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Mie Afify
Department of Biochemistry,
National Research Center,
Cairo, Egypt

Tel: 002-012-7889660

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Clinical Evaluation of Some Biochemical Markers in Multiple Myeloma among Egyptian Patients

[1]Magda Sayed, [1]Mie Afify Mohammed and [2]Ola Khorshid

The objective of the current study was to evaluate the circulating levels of mediators such as interleukin-6 (IL-6), β-2 microglobulin (β2-M), lactate dehydrogenate (LDH), total alkaline phosphatase (TALP), bone alkaline phosphatase (BALP) and C-reactive protein (CRP) before and after treatment in patients with MM. Testing these biochemical markers might help in the identification, staging, determining its severity and monitoring the response to treatment of the myeloma patients. It was determined serum levels IL-6, β2-M, LDH, TALP, BALP and CRP in 28 newly diagnosed (group I) and 23 after treatment (group II) in patients with MM. The mean age of the patients was 59.63 and 61.55 years, respectively. All patients were in stage III (classified according to the Durie-Salmon classification). The same parameters were measured in 15 healthy controls with a mean age of 58.06 years. The results showed that the serum levels of IL-6 (pg mL^{-1}), beta-2 microglobulin (μg mL^{-1}), CRP (mg mL^{-1}) and LDH (U L^{-1}) were increased in-group (I) and this increment were highly significant ($p<0.001$) on comparison with the control group and group (II). The serum level of TALP and BALP (U L^{-1}) were increased in-group (I) and this increment was slightly significant ($p<0.05$) on comparison with the control group and group (II). All of the parameters were found to be significantly reduced after chemotherapy treatment. The serum level of T ALP in-group (II) decreased but did not reached the normal value and there was significant increased as compared to the control group ($p<0.05$). There were a significant positive correlation ($p<0.05$) between serum level of IL-6 and beta-2 microglobulin, CRP, LDH, TALP and BALP, the r-values were 0.7319, 0.6875, 0.7528, 0.8119 and 0.7072, respectively in-group (I). In conclusion, It was found that after the therapy, the levels of these parameters, which are thought to play an important role in the pathogenesis of MM, were significantly suppressed denoting their importance in the prognosis and monitoring the MM disease.

Key words: Multiple myeloma, biochemical markers, interleukin-6, β2-microglobulin, LDH

[1]Department of Biochemistry, National Research Center, Dokki, Egypt
[2]Department of Medical Oncology, National Cancer Institute,
Cairo University, Cairo, Egypt

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INTRODUCTION

Multiple Myeloma (MM) (also known as plasma cell myeloma) is a progressive hematological disease. It is a cancer of the plasma cell, which is considered as an important part of the immune system that produces immunoglobulin (antibodies) to help fight infection and disease. MM is characterized by excessive numbers of abnormal plasma cells in the bone marrow and overproduction of intact monoclonal immunoglobulin (IgG, IgA, IgD, or IgE) or Bence-Jones protein (free monoclonal κ and λ light chains). Plasma cells usually make up less than 5% of the cells in the bone marrow. However, in MM, a group of abnormal plasma cells (myeloma cells) multiplies, raising the percentage of plasma cells to more than 10% of the cells in the bone marrow (Kuku *et al*., 2005).

The etiology of MM is unknown; however, a multi-step process has been suggested (mutation-induced genetic abnormalities, chromosomal translocations, viral triggers). Common clinical manifestations of MM are bone pain, tenderness (especially back, ribs; made worse by movement), fracture, hypercalcaemia, anemia, renal damage, increased susceptibility to bacterial infection and impaired production of normal immunoglobulin. It is often also characterized by diffuse osteoporosis. MM is the second most prevalent blood cancer after non-Hodgkin's lymphoma. It represents approximately 1% of all cancers and 2% of all cancer deaths. The male-to-female ratio is 3:2; the median age of patients is 68 years for men and 70 years for women, only 3-5% of patients with MM are younger than 45 years. MM has a general median survival of three years. The disease is rare in children (Rajkumar and Kyle, 2005).

