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Prognostic Value of B-2 Microglobulin and Interleukin-6 in Multiple Myeloma

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ABSTRACT

Background: Prognostic markers of multiple myeloma (MM) are still in the process of research, so therapeutic approach is still inadequate for an individual patient. Other prognostic markers for example hemoglobin level, serum calcium, serum creatinine, and severity of bone lesions are of exceeding importance, but are not as simple as standard methods. Worldwide interleukin-6 is known major growth factor for myeloma cells. β2–M is present on the surface of plasma cells. It is also found in increased quantities in the serum and in other fluids from individuals with myeloma.
Methods: A survival analysis carried out in 244 MM patients at diagnosis, samples were collected from all over Pakistan. Serum IL-6 levels were evaluated on commercially available quantikine ELISA human IL-6 immunoassay (Thermo scientific) (USA). Serum β2-M levels were evaluated on commercially available enzyme immunoassay (quantitative) β2-M (Bio Check, Inc) (USA).
Results: Mean serum β2-M level was 4.02 µg/l (Reference range 0-2.0 µg/l) and mean IL-6 level was 269.3 (Reference range 0-149 pg/ml). According to results and outcome of disease process three groups suggested, patients with β2-M ranges in between 1-2 and IL-6 10-100 (Group I), patients with β2-M ranges in between 2.1-3 and IL-6 100.1-200 (Group II), patients with β2-M ranges in between 3.1-4 or more and IL-6 200.1-300 or more (Group III).
Conclusion: β2-M correlates with tumor mass and IL-6 correlates with tumor cell growth, their lower serum levels correlates with a better prognosis, with a longer remission and survival, and higher serum level correlates with poor prognosis.
Keywords: Immunoglobulin, plasmacytosis, osteolytic leision, monoclonal gammopathy, tyrosine phosphorylation.

INTRODUCTION

Multiple myeloma (MM) is the most common B–cell malignancy of neoplastic plasma cells in bone marrow compartment and in extra osseous tissue in a multifocal fashion that synthesize abnormal amount of immunoglobulin (Ig)[1,2]. The unique triad of osteolytic lesions, atypical marrow plasmacytosis, and a monoclonal gammopathy is characteristic[3,4]. The disease spans a spectrum from localized, indolent to disseminated, aggressive forms[5]. IL–6 is produced by variety of cells and B–cells[6]. IL–6 induces (a) terminal differentiation and Ig secretion of B–cells; (b) growth promotion of B–cells, plasma cells, and myeloma cells; (c) support of colony formation by stem cells; (d) induction of acute phase response–proteins (APR–proteins); (e) differentiation and activation of T–cells and macrophages; and (f) neural differentiation[7,8]. It is involve in pathology of MM. It is potent stimulator of terminal B–cell differentiation and antibody formation. The IL–6 is involved in proliferation of plasmablasts and differentiation in to mature plasma cells, acting by an autocrine or paracrine mechanism. In addition to its proliferative activity, it blocks the apoptotic pathway. The glycoprotein 130 (gp130) is activated sometimes in absence of direct ligand binding, i.e., independent of IL–6R and IL–6. IFN–α induce tyrosine phosphorylation of gp130 in myeloma cell lines that are responsive to IL–6[9,13]. β2–M is present on the surface of plasma cells. It is also found in increased quantities in the serum and in other fluids from individuals with myeloma[10,11,12]. Serum β2–M levels reflect tumor cell (plasma cells) burden and is the strongest and most reliable marker for MM[13,14].

MATERIAL AND METHODS

This is descriptive and analytical cross–sectional study performed on serum from MM patients in Hematology Department, of Sheikh Zayed Hospital lahore and in other hospitals of Pakistan. A total number of 244 patients included in the present study. Patients those included in study were diagnosed patients of MM who did not receive treatment are of either sex, and patients those excluded in this study who have associated acute infection, cardiovascular

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disease, and renal disease before diagnosis of MM. Patients with asymptomatic (smoldering) myeloma, IgM related disorders or with primary amyloidosis were not included. The test requires 5ml of blood. All sera were stored at –20°C before measurement. The Sample size was calculated by using the software “Power and sample size” at 5 % level of significance and 80% power of test. The calculated sample size at 20 % expected exposure and with 1.8 expected odd ratio of having patients with conventional prognostic factors as compare to proposed IL-6, and β2–M. Sampling technique was non probability purposive sampling. The data entered and analyzed by using Statistical Package for the Social Sciences (SPSS) Version 15.0. The study variables will be severity and patient life span. Quantitative variables IL-6, β2–M described by using mean ± S.D. Qualitative variables, symptoms, sign described by using frequencies and percentages. Comparison of means of IL-6, β2–M were analyzed by using analysis of variance (ANOVA). Qualitative variables like symptoms and signs compared with IL-6, β2–M by using chi–square test. A p–value of ≤ 0.05 will be considered as statistically significant.

Serum IL-6 levels were evaluated on commercially available quantikine ELISA human IL-6 immunoassay (Thermo scientific) (USA) This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped and the intensity of the color absorbance is measured at 540 nm or 570 nm[15].

