

Predictive and Prognostic Significance of Vascular Endothelial Growth Factor (VEGF) and Response to VAD Protocol of Chemotherapy In Multiple Myeloma Patients

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ABSTRACT

Angiogenesis played a crucial role in the pathogenesis of hematological malignancies including multiple myeloma.

AIM: This study aimed to assess angiogenesis activators such as vascular endothelial growth factor and its soluble receptor-2 in blood plasma of multiple myeloma patients and response to VAD protocol of therapy

PATIENTS AND METHODS: fifty six patients with multiple myeloma were enrolled in our study, there age range (45-65) with median 53 years old, forty one were males and rest were females, routine hematological and biochemical tests, serum monoclonal protein concentration and/or urinary light chain excretion and beta 2-microglobulin were performed at the beginning of treatment and four months after VAD chemotherapy with serial measurements of vascular endothelial growth factor (VEGF) and its soluble receptor-2 in the peripheral blood plasma of patients at diagnosis and after treatment by VAD protocol of chemotherapy.

RESULTS: There was significant decrement of vascular endothelial growth factor and its soluble receptor-2 levels in the plasma of patients who achieved complete or very good partial response ($p=0.01$ and $p=0.04$ respectively), also a significance association between vascular endothelial growth factor, its soluble receptor and

other bad prognostic factors such as stage, high B2 macroglobulin level in multiple myeloma patients, and when multi analysis was applied a high significant association were observed reflecting an independent risk of vascular endothelial growth factor, or its soluble receptor-2, ten myeloma patients (17.8%) showed complete response, while twenty seven (48.2%) patients showed either very good or partial response and rest patient showed no response with tolerated toxicity according to WHO criteria of toxicity and the median time to progression for responders was 22.5 months while 95% confidence interval (CI) was 1.71-3.09.

CONCLUSION: Vascular endothelial growth factor and its soluble receptor -2 had a prognostic and predictive utility in responded multiple myeloma patient indicating that the rate of angiogenesis was decreased after successful treatment and might open a future direction for target therapy

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Key words: Multiple myeloma; Vascular endothelial growth factor; Chemotherapy; Angiogenesis

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INTRODUCTION

Angiogenesis was defined as the formation of new blood vessels from pre-existing ones and played a crucial role in the pathophysiology of solid tumors and hematological malignancies, in the past decade, several groups had demonstrated a significantly elevated angiogenic activity in the bone marrow of patients with various hematological malignancies such as multiple myeloma, myelodysplastic syndromes,

acute myeloid leukemia and B cell chronic lymphocytic leukemia^[1,2].

Multiple myeloma was characterized by proliferation of malignant plasma cells that accumulate in the bone marrow and often produce a monoclonal immunoglobulin and it was the first hematological malignancy in which increased angiogenesis rate was detected and its activators as vascular endothelial growth factor played a role in myeloma pathogenesis and had shown to stimulate the proliferation of malignant plasma cells as well as nonmalignant stromal cells through activation of different type of receptors of tis factors^[3,4].

Vascular endothelial growth factor (VEGF) was an important positive regulator of angiogenesis and the biological activity of it was mediated by different receptors of tyrosine kinase activity, vascular endothelial growth factor receptor -1(fms-like tyrosine kinase1 - FLT-1) and vascular endothelial growth factor receptor-2 (kinase domain receptor) both receptors had a high affinity for factor, and were expressed on vascular endothelial cells as well as on malignant cells and receptor -2 had 1,356 amino acids in humans and can be separated into 4 regions the extra cellular ligand-binding domain, transmembrane domain, tyrosine kinase domain, and downstream carboxy terminal region also it is the major positive signal transducer for both physiological and pathological angiogenesis^[5,6].

The aim of this study was to evaluate the levels of vascular endothelial growth factor and its soluble receptor 2 in the platelet-poor blood plasma of myeloma patients at diagnosis and after treatment by four courses of VAD protocol of chemotherapy.

PATIENTS AND METHODS

This study had been carried out at inpatient and outpatient clinic of hematology/ oncology unit of internal medicine department, clinical pathology department, faculty of medicine, zagazig university and zagazig insurance hospital in period between January 2011 and July 2014, fifty six patients with multiple myeloma were enrolled in the study and there age range (45-65) with median 53 years old, forty one were males and rest were females, routine hematological and biochemical tests, serum monoclonal protein concentration and/or urinary light chain excretion and β 2-microglobulin were performed at the beginning of treatment and 3-4 months after VAD chemotherapy. Bone marrow aspirate and/or bone marrow biopsy were performed before the first course of treatment and after administration of the fourth course of treatment, all patient were entered in a study received VAD bolus protocol of chemotherapy after approval of local board committee and it consisted of vincristine 0.4 mg i.v., doxorubicin 9 mg/m² i.v. and dexamethasone 40 mg per oral daily for four consecutive days for three to four months then reevaluated and categorized according to response criteria ,

Response Criteria

Response to treatment was documented according to International Myeloma Working Group. Compleat response(CR)requires negative immunfixation on the serum and urine and less than 5%plasma cell in bone marrow. Very good partial response (VGPR) was defined as reduction of serum of monoclonal (M) protein by at least 90% and urine M protein level less than100 mg per 24 hour. Partial response (PR) was defined as decrease in serum M protein by 50% to 90% and urine M protein level less than 200 mg per 24 hour.insufficient response (IR) as decrease in M-protein by less than 50%^[7].