Bone marrow stromal cells and myeloma cells produce several proinflammatory cytokines that play an important role in the pathogenesis of MM. Of these, Interleukin-6 (IL-6) is known as a growth and survival factor in MM via activation of extracellular signal-regulated kinase and phosphatidylinositol 3-kinase signaling cascade. Feng *et al*. (2006) reported that, human interleukin-6 is involved in the maintenance and progression of several diseases such as MM, rheumatoid arthritis, or osteoporosis. Another proinflammatory marker such as C-Reactive Protein (CRP) is produced by hepatocytes in response to inflammation and infection and strongly correlated with proinflammatory cytokines particularly IL-6, also may be used to determine prognosis in patients with MM (Hsu *et al*., 2004).

The most significant factor among all parameters is beta 2 microglobulin levels (β2-M), which correlate with the Durie and Salmon (1975) clinical staging system for assigning prognosis, as it was proved that β2-M is a protein on the cell surface of myeloma and other cells (Rajkumar and Greipp, 1999). There are several important prognostic factors identifying patients with poor outcomes: Serum β2-M, bone marrow Plasma Cell Labeling Index (PCLI), cytogenetics, plasmablastic morphology, lactate dehydrogenase (LDH) and C-Reactive Protein (CRP) (Biro *et al*., 1998).

Osteolytic bone disease is a major clinical feature of MM. Mechanisms of bone destruction are related to increased osteoclastic activity, which is not accompanied by a comparable increase in bone formation, as osteoblasts are functionally exhausted ALP is a sensitive and reliable indicator of bone metabolism. Although a direct product of the osteoblast, the cell that forms bone during bone remodeling, bone ALP reflects overall bone turnover when the bone resorption and formation processes remain coupled (Terpos, 2006).

The objective of the current study was to evaluate the circulating levels of mediators such as IL-6, LDH, TALP, BALP, β2-M and CRP before and after treatment in patients with MM. Testing these biochemical markers might help in the identification, staging, determining its severity and monitoring the response to treatment of the myeloma patients.

MATERIALS AND METHODS

After approval a medical and ethical committee of the National Cancer Institute, this study was conducted on 28 patients suffering from MM selected from the out-patients of Medical Clinic and in-patients sections of Medical Oncology Department of National Cancer Institute, Cairo University during the period between February and August 2006, their age ranged from 48-72 years. An informed consent was taken from all patients included in the study. They were diagnosed as MM patients stage II according to Durie and Salmon Classification, as the criteria of complete blood picture, % of plasma cells in bone marrow examinations, histopathology, protein electrophoresis and immuno-electrophoresis. Those patients were classified into two groups:

Group I (G I): Included 28 newly diagnosed and before treatment patients with MM, their age ranged from 48-71 years with a mean of 59.63 years, they were 18 males and 10 females.

Group II (G II): Included 23 patients with MM after the end of treatment and coming for follow up. There were 5 patients missed in the study at the end of treatment

(three patients were died during therapy and two patients did not continue the treatment), their age ranged from 50-71 years with a mean of 61.55 years, they were 14 males and 9 females.

Besides, 15 healthy, age and sex-matched persons were also included as a control group, their age ranged from 49-70 years with a mean of 58.06 years, they were 10 males and 5 females.

Durie-Salmon Staging system for MM

Stage I: MM cell mass <0.6 cells × 10^{12} m^{-2}
All of the following features are present:

- Hemoglobin value >10 g dL^{-1} (100 g L^{-1})
- Serum calcium value normal (<12 mg dL^{-1} [2.99 mmol L^{-1}])
- Normal bone structure on radiographs (grade 0) or solitary plasmacytoma only
- Low M-component production rates (IgG value <50 mg dL^{-1} [0.5 g L^{-1}], IgA value <30 mg dL^{-1} [0.3 g L^{-1}], urine light chain M component on protein electrophoresis <4 g per 24 h)

Stage II: MM cell mass 0.6 to 1.2 cells × 10^{12} m^{-2}
Features fitting neither stage I or stage III

Stage III: MM cell mass >1.2 cells × 10^{12} per m^2
One or more of the following features are present:

