Insulin-Like Growth Factor-1 and Interleukin-6 as Key Cytokines in Pathogenesis of Multiple Myeloma

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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 induction therapy

Material and Method: This study includes 40 newly diagnosed MM patients referred to Kasr AL-Ainy Centre of Clinical Oncology and Radiation-Cairo University during the period 2011-2012. 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 ± 61ng/ml before vs. 65.7 ± 51.4ng/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 and therapeutic targets for MM.

Key Words: IL6 – IGF1 – MM – Thalidomide.

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 especially λ chain 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, IL-8 and tumour necrosis factor alpha (TNFa) 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 et al suggest that IGF-I significantly contributes to the expansion of MM cells [6].

In spite of the progresses recently registered in the therapy of Multiple Myeloma (MM), the prognosis for patients affected by this disease remains 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 thalidomide 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 the 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 2010-2012 among cases referred to the Clinical Oncology Department, Cairo University after taking their informed consent. 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 leukocytic 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 β2-microglobulin, CRP, LDH and BM aspirate, (3) Skeletal bone survey including: Plain X-ray of spine, pelvis, skull, humeri, and femurs, (4) Specific investigations including: Quantitative assessment of IL6 using AviBion Human IL-6 ELISA Kit that

was provided by (Orgenium Laboratories, Helsinki FinlandI) and IGF-1using DRG IGF-I 600 ELISA kit (DRG Instruments GmbH, Germany).

Treatment protocol:

Oral Thalidomide (100mg) given for 28 continuous days (one cycle). After 2 cycles, the patients were referred for Lab. Assessment.

Statistical data analysis:

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. Patients were treated with thalidomide (100mg) and dexamethasone (Thaldex.). They were 29 males (72.5%) and 11 females (27.5.5%). Their ages ranged between 35 and 69 years with median value of 49 years.

MM patients were compared before and after therapy regarding WBCs count, ESR and plasma cells count in BM; all were significantly higher in MM patients before treatment. Other laboratory findings, creatinine, calcium, uric acid, LDH and B2M levels were also significantly higher in MM patients before treatment. As regards Ig subtypes, twenty six patients (65%) had IgG monoclonal band, 14 patients (35%) had IgA. Twenty eight (70%) were Kappa chain positive and 12 patients (30%) 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): Comparison between laboratory findings pre and post treatment in 40 MM patients.

Variable	Pre treatment	Post treatment	*p*-value
WBCs X 10^9/L (N: 4-11 X 10^9/L)	5.123±2.7504	4.420±5.001	0.033*
Hb g/dl (Male: 13-17g/dL) (Female: 12-15 g/dL)	10.4±1.8	10.6±1.5	0.444
Platelets X 10^9/L (N: 150-450 X 10^9/L)	32.700±37.035	316.600±99.757	<0.00*1
ESR (Female: 1-25mm/hr) (Male: 0-17mm/hr)	73±21.3	43.4±12.5	<0.001*
Plasma cells count in BM (N: 0-1%)	49.8±15.2	11.3±5.6	<0.001*
Uric acid mg/dl (Female: 2.5-7.5mg/dl) (Male: 3.5-7.5mg/dl)	5.7±2.1	4.4±1.1	<0.001*
Creatinine mg/dl (Female: 0.6-1.2mg/dl) (Male: 0.5-1.1mg/dl)	1.8±1.5	1.1±0.3	0.001*
Calcium mg/dl (N: 8.2-10.2mg/dl)	8.2±0.5	7.8±0.6	0.016*
LDH U/l (N: 0-250U/L)	330.5±81.7	264.8±58.3	0.006*
Albumin g/dl (N: 3.2-5g/dl)	2.8±0.6	3.3±0.5	0.001*
Total protein g/dl (N: 6.0-8.5g/dl)	9.1±2.2	8.4±1.5	0.139
B2M μg/mL (N: 0-3μg/mL)	4.2±3.4	1.9±0.9	<0.001*
IL-6 pg/ml (N: 0-500pg/ml)	38.9±64.7	22.5±36.1	0.296
IGF-1 ng/ml (N: 0-350ng/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 patient were included and we demonstrated that IL-6 levels posttreatment are lower than pretreatment levels but with no significant statistical 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 reflects 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 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 immuno-deficiency 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 millieu. We suggest that IL6 and IGF-I should be further studied in future clinical trials as possible monitoring markers and possible therapeutic targets for MM.

Conclusion:

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

Conflicts of interest:

None

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