

Insulin-Like Growth Factor -1- And Interleukin -6- As Key Cytokines In Pathogenesis Of Multiple MyelomaNeemat M. Kassem, MD¹, Magda Erian, BSc², Olfat G. Shaker MD³, Rasha Salama MD⁴¹Clinical pathology department, Cairo University,
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Abstract: Rationale: Increased angiogenesis has recently been recognized in active multiple myeloma (MM) and is associated with poor prognosis. The underlying mechanism for increased angiogenesis in MM remains unclear, with various factors implicated such as interleukin-6 (IL6) and insulin Growth Factor-1 (IGF1) which promote the proliferation and survival of myeloma cells. **Purpose:** The present work is intended to study the level of IL-6 and IGF-1, their role in the pathogenesis of MM and to define the effect of thalidomide on BM angiogenesis and angiogenic cytokines when used as initial therapy as a part of treatment. **Materials and method:** This study includes 40 newly diagnosed MM patients referred to Kasr AL Aini Centre of Clinical Oncology and Radiation-Cairo University during the period 2012-2014. ELISA technique was used to measure IGF-1 and IL-6 in subjects' sera. **Results:** This study showed that IGF-1 and IL-6 post treatment were lower than pretreatment levels but IGF-1 level was more significantly responding to treatment (102.8 ± 61 ng/ml before vs. 65.7 ± 51.4 ng/ml after, P value 0.028). **Conclusion:** Our study delineates the importance of anti-angiogenic drugs such as thalidomide against MM and further suggests the clinical utility of novel treatment paradigms targeting not only the tumor cell directly, but also cellular interactions and cytokine secretion in the BM milieu. We suggest that IL6 and IGF-I should be further studied in future clinical trials as useful monitoring biomarkers for MM.

[Neemat M. Kassem, MD, Magda Erian, BSc, Olfat G. Shaker MD, Rasha Salama MD. **Insulin-Like Growth Factor -1- And Interleukin -6- As Key Cytokines In Pathogenesis Of Multiple Myeloma.** *Rep Opinion* 2017;9(12):79-82]. ISSN 1553-9873 (print); ISSN 2375-7205 (online). <http://www.sciencepub.net/report>. 11. doi:[10.7537/marsroj091217.11](https://doi.org/10.7537/marsroj091217.11).

Key words: IL6- IGF1-MM-anti-angiogenic drugs.

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 pain and fractures. Occasionally, plasma cells infiltrate multiple organs and produce a variety of symptoms. The plasma cell clone produces a monoclonal (M) protein that can lead to renal failure caused by light chains (Bence Jones protein) or hyperviscosity from excessive amounts of M protein in the blood. The diagnosis depends on the identification of abnormal monoclonal plasma cells in the bone marrow, M protein in the serum or urine, evidence of end-organ damage and a clinical picture consistent with MM [1].

Recent evidences indicate that angiogenic processes are increased and are fundamental not only in solid tumours but also in hematologic diseases, including MM, as well [2, 3]. The underlying mechanism for increased angiogenesis in MM remains unclear, with various cytokines, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin (IL)-6 and IL-8 and

tumour necrosis factor alpha (TNF α) being implicated. [4].

IGF-I is a mitogen and anti-apoptotic cytokine/growth factor/hormone produced by several types of cells (fibroblasts, hepatocytes, chondroblasts...etc.) [5]. Its potential role as a growth factor for myeloma cells has been deeply analyzed and data of Ge NL et al [6] suggest that IGF-I significantly contributes to the expansion of MM cells.

In spite of the progresses recently registered in the therapy of multiple myeloma (MM), the prognosis for patients affected by this disease remains still poor [7]. Among innovative treatments, antiangiogenic therapy seems to represent a promising approach, which is based on tumor growth inhibition by starving cancer cells of vital nutrients [8].

The present work is intended to study the level of IGF-1 and IL-6 and their role in the pathogenesis of MM and to define the effect of anti angiogenic drugs such as thalidomide (with or without proteasome inhibitors) on BM angiogenesis and angiogenic cytokines when used as initial therapy. We measured the levels of these prognostic biomarkers in Egyptian MM patients to help in defining additional therapeutic lines for this disease.

Patients and Methods

Study Population

The current study was carried out on 40 Egyptian patients with MM. Patients were chosen during the period of 2012-2014 among cases referred to the Clinical Oncology Department, Cairo University after taking their informed consents. The research was approved by the IRB of the Clinical Oncology Department, Cairo University. Patients were 29 males and 11 females. Their ages ranged between 35 and 69 years with a median value of 49 years.

All patients were subjected to: 1. *Routine laboratory investigations: including*, complete blood count with differential leukocyte count, metabolic panel (calcium, albumin, and creatinine) and coagulation tests. 2. *Myeloma-specific investigations: including*, serum protein electrophoresis, monoclonal protein analysis by immune-electrophoresis, urine protein electrophoresis, serum β 2-microglobulin, CRP, and LDH, BM aspirate, 3. *Skeletal bone survey: including*, plain x-ray of spine, pelvis, skull, humeri, and femurs, 4. *Specific laboratory investigations: including* quantitative assessment of IL6 using AviBion Human IL-6 ELISA Kit that was provided by (Orgenium Laboratories, Helsinki Finland) and IGF-1 using DRG IGF-I 600 ELISA kit (DRG Instruments GmbH, Germany).

Statistical Method

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data of scores were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative

variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. A p-value < 0.05 was considered significant.

Results

This study included 40 newly diagnosed multiple myeloma (MM) patients and 20 healthy volunteers.. They were 29 males (72.5%) and 11 females (27.5%). Their ages ranged between 35 and 69 years with median value of 49 years.