- Hemoglobin value <8.5 g dL^{-1} (85 g L^{-1})
- Serum calcium value >12 mg dL^{-1} (2.99 mmol L^{-1})
- Advanced lytic bone lesions (grade 3)
- High M-component production rates (IgG value >70 mg dL^{-1} [0.7 g L^{-1}], IgA value >50 mg dL^{-1} [0.5 g L^{-1}], urine light chain M component on protein electrophoresis >12 g per 24 h)

Sub classification:

- Relatively normal renal function (serum creatinine <2 mg dL^{-1})
- Abnormal renal function (serum creatinine >2 mg dL^{-1})

Adapted from Durie and Salmon (1975)

Sampling: Fasting blood samples were collected from each patient before and after the treatment. The blood samples were allowed to clot and sera separated by centrifugation at 3000 rpm for 10 min. The serum samples were either analyzed immediately or kept at -20°C until the time of analysis.

All cases were subjected to the following investigations.

- Full clinical history and thorough clinical examination.
- Skeletal X-ray and Bone scan
- **Laboratory investigations:**
  - Hemogram: Included hemoglobin concentration, Total leucocytic count, Platelet count using coulter counter and examination of Lishman or Wrght-stained peripheral blood smears.
  - Bone marrow aspirations and examination of Leishman stained Bone Marrow (BM) films for all the patients.
  - Determination of serum IL-6 levels by a solid phase Enzyme Amplified Sensitivity Immuno-Assay (EASIA) performed on microtitre plate using kit supplied by Biosource Europ S.A., Rue de industries, 8B-1400 Nivelles Belgium (Le Moine *et al*., 1994).
  - Determination of serum β2-microglobulin using quantitative test kit based on a solid phase enzyme linked immunosirbent assay (EIA kit) supplied by Immunos cat. No. KH5005 (Bergard and Beam, 1968).
  - Determination of C-Reactive Protein (CRP) by a high sensitive immunoassay for measuring human CRP which is a two step sandwich ELISA technique using kit supplied by Diagnostic System Laboratories (DSL-10-42100) Webster, Texas, USA (Rifai *et al*., 1999).
  - Serum total alkaline phosphatase (TALP) enzymatic activity was measured kinetically according to (Bowers and Mc Comb, 1966).
  - Determination of bone isoenzyme of alkaline phosphatase (BALP) by using quantitative sandwich enzyme immunoassay technique (ELISA) using kit supplied by Quiedel Com. San Diego. USA (catalog. No. 8012) according to (Price, 1993).
  - Determination of serum total lactate dehydrogenase (LDH) enzymatic activity at 37°C according to (Gay *et al*., 1968).
  - Alkaline phosphatase isoenzyme patterns were performed on vertical 7% (W/V) polyacrylamide slab gel electrophoresis in tris-borate buffer pH 9.5 (0.38 mol L^{-1} tris gel electrophoresis in $MgCl_2$ and adjusted by 2% boric acid till pH 9.5) using a method modified from that of (Akroyed, 1967).
  - Determination of serum creatinine level according to the Jaffe reaction (Husdan and Rapoport, 1968).

Protocol of therapy: Initial therapy is aimed at treating symptoms and reducing the burden of disease. Patients

with MM above the age of 55 years and not eligible for bone marrow transplantation the protocol of treatment given was melphalan plus prednisone to palliate symptoms in these patients who cannot tolerate aggressive therapy (Melphalan (8 mg/m2/d PO) + prednisone (100 mg d^{-1} PO) on days 1-4 every 4-5 weeks). For patients younger than 55 years, a combination of alkylating agents (VAD) which consists of Vincristine, doxorubicin (adriamycin) and dexamethasone were given. Patient were given vincristine 0.4 mg total dose and doxorubicin 9 mg/m2 both given by continuous IV infusion for four days, either through a pump or as an infusion. Patients were also given dexamethasone 40 mg/m2 for days 1-4 and 9-12 and 17-20 every 4 weeks. (Dexamethasone given on days 9-12, 17-20 was given in the first cycle only. .Usually 4-6 cycles are given over a period of 4-6 months, depending on patient's response after the 3rd cycle.