Method of assay

After giving written informed consent to the study, patients had bone marrow aspiration from either the sternum or iliac crest, peripheral

venous blood sample was taken on the same day. All samples were immediately mixed with EDTA and centrifuged at 3,800 rpm for 15 min at room temperature to give plasma poor platelet sample. Vascular endothelial growth factor (VEGF) measurements were done using ELISA kits according to the manufacturers' instructions. The following ELISA kits were used: human VEGF (R&D systems, Minneapolis (USA). For each patient, level of vascular endothelial growth factor (VEGF) and its soluble receptor-2(sVEGFR-2) were measured at diagnosis and at the time of maximum treatment response, four months after treatment^[8].

Statistical analysis

Data was 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. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For not normally distributed 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. Cox-regression method was used to test the relation between numerical variables and survival. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation. P value less than0.05 was significant

RESULTS

The fifty six multiple myeloma patients had evaluated by plasma samples taken at diagnosis and after four courses of therapy and patient's characteristics were shown in Table 1.

Table 1 Patient characteristics of multiple myeloma patients.

Variables	Number
Age (year)	
Range (45-68)	56
Median 56	
Gender	
Male	41
Females	15
Durie-Salmon stage	
IA	9
IIA	10
IIIA	28
IIIB	9
Immunoglobulin type	
Ig G	44
Ig A	8
Ig D	14
Lytic bone lesion	
1-3	35
>3	21
Complete Response	10/56
Very good/ partial Response	27/56
No Response	19/56
Albumin (g/dl)	3.1 (1.2-5.1)
Creatinine (mg/ dl)	1.1 (0.4- 8.7)
Calcium (mg/ dl)	12.1 (4.7-17.4)
Hemoglobin (g/ dl)	9.7(4.0-17.2)
LDH (IU/L)	242 (20-2286)
β 2-microglobulin (μ g/ mL)	15.50 (0.75-65.80)
Serum M protein (g/ dl)	11.24 (9.09-20.49)
Bence-Jones protein (mg/24h)	805.5 (5.1-11534.0)
Bone Marrow plasma cells (%)	31% (1.0-97.0)
VEGF(pg /mL) at diagnosis	43.2 (10.3-80.9)
VEGFR-2 (pg/ mL) at diagnosis	11.3 (6.5-18.2)

Vascular endothelial growth factor (VEGF). Vascular endothelial growth factor receptor-2 (VEGFR-2), Lactate dehydrogenase (LDH), Immunoglobulin G (Ig G).

The plasma level of vascular endothelial growth factor as well as its soluble receptors were decreased significantly in responded patient either complete response (CR) or very good and partial response patients ($p=0.019$, $p=0.043$ respectively) while there was not any significant reduction in Non-responded patients for chemotherapy table 2.

When multi variant cox analysis was applied to detect the significance association between vascular endothelial growth factor, its soluble receptor and other bad prognostic factors in multiple myeloma patient, a high significant association were detected reflecting independent risk of vascular endothelial growth factor, or soluble receptor table 3.

All myeloma patients are treated by VAD bolus protocol, ten patient (17.8%) showed complete response, while twenty seven (48.2%) patients showed either very good or partial response and rest patient showed no response with tolerated toxicity according to WHO criteria of toxicity (Tables 1 and 4).

The median time to progression for responded patients was 22.5 months (95% confidence interval 1.71-3.09) by VAD bolus protocol (Figure 1).

DISCUSSIONS

Vascular endothelial growth factor (VEGF) considered a major regulator for angiogenesis and it had five tyrosine kinase receptors and activation of these receptors regulate either physiological or pathological angiogenesis according to its pathway, vascular

Table 2 Vascular endothelial growth factor and its receptors before and after therapy patients groups.

Variables	Responded patient No (37)	Non responded patients No (19)
VEGF(pg/mL)		
Before Therapy	43.2 (10.3- 80.9)	43.2 (10.3- 80.9)
After Therapy	11.5 (7.6-15.7)	42.9 (12.2-85.3)
P value	0.019*	NS
VEGFR-2(ng/mL)		
Before Therapy	11.3 (6.5-18.2)	11.3 (6.5-18.2)
After Therapy	7.3 (4.2-6.7)	10.9 (7.3-17.6)
P value	0.043*	NS

* Significant.

