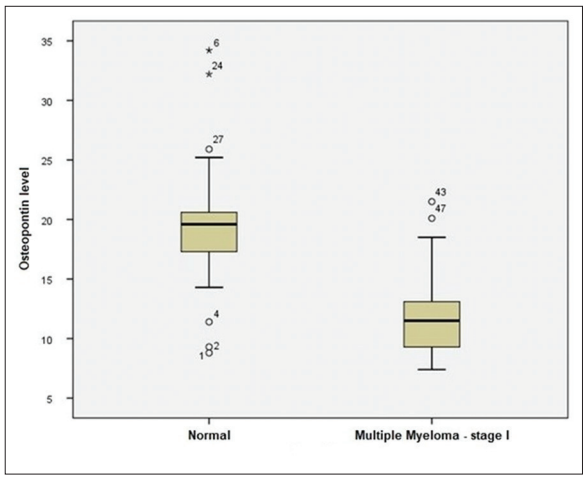

Figure 2: Changes of osteopontin level in the multiple myeloma-I patients and normal people and normal people

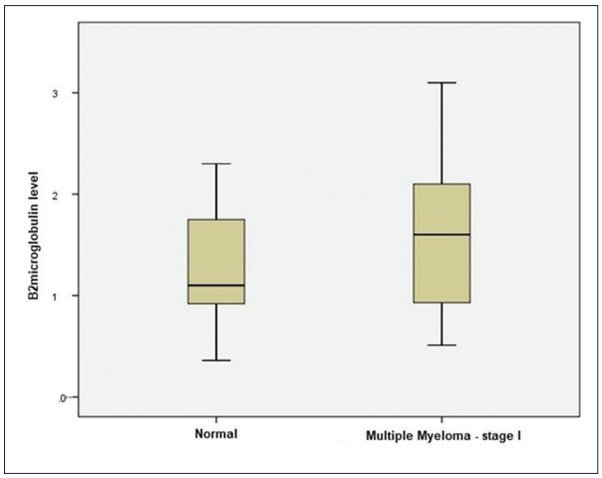

Figure 3: Changes of β2-microglobulin levels in the multiple myeloma-I patients and normal people

β2M and the stage I of MM. Woitge *et al*.[13] showed that BSP levels correlated with the bone marrow plasma cell content, and MM patients with normal baseline BSP levels survived longer than patients with initially elevated BSP values that indicate that BSP has an important role in early diagnosis and treatment. BSP has also been related to a higher risk for development of bone metastases and has been implicated in cancer-mediated changes in bone metabolism.[14] Significantly higher serum BSP values were found in tumor patients with bone metastasis than in those without bone metastasis. Compared with other bone matrix proteins, BSP is relatively restricted to the bone. However, BSP immunoreactivity has also been found in several other tissues.[15] Studies about cancer cell of bone suggest that BSP and OPN have a close relationship. Carlinfante *et al*.[14] showed that the pattern of OPN/BSP expression could be an important determinant for the different characteristics of two types (breast tumors and prostate tumor) of bone metastasis.[14] OPN was first characterized as a transformation-related phosphoprotein, and its expression is increased in several forms of cancer. In our research myeloma cells may preferentially grow in the bone marrow, and it is reasonable to believe that the myeloma cells in some ways instruct the bone marrow environment to help the expansion of the malignant clone. By causing the stromal cells to produce more OPN, the myeloma cells possibly make the bone marrow environment favorable for retention and growth of the tumor cells.[16] Tanaka *et al*.[17] showed that osteoclast-derived OPN and vascular endothelial growth factor from myeloma cells cooperatively enhance angiogenesis and also induce osteoclastogenic activity by vascular endothelial cells. These observations suggest the presence of a close link between myeloma cells, osteoclasts, and vascular endothelial cells to form a vicious cycle between bone destruction, angiogenesis, and myeloma expansion.[17] Expansion of MM bone disease is associated with an enhancement of angiogenesis around MM cells. OPN is a well-known multifunctional factor involved in various aspects of cancer progression, including MM. These factors trigger growth, survival, and migration of multiple myeloma cells[18] increase angiogenesis as well as osteoclastic bone resorption.[19] Abnormalities in the expression and signaling pathway of OPN can, therefore, play an important role in the development and progression of MM.[17]

Based on the observed levels of OPN, it could be an important protein in MM. We also demonstrated that the plasma BSP and OPN levels of the patients with MM correlated with both the progression and bone destruction of the disease. Serum β2M has recently been shown to be a powerful, although nonspecific, marker of MM disease activity. The nature of cells which produce high levels of β2M remains obscure in MM. Nakao *et al*.,[20] showed serum level of β2M increased in broad spectrum of patients with haematological malignancies, however the incidence of elevated serum β2M was higher in patients with neoplasms of B cell lineage such as MM, B cell chronic lymphocytic leukemia and non-Hodgkin's B cell lymphoma..[20]

In predicting the progression of the disease and better identifying the MM, mixing the above factors with molecular pathways will be more interesting and useful, and relevant researches are widespread.