Patients achieving complete remission and have a donor were sent for reduced intensity allogenic stem cell transplantation; if no donor is available they were to go for tandem autologus stem cell transplant. Plasmapheresis can be used to treat protein proliferation (Hyperviscosity syndrome).

Statistical methods: Data were presented as mean±standard deviation. Variable normality analysis was performed. Comparison of means was calculated by parametric unpaired t-test. 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 $p<0.05$. All the above-mentioned analysis was performed using the Standard Package for Social Sciences (SPSS) version 9.0 for Windows (SPSS, Chicago, Ill).

Notice that, there is decrease in the band of bone alkaline phosphatase in the MM patients than the control. The anodal situated zone is traces of LALP, while the less anodal is the BALP with IALP (as one band due to the IALP is not affected by neuraminidase treatment).

RESULTS

Table 1 Summarizes the characteristics of patients with MM (before and after treatment) and the control group, all groups well matched for age and sex. As regards to the manifestation of the disease, bony pains represent 32.14 and 8.69%, generalized malaise present in 50 and 21.74%, infection 28.57 and 4.35%, fever 46.42 and 0%, bleeding 21.43 and 0%, peripheral neuropathy 3.57 and 0% in group I and group II, respectively. The incidence of anemia were 75 and 8.69% in group (I) and (II) with mean value of Hb% 8.2 and 10.7, respectively, while there is no renal insult in all patients as evident by normal range of creatnine in the all studied groups.

Table 2 showed the descriptive statistic (mean±SD) of serum levels of IL-6 (pg mL^{-1}), β2-microglobulin (μg mL^{-1}), CRP (mg mL^{-1}), LDH (U L^{-1}), TALP (U L^{-1}) and BALP (U L^{-1}) in the various studied groups.

There are marked increased in the serum levels of IL-6 (pg mL^{-1}), β2-microglobulin (μg mL^{-1}), CRP (mg mL^{-1}) and LDH (U L^{-1}) in-group (I) and this increment were highly significant ($p<0.001$) on

Table 1: Patients characteristic and clinical manifestation of MM

Characteristics	Group (I) n = 28	Group (II) n = 23	Control group n = 15
Age			
Range	48-72	50-71	49-70
Mean	59.63	61.55	58.06
Sex			
M:F	18:10	14:9	10:5
Symptoms			
Anemia	21 (75)	2 (8.7)	----
Bony pains	9 (32.1)	2 (8.7)	----
Generalized malaise	14 (50)	5 (21.7)	----
Infections	8 (28.6)	1 (4.4)	----
Fever	13 (46.4)	----	----
Bleeding	6 (21.4)	----	----
Neurological symptoms			
Peripheral neuropathy	1 (3.57)	----	----
Meningitis	----	----	----
Hb % (g %)			
Range	5.4-10.8	9.1-11	10-14
Mean±SD	8.2±1.2	10.07±1.6	11.913±1.89
Creatinine (mg mL^{-1})			
Range	0.6-0.92	0.4-0.8	0.5-0.95
Mean±SD	0.8±0.13	0.61±0.09	0.7±0.12

M/F male over female ratio, Group (I) patients at diagnosis and before the treatment, Group (II) patients after the end of treatment and during follow up, Values in parentheses show percentage

Table 2: Serum levels of the biochemical markers and it's significant in the studied groups

