Original Article

Interleukin-6 and interleukin-4 levels in multiple myeloma and correlation of interleukin-6 with β2 microglobulin and serum creatinine

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ABSTRACT

Background: Multiple myeloma (MM) is a very common hematological malignancy in elderly person throughout the world. Cytokine network have been implicated in the pathogenesis of MM especially in bony lesions, which are the predominant features of plasma cell dyscrasia. **Aims and Methods:** The aim of this study was to investigate level of serum interleukin 6 (IL-6) and its correlation with β2 microglobulin and serum creatinine and level of IL-4 in MM. Both cytokines and β2 microglobulin were estimated by ELISA kit. **Results:** Serum IL-6 level was done in 34 patients of MM and 75 healthy controls. It was found to be elevated (>11 pg/ml) in 69.7% cases, whereas none of the control cases had this value. There was no correlation of serum IL-6 with age of the patient, serum β2 microglobulin or serum calcium but serum IL-6 correlated positively with serum creatinine. Rise of IL-6 in MM as compared to control was highly significant. Contrary to this serum IL-4 level in 60% MM cases were below 2.5 pg/ml and rest 40% patients had it between 2.5 and 12 pg/ml. Contrary to this, 86.67% healthy control had IL-4 between 2.5 and 12 pg/ml and only 13.33% had its value below 2.5 pg/ml. Statistically reduction of IL-4 when compared to control was significant. **Conclusions:** Our study concludes that MM is associated with increase in IL-6 and decrease in IL-4 in majority cases. In future, treatment with monoclonal antibody to IL-6 and recombinant IL-4 can be used to treat the patients of MM.

Key words: Interleukin 4, interleukin 6, interleukin-4 in myeloma, interleukin-6 in myeloma, multiple myeloma, plasma cell dyscrasia

INTRODUCTION

Multiple myeloma (MM) is a malignant tumor of plasma cells. It accounts for 1% of all cancer death in Western countries.[1] Clinically it is characterized by severe bone pain, spontaneous fractures due to lysis of bone, which give rise to spinal cord compression, bone pain, numbness, weakness and paraplegia.[2] Besides this patients may manifests with symptoms related to hypercalcemia, and hyperviscosity syndrome (dizziness, confusion, purpura, etc.), renal failure and infection. Most of these symptoms are due to monoclonal protein in serum and urine.[3,4] It is predominantly a disease of seventh and eighth decades, but 7.86% cases can be seen under 40 years of age.[5]

Bone disease is the major feature of MM. This is due to increased activity of osteoclast and decreased activity of osteoblast.[6,7] Cytokines secreted by MM cells and stromal cells are the chief factor for producing bone lysis, fracture and bony pain. Increased production of many cytokines such as interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-2, soluble IL-2 receptor, IL-3, IL-6, IL-10, IL-8, granulocyte-macrophage colony-stimulating factor, vascular endothelial growth factor, macrophage inflammatory protein 1-alpha, dickkopf-related protein-1 protein, RANKLE and decreased production of osteoprotegerin are involved for bony lesion.[8-11] These cytokines in addition to bone lesion also produces proliferation of malignant plasma cells.[12-14]

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Among these cytokines IL-6 cytokine is important. It belongs to cytokine superfamily which includes leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor and IL-11.[12,15] In normal person it is secreted mainly by TH2 cells, monocyte, macrophages, activated B cells and endothelial cells. It has synergistic action with IL-1, TNF-α and produces acute phase response, B cell proliferation and differentiation, immunoglobulin production and hematopoiesis. It has very little direct effect on immune cells.[15]

In MM adherent bone marrow stromal cells are responsible for increased synthesis of IL-6 and some MM cells directly produce IL-6. This cytokine is not only responsible for proliferation of MM cells but also produces destruction of bone. Contrary to it IL-4, cytokine, secreted by TH2 cells have been found to be decreased in myeloma.[16] In normal person IL-4 produces proliferation of TH2 cell, B-cells, mast cell, eosinophil and isotype switching for IgE production.[14] Some studies have also found that IL-4 addition in culture of peripheral blood cell produces differentiation of precursor cells of MM into plasma cells.[16]

Studies in India on IL-4 and IL-6 in MM are very sparse hence this study has been done to see the levels of IL-6 and IL-4 in MM and its correlation with β2 microglobulin and serum creatinine.

MATERIALS AND METHODS

Totally 34 diagnosed cases of MM were taken from outdoor and indoor of the Department of Medicine. About 75 healthy aged and sex matched persons who came as blood donor were taken for controls. Peripheral blood sample was taken from the diagnosed cases of MM as well as from the controls and the detailed clinical history and radiological findings were noted. Routine, hematological tests and urine examination were done for all patients.

In all cases 5 ml blood was taken in plain vial and serum was separated and stored at −20°C till tests were performed. Serum electrophoresis and urine electrophoresis were done by cellulose acetate electrophoresis on RALCDIN electrophoresis apparatus.