Serum β2-M levels were evaluated on commercially available enzyme immunoassay (quantitative) β2-M (BioCheck, Inc) (USA). The BioCheck β2-M ELISA test is based on the principle of a solid phase ELISA. The assay system utilizes a monoclonal anti-β2-M antibody for solid phase immobilization (on the microtiter wells). A sheep anti-β2-M antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The diluted test sample is allowed to react first with the immobilized antibody for 30 minutes at 37° C. The sheep anti-β2-M HRPO conjugate is then added and reacted with the immobilized antigen for 30 minutes at 37° C, resulting in the β2-M molecules being sandwiched between the solid phase and enzyme linked antibodies. The wells are washed with water to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes at room temperature, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, changing the color to yellow. The concentration of β2-M is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm[16].

RESULTS

Mean age of the 244 patients was 56.68 years, 147/244 (60.25%) of patients were male and 97/244 (39.75%) of patient were female. Male female ratio was 1.52:1. 163/244 (66.8%) had IgG isotype, 53/244 (21.72%) had IgA isotype, 6/244 (2.45%) had light–chain isotype, 20/244 (8.19%) had IgD isotype, and 2/244 (0.81%) had biclonal or other isotype. Mean serum M–protein level was 4.54g/dl. 190/244 (77.86%) of patients had sign and symptoms of hyperviscosity. Mean Hb level in male was 10.26 g/dl and in females was 10.36 g/dl, 233/244 (95.49 %) of patients had anemia. Mean creatinine level was 2.68mg/dl, renal function was compromised in 240/244 (98.4 %) of patients and only 4/244 (1.63 %) patients has normal renal function. Mean Ca level was 10.85 mg/dl, 189/244 (63.21 %) of patients had sign and symptoms of hypercalcemia. Mean bone marrow plasma cell percent was 14.18. Total 137/244 (56.14 %) patients had pathologic fractures out of which 13/137 (9.48 %) patients had three or more bone lesions (pathological fractures), 81/137 (59.12 %) patients had two bone lesions (pathological fractures), 43/137 (31.38 %) patients had one bone lesion (pathological fracture), and 107/244 (43.85 %) patients had no bone lesion (pathological fractures), All patients (244) had complain of bone pain out of which 20/244 (8.19 %) had moderate and 224/244 (91.8%) had severe bone pain. Bleeding diathesis was found in 11/244 (4.5 %) patients, out of which 2/244 (0.81%) patients in stage II and 9/244 (3.68 %) patients in stage III. Mean serum β2-M level was 4.02 µg/l (Reference range 0-2.0 µg/l) and mean IL-6 level was 269.3 (Reference range 0-149 pg/ml)

According to results of β2-M IL-6 and their life span MM pts distributed in to three groups

Groups	β2-M µg/l	IL-6 µg/l	Life span (Mean)
Group I	1-2	10-100	5.2 Y (62 M)
Group II	2.1-3	100.1-200	3.5 Y (41 M)
Groups III	3.1-4 or more	200.1-300 or more	2.1 Y (25 M)

DISCUSSION

The existence of β2-M on the surface of the myeloma cells categorize tumor mass. Its relationship can be limited to the estimation of certain parameters: the degree of bone marrow plasmacytosis, synthesis rate of myeloma immunoglobulins, and the extension of bone lesions. The higher levels of β2-M showed a direct strong correlation with marrow plasma cells, and monoclonal immunoglobulin. [10] IL-6 is a pleotropic cytokine with a broad range of biologic effects. The well known function of IL-6 is; it work as growth factor for myeloma cells, through autocrine or paracrine fashion. [9]

To assess the growth of myeloma cells we perform β2-M, IL-6, and survival times of myeloma patients with regard to their serum levels of β2-M, and IL-6 at the time of diagnosis and after chemotherapy. 244 patients with multiple myeloma or plasma cell leukemia were studied. Survival was 62, 41, and 25 months, respectively *($P < .0001$)*. We thus propose a new and powerful myeloma prognostic factors based on simple and reliable laboratory evaluations. So according to results and outcome of disease process three groups suggested, patients with β2-M ranges in between 1-2 and IL-6 10-100 (Group I), patients with β2-M ranges in between 2.1-3 and IL-6 100.1-200 (Group II), patients with β2-M ranges in between 3.1-4 or more and IL-6 200.1-300 or more (Group III). From these results it is obvious that patients whose serum levels of β2-M and IL-6 are increased their life expectancy is less.

CONCLUSION

β2-M significantly correlates with tumor mass both at the time of diagnosis and at the end of therapeutic protocol. β2-M together with the clinical criteria for the disease staging enable a better prognostic orientation; lower serum levels correlates with a better prognosis, with a longer remission and survival, and higher serum level correlates with poor prognosis, with less survival expectancy. IL-6 significantly correlates with tumour cell growth at the time of diagnosis and at the end of therapeutic protocol. IL-6 together with the clinical criteria for the disease staging enable a better prognostic orientation; lower serum levels correlates with a better prognosis, with a longer remission and survival, and higher serum level correlates with poor prognosis, with less survival expectancy.

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