	Control	Group (I)	Group (II)	p-value
IL-6 (pg mL^{-1})				
Range	7.1-16.2	316-565	11-15.1	C vs. (I) <0.0001†
Mean±SD	11.64±2.949	358±55.90	12.1521±1.2862	C vs. (II) 0.639486
				(I) vs. (II) <0.0001†
β2-M (μg mL^{-1})				
Range	2.3-5.8	19-28	2.8-12	C vs. (I) <0.0001†
Mean±SD	4.1166±1.354	22.392±2.793	5.813±1.9449	C vs. (II) 0.05866
				(I) vs. (II) <0.0001†
CRP (mg mL^{-1})				
Range	0.2-0.9	20-45	0.19-1.4	C vs. (I) <0.0001†
Mean±SD	0.4866±0.2012	28.025±4.8981	0.4773±0.2997	C vs. (II) 0.37037
				(I) vs. (II) <0.0001†
LDH (U L^{-1})				
Range	90-130	90-420	98-136	C vs. (I) 0.00103†
Mean±SD	101.6±9.832	192.678±56.5409	107.913±9.7789	C vs. (II) 0.2124
				(I) vs. (II) 0.00164†
TALP (U L^{-1})				
Range	45-89	41-58	34-67	C vs. (I) 0.1000
Mean±SD	56.73±12.91	50.347±6.38	49.93±11.62	C vs. (II) 0.0916
				(I) vs. (II) 0.871
B. ALP (U L^{-1})				
Range	30-55	30-45	30-45	C vs. (I) 0.12689
Mean±SD	40.266±5.54	37.521±4.17	37.5±5.43	C vs. (II) 0.1139
				(I) vs. (II) 0.9871

Il-6 = Interleukin-6, β2-M = β2-microglobulin, CRP = C Reactive protein, LDH = Lactate dehydrogenase, TALP = Total alkaline phosphatase, BALP = Bone alkaline phosphates. † = Highly significant p<0.001

Table 3: Correlations between serum IL-6 and β2-microglobulin with the other parameters in group I (r- value)

Variables	β 2-microgl (μg mL^{-1})	CRP (mg mL^{-1})	LDH (U L^{-1})	TALP (U L^{-1})	BALP (U L^{-1})
IL-6 (pg mL^{-1})	0.7319*	0.6875*	0.7528*	0.05503	0.01465
β2-microgl. (μg mL^{-1})	--------	0.5191*	0.3595*	0.03992	0.09790

* Significant p<0.05

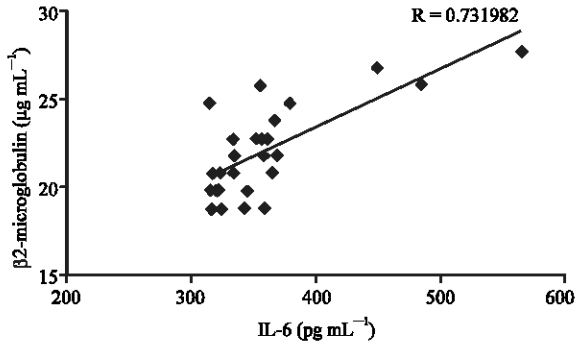

Fig. 1: Correlation between serum IL-6 pg mL^{-1} and β2-microglobulin μg mL^{-1} at the diagnosis

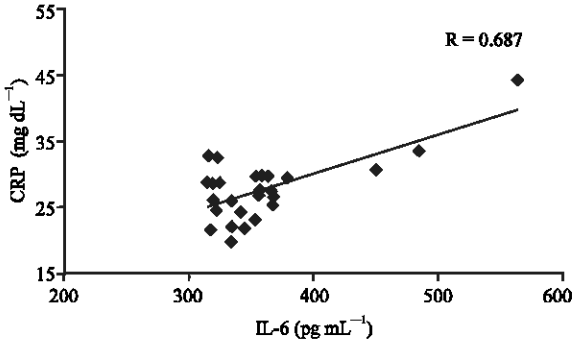

Fig. 2: Correlation between serum levels of IL-6 pg mL^{-1} and CRP μg mL^{-1} in MM at the diagnosis

comparison with the control group and group (II), while the serum levels of TALP and BALP decreased but not significantly (p>0.05) on comparison with the control group. After therapy in group (II) the serum levels of IL-6, β2-microglobulin, CRP and LDH were decreased to nearly normal so, there were non significant difference (p>0.05) between the levels of these markers as compared to the control group, while the serum levels of TALP and BALP decreased below the normal levels but these decrement were not significant as compared to the control group (p>0.05) as shown in Table 2.