Table 3 the multivariate cox proportional hazards model to assess the association between the covariates and the overall survival.

Variables	Hazard Ratio	95% CI	P
albumin<3.5 g/dL	1.53	1.10-1.25	0.02*
β 2microglobulin $\geq 5.5\mu\text{g/mL}$	1.50	1.04-2.15	0.03*
Creatinine $\geq 2\text{ mg/dL}$	1.94	1.41-2.67	0.02*
VEGF (pg/mL) at diagnosis ≥ 40	2.30	1.71-3.09	0.017*
VEGFR-2 (ng/mL) at diagnosis ≥ 10	1.84	1.35-2.50	0.016*

* Significant.

Table 4 Toxicity of VAD protocol according to WHO criteria.

Toxicity	No.
Neutropenia ≥ 2	3
Thrombocytopenia ≥ 2	5
Nausea/vomiting ≥ 2	10
Alopecia ≥ 1	45
Mucositis ≥ 2	7
Neurotoxicity ≥ 2	8

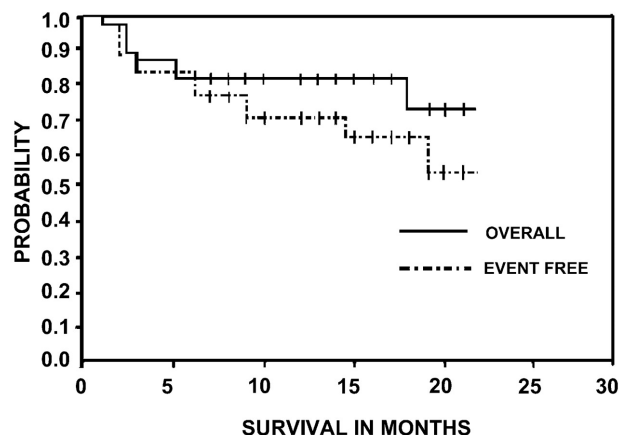


Figure 1 Overall survival for 2 years for VAD protocol in responded patients (log rank test, p value 0.01).

endothelial growth factor receptor -2 had strong tyrosine kinase activity, and transduced the major signals for angiogenesis. However, unlike other representative tyrosine kinase receptors which used the Ras pathway and this soluble receptor 2 mostly used the Phospholipase-C γ -Protein kinase- C pathway to activate MAP-kinase and DNA synthesis so this receptor had a direct signal transducer for pathological angiogenesis including cancer and diabetic retinopathy by itself and the signaling appeared to be critical targets for the suppression of these diseases^[9,10].

Vascular endothelial growth factor (VEGF) was an important positive regulator of angiogenesis and the biological activity of it was mediated by different receptors of tyrosine kinase activity^[11].

In multiple myeloma, vascular endothelial growth factor was produced by malignant plasma cells and stimulates the proliferation of endothelial cells, also at the same time, vascular endothelial growth factor had secreted by endothelial cells in the tumor microenvironment it acted in an autocrine and paracrine way on both endothelial and myeloma cells, so there was a reciprocal stimulation between endothelial cells and myeloma cells to produce it^[12].

A significant increment in plasma level of vascular endothelial growth factor and its soluble receptor -2 in myeloma patients at diagnosis before starting chemotherapy protocol with significant decrement in there plasma levels after four courses of VAD protocol of therapy in responded patients and this was consistent with same data observed by Mileskhin et al. who reported that high levels of vascular endothelial growth factor were associated with response rate in patients treated chemotherapy^[13,16].

Anther contradictory results were detected by Cibeira and collaborators who noticed that there was not a significant correlation between response rate and vascular endothelial growth factor level or its receptor-2^[14,15] and this might attributed to different in selection criteria of patients or method of assessment as the concentrations of angiogenesis factors in the plasma rather than in the serum because platelets contain significant concentrations of these cytokines, released during preparation of serum^[6,17].

Vascular endothelial growth factor and its soluble receptor -2 in myeloma patients had associated with other poor prognostic factors and both of them had independent risk factors with a predictive value due to significant decrement in there plasma level after four courses of VAD protocol of therapy in responded patients reflecting decrement of angiogenesis rate in myeloma patients after therapy^[16].

The aim of front-line therapy for multiple myeloma was to substantially decrease tumor burden and the degree of disease

reduction is associated with improved outcome, including prolonged progression-free survival (PFS) and overall survival (OS)^[17].