In all cases 24 h urine sample was taken for Bence Jones protein estimation which was estimated by two laboratory methods called as P-toluene sulfonic acid and by Haskins and Osgood method.[17,18] In all serum β2 microglobulin was done by ELISA kit of LDN Co. supplied by M/s Biogenix Inc., K-796, Aashiyana Colony Lucknow, India. Serum IL-4 and IL-6 estimations were done by kit of Beckman Counter supplied by M/s Anand Brothers Ab Diachem Systems Pvt. Ltd., Anand House: 5, Local Shopping Centre, Karampura, New Delhi.

All data were analyzed using Statistical Package for Social Sciences (SPSS, Chicago, Illinos, USA), version 16. Pearson's Chi-square and Fisher exact test were used to compare differences between the frequencies as per the requirement. Student's *t*-test and one-way ANOVA were done to compare the mean values of different groups. Fisher's exact test was performed if expected frequency of an antigen was <5.

$P < 0.05$ was considered as significant for all analysis.

RESULTS

Out of 34 cases of MM about 29.41% cases had IL-6 within normal range while 70.59% had elevated IL-6 level. In healthy control, all had serum IL-6 within range of 2–11 pg/ml. Rise of IL-6 was statistically significant [Table 1]. Age wise analysis showed that maximum cases of myeloma were seen in sixth decades of life (75.0%) followed by fifth decades (25.0%). About 28.0% MM of sixth decades and 25% cases of fifth decades had normal IL-6 level. There was no significant correlation of IL-6 with age of the patients ($P = 0.708$) [Table 2]. Correlation of serum IL-6 with β2 microglobulin was done

Table 1: IL-6 in myeloma and healthy controls

Groups (number of cases)	IL-6 (pg/ml) (number (%))			Mean±SD (pg/ml)	*t* A versus S	*P*	χ^2
	<2 (pg/ml)	2-11 (pg/ml)	>11 (pg/ml)				
A. Myeloma (34)	0 (0)	10 (29.41)	24 (70.59)	157.85±184.71	7.180	0.0001	67.889
S. Healthy controls (75)	0 (0)	75 (100)	0 (0)	5.55±2.31			

IL-6: Interleukin-6, SD: Standard deviation

Table 2: Correlation of age with IL-6

Age of patients in years	Total number of cases (number (%))	IL-6 level (pg/ml) (number (%))		χ^2 B versus C	*P*
		2-11 (pg/ml)	>11 (pg/ml)		
<40	1 (2.94)	1 (100)	0 (0)	0.141	0.708
40-50	8 (23.53)	2 (25.0)	6 (75.0)		
>50	25 (73.53)	7 (28.0)	18 (72.0)		
Total	34	10	24		

IL-6: Interleukin-6

but it was not significant [Table 3]. Correlation of serum IL-6 with serum creatinine showed that with rise of serum creatinine, level of IL-6 rises but when it is above 2.5 mg/dl then rise of IL-6 is not so prominent [Table 4]. Rise of IL-6 with increasing serum creatinine is significant [Table 4]. There was no significant correlation between serum IL-6 with serum calcium [Table 5].

About 86.7% healthy control had IL-4, between 2.5 and 12 pg/ml and only 13.3% had low level. Contrary to it 60% (MM) patient had low level of serum IL-4 below 2.5 pg/ml. Correlation of serum IL-4 in patients and healthy control were statistically significant [Table 6]. Since IL-4 was done in small number of cases hence statistical correlation with other variable was not done.

DISCUSSION

Interleukin-6 is a principle cytokine responsible for growth of myeloma cells.[12,14,15] IL-6 stimulate growth of myeloma cells by inhibiting apoptosis and by down regulating dephosphorylated retinoblastoma protein.[3,19]

In our study, about 70% myeloma patients had elevated IL-6 whereas none of the controls had higher value above 12 pg/ml.[12,16] This supports the view of earlier studies who also found that IL-6 was elevated in 66% patients of MM.[16]

Nachbaur *et al*.[19] found that 42% patients of MM had elevated IL-6 while only 5–15% patients of myeloproliferative, and 16% patients of monoclonal gammapathy of undermined significance (MGUS) had elevated value. They suggested that this cytokine may help in distinguishing MM from MGUS.

Earlier studies[12,20,21] have found that level of IL-6 are higher in stage II and III of myeloma as compared to stage I of Durie and Salmon criteria.[22] and it correlated well with bone marrow plasmacytosis, serum lactate dehydrogenase (LDH), serum β2 microglobulin, serum calcium and serum neopterin. Some worker proposed that IL-6 is a prognostic marker for survival of MM patients.[12,22]

Table 3: Correlation of IL-6 with β2 microglobulin

β2 microglobulin (mg/l)	β2 microglobulin (mg/l) mean±SD	IL-6 level (pg/ml) (number (%))		Serum IL-6 (pg/ml) mean±SD	*F*	*P*
		2-11 (pg/ml)	>11 (pg/ml)			
<3 (3)	2.17±0.29	1 (33.33)	2 (66.67)	238.68±242.76	0.310	0.736
3-10 (14)	5.90±2.44	3 (21.43)	11 (78.57)	145.24±166.10		
>10 (17)	12.64±1.53	6 (35.29)	11 (64.71)	153.98±198.00		