Table 3 and Fig. 1 and 2 showed the correlations between serum IL-6 and beta-2 microglobulin with the other parameters in-group (I). There were significant positive correlation (p<0.05) between serum level of IL-6 and each of beta-2 microglobulin, CRP and LDH with the r-values of 0.7319, 0.6875, 0.7528, respectively, but there were no significant correlation (p>0.05) with TALP and BALP with r-values of 0.055 and 0.01465, respectively. There are a significant positive correlation (p<0.05) between serum levels of beta-2 microglobulin and each of CRP and LDH with r-values of 0.5191 and 0.395,

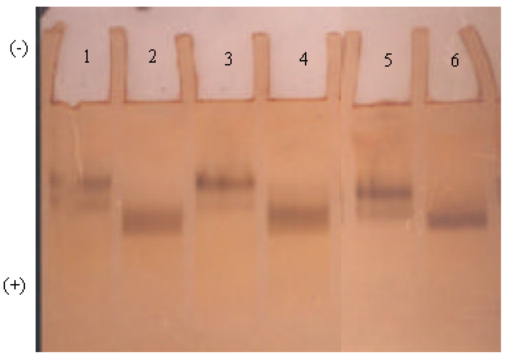

Fig. 3: Polyacrylamide gel electrophoresis of alkaline phosphates isoenzymes: Effect of partial digestion by neuraminidase on electrophoretic mobilities of Liver ALP (LALP) and Bone ALP (BALP). Lane (1): Serum sample from MM patient at diagnosis after partial digestion of the sialic acid residue by neuraminidase treatment. Lane (2): The serum of the same patient with MM before therapy (without neuraminidase treatment), they consist mainly of BALP. Lane (3): Serum sample from MM patient at end of therapy after partial digestion of the sialic acid residue by neuraminidase treatment. Lane (4): The serum of patient with MM at end of therapy (without neuraminidase treatment), they consist mainly of BALP. Lane (5): Serum sample from control after partial digestion of the sialic acid residue by neuraminidase treatment. Lane (6): The serum of the same control person (without neuraminidase treatment), they consist mainly of BALP

respectively, but there were no significant correlation ($p>0.05$) with TALP and BALP with r values of 0.03992 and 0.09796, respectively. Figure 3 showed the decrease in the bone fraction of ALP by polyacrylamide gel electrophoresis of alkaline phosphates isoenzymes in healthy controls and some serum samples of patients with MM.

DISCUSSION

MM is a β-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 and markers that play an important role in the pathogenesis of MM (Rajkumar and Kyle, 2005).

The most significant finding in this study is that serum level of proinflammatory cytokine IL-6 in newly diagnosed (group I) patients was significantly high compared to the normal controls and significantly decreased after the treatment in (group II) patients indicating that IL-6 can be considered as a prognostic marker for evaluation of the response to treatment in MM patients. The predominant source of IL-6 was found to be paracrine in nature, namely bone marrow stromal cells due to adhesion molecules present on myeloma plasma cells that bind to their respective receptors on bone marrow stromal cells (Silvestris *et al*., 2004).

These results were in accordance with several reports; Lauta (2003) stated that IL-6 is a normally occurring growth factor and is required in the development of normal plasma cells. Serum and bone marrow IL-6 levels were found to be elevated in myeloma patients and levels correlate with disease activity and disease status. IL-6 is an important multifunctional proinflammatory cytokine involved in tumor growth and metastasis. Other studies concluded that IL-6 is the major growth and survival factor for MM and has been shown to protect MM cells from apoptosis induced by a variety of agents (Wang *et al*., 2006; Michalaki *et al*., 2004; Klein *et al*., 2003).

However, in the presence of malignant plasma cells, IL-6 induces a significant proliferative response, driving a rapid increase in the number of malignant cells. At elevated levels, soluble interleukin-6 receptors (sIL-6R) amplify the stimulatory effect of IL-6 on malignant plasma cells by a factor of up to 10-fold. High levels of either IL-6 or sIL-6R are predictors of poor outcomes (Rajkumar and Greipp, 1999).

Sfiridaki *et al*. (2005) found that, MM is characterized by accelerated production of the proteolytic enzyme matrix metalloproteinase (MMP)-9. They hypothesized that myeloma-produced MMP-9 may influence the rate of bone turnover in a paracrine manner. Levels of MMP-9 and of IL-6 in MM correlate well with bone turnover rate and may be useful in disease evaluation.