CONCLUSION

The present findings suggest that the plasma OPN, BSP, and β2M levels may be useful biomarkers for both assessment of bone destruction and prognosis of MM stage I, although further investigations are necessary.

Acknowledgments

This work is derived from a Master of Science thesis in Biochemistry and supported by a research grant from the UMSU, Urmia, Iran. We would like to acknowledge the individuals that participated in this research. We thank our colleagues for their helpful.

Financial support and sponsorship

This work is supported via Research Administration in UMSU.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia 2009;23:3-9.
2. Terpos E. Biochemical markers of bone metabolism in multiple myeloma. Cancer Treat Rev 2006;32 Suppl 1:15-9.
3. Fisher LW, McBride OW, Termine JD, Young MF. Human bone sialoprotein. Deduced protein sequence and chromosomal localization. J Biol Chem 1990;265:2347-51.
4. Fisher LW, Fedarko NS. Six genes expressed in bones and teeth encode the current members of the SIBLING family of proteins. Connect Tissue Res 2003;44 Suppl 1:33-40.
5. Fisher LW, Torchia DA, Fohr B, Young MF, Fedarko NS. Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. Biochem Biophys Res Commun 2001;280:460-5.
6. Xu L, Anderson AL, Lu Q, Wang J. Role of fibrillar structure of collagenous carrier in bone sialoprotein-mediated matrix mineralization and osteoblast differentiation. Biomaterials 2007;28:750-61.
7. Oldberg A, Franzén A, Heinegård D. Cloning and sequence analysis of rat bone sialoprotein (osteopontin) cDNA reveals an arg-gly-asp cell-binding sequence. Proc Natl Acad Sci U S A 1986;83:8819-23.
8. Soriano P, Montgomery C, Geske R, Bradley A. Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 1991;64:693-702.
9. Bataille R, Grenier J, Sany J. Beta-2-microglobulin in myeloma: Optimal use for staging, prognosis, and treatment – A prospective study of 160 patients. Blood 1984;63:468-76.
10. Greipp PR, Katzmann JA, O'Fallon WM, Kyle RA. Value of beta 2-microglobulin level and plasma cell labeling indices as prognostic factors in patients with newly diagnosed myeloma. Blood 1988;72:219-23.
11. Durie BGM, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer 1975;36:842-54.
12. Bataille R, Chappard D, Marcelli C, Dessauw P, Sany J, Baldet P, *et al.* Mechanisms of bone destruction in multiple myeloma: The importance of an unbalanced process in determining the severity of lytic bone disease. J Clin Oncol 1989;7:1909-14.
13. Woitge HW, Pecherstorfer M, Horn E, Keck AV, Diel IJ, Bayer P, *et al.* Serum bone sialoprotein as a marker of tumour burden and neoplastic bone involvement and as a prognostic factor in multiple myeloma. Br J Cancer 2001;84:344-51.
14. Carlinfante G, Vassiliou D, Svensson O, Wendel M, Heinegård D, Andersson G, *et al.* Differential expression of osteopontin and bone sialoprotein in bone metastasis of breast and prostate carcinoma. Clin Exp Metastasis 2003;20:437-44.
15. Withold W, Armbruster FP, Karmatschek M, Reinauer H. Bone sialoprotein in serum of patients with malignant bone diseases. Clin Chem 1997;43:85-91.
16. Standal T, Hjorth-Hansen H, Rasmussen T, Dahl IM, Lenhoff S, Brenne AT, *et al.* Osteopontin is an adhesive factor for myeloma cells and is found in increased levels in plasma from patients with multiple myeloma. Haematologica 2004;89:174-82.
17. Tanaka Y, Abe M, Hiasa M, Oda A, Amou H, Nakano A, *et al.* Myeloma cell-osteoclast interaction enhances angiogenesis together with bone resorption: A role for vascular endothelial cell growth factor and osteopontin. Clin Cancer Res 2007;13:816-23.
18. Podar K, Tai YT, Davies FE, Lentzsch S, Sattler M, Hideshima T, *et al.* Vascular endothelial growth factor triggers signaling cascades mediating multiple myeloma cell growth and migration. Blood 2001;98:428-35.
19. Asou Y, Rittling SR, Yoshitake H, Tsuji K, Shinomiya K, Nifuji A, *et al.* Osteopontin facilitates angiogenesis, accumulation of osteoclasts, and resorption in ectopic bone. Endocrinology 2001;142:1325-32.
20. Nakao Y, Matsumoto H, Miyazaki T, Watanabe S, Masaoka T, Takatsuki K, *et al.* Genetic and clinical studies of serum beta 2-microglobulin levels in haematological malignancies. Clin Exp Immunol 1981;46:134-41.