IL-6: Interleukin-6, SD: Standard deviation

Table 4: Correlation between serum creatinine and IL-6

Serum creatinine (mg/dl)	Serum creatinine (mg/dl) mean±SD	IL-6 level (pg/ml) (number (%))		Serum IL-6 (pg/ml) mean±SD	*F*	*P*
		2-11 (pg/ml)	>11 (pg/ml)			
<1.2 (8)	1.02±0.09	3 (37.50)	5 (62.50)	49.53±68.86	3.817	0.022
1.2-1.96 (12)	1.47±0.22	5 (41.67)	7 (58.33)	113.99±165.99		
1.97-2.5 (3)	2.3±0.17	0 (0.00)	3 (100.0)	388.12±95.48		
>2.5 (6)	5.05±2.13	2 (33.33)	4 (66.67)	132.07±200.72		

F=Ratio of two variances SSB and SSW. SSB: Sum of square between sample, SSW: Sum of square within sample, IL-6: Interleukin-6, SD: Standard deviation

Table 5: Correlation between serum calcium and IL-6

Serum calcium	Serum calcium (mg/dl) mean±SD	IL-6 level (pg/ml) (number (%))		Serum IL-6 (pg/ml) mean±SD	*t* A versus B	*P*
		2-11 (pg/ml)	>11 (pg/ml)			
<9 mg/dl (10)	7.2±2.10	4 (40.0)	6 (60.0)	164.11±179.15	0.724	0.480
9-12 mg/dl (19)	9.82±0.61	6 (31.58)	13 (68.42)	115.18±169.92		

IL-6: Interleukin-6, SD: Standard deviation

Table 6: Serum IL-4 in healthy controls and myeloma

Groups (number of cases)	IL-4 (pg/ml) (number (%))		Mean±SD (pg/ml)	χ^2 A versus B	*P*
	<2.5	2.5-12			
Myeloma (15)	9 (60.0)	6 (40.0)	3.44±1.89	6.081	0.024
Healthy controls (75)	10 (13.3)	65 (86.67)	3.97±1.96		

IL-6: Interleukin-6, SD: Standard deviation

Suppression of MM cell growth after treatment with osteoproteogein and monoclonal antibody to IL-6 have also been reported.[14] β 2 M forms nonpolymorphic chain of class I HLA Ag. It is also marker of renal failure and transplant rejection.[23,24]

In the present study, we did not find any significant correlation of IL-6 with serum β2 microglobulin and serum calcium but a significant positive correlation was found between IL-6 and rising serum creatinine level.

Terpos *et al*. 2010 also studied multiple clinical and laboratory variable as prognostic factor for MM.[25] they found significant prognostic association with low hemoglobin, high serum LDH, monoclonal protein and high N-terminal cross linked telopeptide of Type I collagene and not with any other laboratory parameter.

Earlier studies have found that IL-6 level correlates with survival of the patients.[3,12] Mean survival was 53.7 months in 50% patients when IL-6 was below 7 pg/ml and 2.7 months when IL-6 level were above 7 pg/ml at the time of diagnosis.[12] In our study, we could not correlate with survival because many patients went to some other places for the treatment.

In the present study, we found that 60% patients of MM have low IL-4 (<2.5 pg/ml) when compared to healthy controls where only 13.3% had IL-4 below 2.5 pg/ml. More or less similar to our study some groups from Greece also found that 75% patients of MM had low IL-4 below 4 pg/ml and after treatment value of IL-4 increased.[16]

Herrmann *et al*. found the therapeutic role of IL-4. They treated the MM cases with IL-4 varying from 50 to 250 U/ml and found that IL-4 blocked the endogenous synthesis of IL-6 and reduced the plasma cell growth.[26]

Interleukin-4 has been found to promote differentiation of MM cell precursor in peripheral blood to plasma cell.[27]

One more study reported that in MM there is increase in TH3 cytokine (transforming growth factor beta 1) which suppresses both TH1 and TH2 cell cytokines hence both IL-2 and IL-4 are found in low quantity and this can be reversed by treatment with interferon alpha.[28]

Function of both IL-4 and IL-6 are related with each other in plasma cell differentiation. Transcription factor study done by Klein *et al*. 2003.[29] have shown that CD40 and IL-4 activation triggers bcl-6 expression while lack of CD40 and IL-4 activation down regulate bcl-6 expression and simultaneously elevated IL-6 by up regulating blimp-1 transcription factor through STAT3 activation.[27] Blimp-1 further down regulates bcl-6 and pax5 expression which caused increased differentiation of B cells into plasma cells. Plasma cell transcription factor blimp1 and xBP-1 are up regulated and B cell transcription factor bcl-6 and pax5 are down regulated in malignant plasma cells when compared to B cell. Hence IL-6 is elevated in MM.

Thus, our study concludes that MM shows elevated IL-6 and decreased IL-4. In future treatment with anti-IL-6 and recombinant IL-4 can be tried to treat these MM cases. Authors also conclude that serum creatinine should also be kept in prognostic factor of myeloma.

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