The results of the current study showed a significant positive correlation ($p<0.05$) between serum level of IL-6 and each of beta-2 microglobulin, CRP and LDH, but there were no significant correlation ($p>0.05$) with TALP and BALP. These results were in agreement with Solary *et al*. (1992) who reported that, IL-6 serum level in MM at advanced stages (II/III) and in progressive disease is significantly higher than in patients with MM stage I or at the plateau phase Furthermore, IL-6 serum levels strongly correlate with such disease parameters as bone marrow plasmacytosis, serum lactate dehydrogenase and serum beta-2 microglobulin. These findings establish the serum level of IL-6 as a significant prognostic marker in MM.

Shen *et al.* (2005) found that, serum VEGF and IL-6 concentrations in patients with MM were significantly higher than the healthy controls. There was significant difference in VEGF and IL-6 levels in various clinical stages of MM. IL-6 levels in Stage II were significantly higher than in Stage I. The levels of IL-6 showed great difference according to bone lesion scores. There was a positive correlation between IL-6 and serum calcium or C-reactive protein ($p<0.01$), the IL-6 levels had significant differences between patients with the normal serum CRP, serum calcium and beta-2 microglobulin and patients with abnormal ones ($p<0.05$). They concluded that serum IL-6 levels are helpful to diagnose the clinical stages and understand bone lesion and severity of MM.

Wang *et al.* (2006) reported that, the serum interleukin-6 level of patients with MM was significantly higher than that of normal control ($p<0.01$). The serum interleukin-6 levels of MM patients in second and third stage were also significantly higher than that of patients in first stage ($p<0.05$). They concluded that the serum interleukin-6 level can be considered as one of indexes to judge the patient severity and the prognosis as one of comprehensive multiparameters to evaluate the curative efficiency and guide the clinical application of drugs. Also, Tienhaara *et al.* (1994) and Nachbaur *et al.* (1991) found that, IL-6 levels have been reported to rise in leukemic phases of the disease, to fall in response to treatment and to rise during relapse.

Studies that are not in concordance with present results include; a research performed by Kiss *et al.* (1994) in which they did not find significant difference of IL-6 plasma levels measured by Enzyme-Linked Immunoabsorbent Assay (ELISA) in normal volunteers, patients with MM and benign monoclonal gammopathies. In another study, no correlation was found between IL-6 plasma level and disease activity and high levels of the cytokine were associated with low tumor burden and low growth fraction (Ballester *et al.*, 1994). Brown *et al.* (1991) studied 34 MM patients, in which they concluded that IL-6 levels were not predictive of remission, recurrence, or progression of the disease process.

These differences may be contributed to the different methods that have been used to evaluate IL-6 activity, as in our study, we used the bioassay technique [enzyme amplified sensitivity immuno-assay (EASIA)], while most of the other studies used the ELISA technique. Samples negative by ELISA may prove positive by bioassay. Furthermore, serum IL-6 levels show daily diurnal (morning and afternoon) variations in some patients and thus the time of sampling is one of the important factors that should be considered (Emile *et al.*, 1994).

Regarding serum level of β2-microglobulin, our study revealed significantly high levels in patients with MM and significant fall to the normal values in response to treatment based on the findings mentioned before indicating that β2-M is a protein found on the surface of myeloma cells and could be used as a good indicator for the staging and prognosis of the disease. Also there are significant positive correlation ($p<0.05$) between serum levels of β2-microglobulin and each of CRP and LDH. Present results were in agreement with several studies; Keren *et al.* (1999) reported that, β2-microglobulin is a small, light chain protein that is an established predictor of survival and serum level of β2-microglobulin has a demonstrated correlation with myeloma tumor burden and values >4 g mL^{-1} are considered as a negative prognostic factor. β2-microglobulin is an established predictor of post treatment survival after conventional chemotherapy and an independent predictor of post-transplant survival.