MM patients were compared before and after therapy (with thalidomide 100 mg daily as a part of induction therapy) regarding WBCs count, Platelets count, ESR and plasma cells count in BM; all were significantly higher in MM patients before treatment. Other laboratory findings, creatinine, uric acid, LDH and B2M levels were also significantly higher in MM patients before treatment. However, calcium and albumin were higher after treatment. As regards Ig subtypes, twenty six patients (65%) had IgG monoclonal band, 10 patients (25%) had IgA, 4 patients (10%) had Waldenstrom's macroglobulinemia (IgM monoclonal band) and 38 patients (95%) were light chain myeloma. Twenty eight (73.7%) were Kappa chain positive and 10 patients (26.3%) were lambda positive. No statistically significant difference was noticed between the two patients groups as regards IL6 levels (p=0.296). However, IGF-1 levels were significantly higher in MM patients before treatment (p=0.028) (table 1).

Table (1): Comparison Between Laboratory Findings pre and Post Treatment In 40 MM Patients

Variables	Pre treatment	Post treatment	P-value
WBCs x 10 ⁹ /L	5.123 ± 2.7504	4.420 ± 5.001	0.033
Hb g/dl	10.4 ± 1.8	10.6 ± 1.5	0.444
Platelets x 10 ⁹ /L	316.600 ± 99.757	32.700 ± 37.035	<0.001
ESR	73 ± 21.3	43.4 ± 12.5	<0.001
Plasma cells count in BM	49.8 ± 15.2	11.3 ± 5.6	<0.001
Uric acid mg/dl	5.7 ± 2.1	4.4 ± 1.1	<0.001
Creatinine mg/dl	1.8 ± 1.5	1.1 ± 0.3	0.001
Calcium mg/dl	7.8 ± 0.6	8.2 ± 0.5	0.016
LDH U/l	330.5 ± 81.7	264.8 ± 58.3	0.006
Albumin g/dl	2.8 ± 0.6	3.3 ± 0.5	0.001
Total protein g/dl	9.1 ± 2.2	8.4 ± 1.5	0.139
B2M ng/l	4.2 ± 3.4	1.9 ± 0.9	<0.001
IL-6 Pg/ml	38.9 ± 64.7	22.5 ± 36.1	0.296
IGF-1 ng/ml	102.8 ± 61	65.7 ± 51.4	0.028

Values are mean ± SD

Discussion

MM evolution has been shown to be strongly conditioned by angiogenic mechanisms in terms of growth and therapy sensitivity. Several authors tried to explain how angiogenic cytokines [9,10] may work influencing the MM cells; consequently, in the recent years, the presence and quantity of several angiogenic factors, their inducers and their signaling mediators have been documented in an effort to explore the possibility to use them as diagnostic, monitoring or prognostic markers of disease evolution and therapy sensitivity.

IL-6 has been demonstrated to be involved in the proliferation of plasmablastic cells in bone marrow and in the differentiation of these cells into mature plasma cells. Apart from its involvement in the development of normal plasma cells, it is clear that IL-6 is a potent myeloma cell growth factor [11].

In this study, 40 MM patients were included and we demonstrated IL-6 levels post treatment are lower than pretreatment levels **but** with no significant difference between them (p value = 0.296). This is in agreement with what was previously reported by Klein et al.1991 that IL-6 serum levels are increased in patients with multiple myeloma and reflect disease severity; the inhibition of myeloma cell proliferation with antitumoral effects was observed in patients who had been given anti-IL-6 murine monoclonal antibodies [12].

Another molecule involved in MM biology is IGF-I, a mediator cytokine known to be a growth promoter for several tumors, including MM, acting through its anti-apoptotic/proliferative [13-15] effects and interaction with angiogenic factors, such as the anti-proliferative TGF-beta1 [16].

Data regarding serum level of IGF-I in MM are very scarce and partially contrasting [17]. The present study clearly shows that the serum IGF-I concentrations significantly decreased after treatment with thalidomide (p value = 0.028). Some hypotheses suggest that thalidomide or its breakdown product (s) inhibits the stimulatory effects of insulin like growth factor 1 (IGF-1) and fibroblast growth factor 2 (FGF-2) on angiogenesis [18,19].

Also, because there is increasing evidence that IGF-1 is also an important growth and survival factor in MM [20] inhibition of IGF-1 signaling by thalidomide may contribute to its anti-MM activity. Specifically, IGF-1 triggers phosphatidylinositol-3-kinase (PI-3K) signaling, with downstream mitogen activated protein kinase (MAPK) activation and proliferation, as well as activation of Akt with downstream phosphorylation and inactivation of the proapoptotic Bcl-2 family member Bad, thereby inhibiting caspase activity. Finally, IGF-1 enhances the growth of the MM cell line OPM-6 in severe

combined immunodeficiency mice, further supporting its role in MM pathophysiology and raising the possibility that inhibition of IGF-1 may account, at least in part, for the anti-MM effects of thalidomide. [21].

Our study delineates the importance of anti-angiogenic drugs such as thalidomide against MM and further suggests the clinical utility of novel treatment paradigms targeting not only the tumor cell directly, but also cellular interactions and cytokine secretion in the BM milieu. So we suggest that IL6 and IGF-I should be further studied in future clinical trials as possible monitoring markers for MM.

Conclusion

The results of this study may be considered as important hypothesis-generating observations and needs further validation with larger numbers of patients. Further research in understanding the role of angiogenesis and other angiogenic cytokines in various stages of MM is needed.

Conflicts of interest

None

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12/25/2017