Durie *et al.* (1995) found that serum β2-microglobulin is the most powerful prognostic factor currently available for MM and it can be used alone or in combination with other variables for pretreatment stratification. Also, Alexondra-kis *et al.* (2002) reported that, β2-microglobulin, free urine Dpd, serum calcium and creatinine were significantly higher in stage III compared to stages I and II ($p<0.05$), whereas differences were not noted between stages I and II. Furthermore, patients with stage III disease had higher levels of CRP in comparison to grouped stages I and II ($p<0.05$). There was no difference in BALP levels among the three MM stages.

A study done by Bettini *et al.* (2005) on a series of 96 patients with MM to verify the prognostic meaning of the β2-microglobulin (β2-M), the serum level of β2-M at the diagnosis was directly correlated with the myelomatous cellular mass. They found besides an inverse correlation between β2-M and survival. In addition, the serum level of CRP was inversely correlated with the survival.

As regards to CRP the results showed that, its serum level is significantly high in patients with MM at diagnosis and fall to the normal level in response to treatment. Also, there were significant positive correlation between CRP and IL-6, β2-M and LDH. CRP is an acute phase reactant that is easily assayed and has been produced by hepatocytes in response to inflammation and infection and strongly correlated with proinflammatory cytokines particularly IL-6. CRP serum levels reflect IL-6 *in vivo* and it may be regarded as a powerful prognostic factor in patients with MM (Biro *et al.*, 1998; Alexandrakis *et al.*, 2003).

Present results were in agreement with Zahlten-Hinguranage *et al*. (2006) who found that patients with an increase of CRP prior to surgery showed inferior survival compared to patients with normal levels. These findings suggest that, in patients with MM serum levels of CRP increase during disease activity and might be significantly correlated with specific disease characteristics including adverse prognostic features such as osteolyses in long weight bearing bones. Thus, preoperative elevated CRP serum levels might be considered as independent predictor of prognosis.

Serum levels of lactate dehydrogenase (LDH) in this study showed a significant increase in patients with MM at diagnosis and decreased to normal level in response to treatment. So it can be used as a marker to monitor the response the therapy.

These high levels have been associated with an aggressive presentation of myeloma. As a rule, the elevations of LDH in patients with cancer are too erratic to be of use in clinical diagnosis, although serum levels have been assayed to monitor changes in tumor burden after chemotherapy. However, elevations of this enzyme are found in only a small number of patients with myeloma and, therefore, the assay has limited application for staging or monitoring myeloma. (Rajkumar and Greipp, 1999).

As regards serum levels of bone formation markers (TALP and BALP) the results showed a non significant decreased in the patients with MM and there is a slight significant positive correlation ($p<0.05$) between serum levels of TALP and BALP, the r-value is 0.36603. Terpos (2006) has demonstrated the increase in osteoclastic bone resorption in myeloma is usually associated with impaired osteoblast function and the rate of new bone formation is often markedly reduced. Parameters of bone formation such as serum alkaline phosphatase are not increased in patients with myeloma, unless the patient has an active fracture undergoing repair.

These results are in agreement with Fonseca *et al*. (2000) who reported that, osteoclasts are involved in the process of resorption. As plasma cell tumors proliferate, substances are released that activate osteoclasts, resulting in areas of bone weakness. As the bone is resorbed at a higher rate than it is formed, high levels of calcium build up in the blood. Serum markers of bone metabolism have been studied to determine whether there is a relationship between the markers, the presence of bone manifestations and survival. The five markers, osteocalcin, carboxy-terminal propeptide of type I collagen, bone alkaline phosphatase (BALP), carboxy-terminal telopeptide of type I collagen and tartrate-resistant acid phosphatase show potential as prognostic markers.

In conclusion, presnt data supported the involvement of several mediators in the pathogenesis of MM. Decreased circulating serum mediators after therapy could provide a valuable additional indicator and a preliminary idea about the assessment of therapy response. Although no marker provides optimal analysis of MM or of its treatment, combinations of markers have at times helped in assessing MM stages and lytic bone disease and in monitoring specific treatment modalities. These parameters help physicians in the identification, staging and monitoring of the myeloma patient. So, more efforts should be directed not only toward understanding the significance of the variability of these markers in patients, but also to their potential role in therapeutic approaches to MM. Further investigations are warranted in larger patient groups to support the present findings.

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