The introduction of immune modulatory drugs and proteasome inhibitors had significantly improved survival of myeloma patients and increasing evidence suggested that these agents also improve outcome of the disease more than traditional protocols^[18]. But in certain situation VAD protocol still used in our country

VAD regimen was introduced many years ago and was shown to be the first effective salvage treatment for multiple myeloma that was resistant to alkylating agents and this combination of vincristine and doxorubicin with intermittent high-dose dexamethasone (VAD) had high response rate in treatment for multiple myeloma with tolerated toxicity^[19,20].

Our study observed that the VAD bolus regimen was effective in patients less or more than 60 years of age and thirty seven (66%) patients showed response rate and this consistent with the data of Segeren et al. study in which administered vincristine and doxorubicin as a rapid i.v. infusion in a large cohort of previously untreated patients ≤ 60 years of age and observed a 67% objective response rate^[21], and well tolerated toxicity without observation of grad four myelosuppression or other toxicity according to WHO criteria with median time to progression for responders was 22.5 months, also the similar results were observed in study of Demopoulos *et al*^[22].

CONCLUSION

Vascular endothelial growth factor and its soluble receptor -2 had a prognostic and predictive utility in responded multiple myeloma patient indicating that the rate of angiogenesis was decreased after successful treatment and it could be used as a target therapy together with its soluble receptor-2 in the future, also VAD protocol was effective and well tolerated regimen for treatment multiple myeloma patients

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

REFERENCES

- Vacca A , Ribatti D , Roncali L, Ranieri G, Serio G, Silvestris F, Dammacco F (1994) Bone marrow angiogenesis and progression in multiple myeloma. *Br J Haematol* 87(3):503–508
- Robert Roskoski Jr.(2007)Vascular endothelial growth factor (VEGF) signaling in tumor progression *Critical Reviews in Oncology/Hematology*, 62, 3(6),179-213
- Vacca A and Ribatti D (2006) Bone marrow angiogenesis in multiple myeloma. *Leukemia* 20(2):193–199
- Rajkumar SV, Leong T and Roche PC (2000) Prognostic value of bone marrow angiogenesis in multiple myeloma. *Clin Cancer Res* 6(8):3111–3116
- Orpana A (2002) Angiogenic and lymphangiogenic molecules in hematological malignancies. *Leuk. Lymphoma* 43(2):219– 224
- Scudla V, Petrova P, Minarik J, Pika T, Bacovsky J.(2011) Analysis of the serum levels of selected biological parameters in monoclonal gammopathy of undetermined significance and different stages of multiple myeloma. *Neoplasma* .; 58 (6):499-506.
- Durie BG, Harousseau JL, Miguel J et al (2006) International myeloma working group: international uniform response criteria for multiple myeloma. *Leukemia* 20(9):1467-1473
- Sezer O, Jakob C, Eucker J et al (2001) Serum levels of the angiogenic cytokines basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) in multiple myeloma. *Eur J Haematol* 66(2):83–88
- Robert Roskoski Jr (2008) VEGF receptor protein–tyrosine kinases: Structure and regulation *Biochemical and Biophysical Research Communications*, 375 (3) 287-291
- Shibuya , M. and Claesson-Welsh, L. (2006) Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp. Cell Res.* 312, 549-560
- Marja L., Maija B., Tuomas T. and Kari A (2009) Vascular endothelial growth factor and receptors involved in angiogenesis versus lymphangiogenesis. *Current Opinion in Cell Biology*, 21, 2(4)154-165
- Vacca A, Ria R, Ribatti D et al (2003) A paracrine loop in the vascular endothelial growth factor pathway triggers tumor angiogenesis and growth in multiple myeloma. *Haematologica* 88(2):176–185
- Mileshkin L, Honemann D, Gambell P et al (2007) Patients with multiple myeloma treated with thalidomide: evaluation of clinical parameters, cytokines, angiogenic markers, mast cells and marrow CD57+ cytotoxic T cells as predictors of outcome.. *Haematologica* 92:1075–1082
- Cibeira MT, Rozman M, Segarra M et al (2008) Bone marrow angiogenesis and angiogenic factors in multiple myeloma treated with novel agents. *Cytokine* 41(3):244–253
- Pour L, Svachova H, Adam Z, et al (2010) Pretreatment hepatocyte growth factor and thrombospondin-1 levels predict response to high-dose chemotherapy for multiple myeloma. *Neoplasma* (57):29-34
- Pour L, Svachova H, Adam Z, Almasi M, Buresova L, et al.(2010) Levels of angiogenic factors in patients with multiple myeloma correlate with treatment response. *Ann of Hemat*, 89 (4)385-389.
- Harousseau JL, Attal M, Avet-Loiseau H. (2009) The role of complete response in multiple myeloma. *Blood* .;114 (15):3139-3146
- Kumar SK, Rajkumar SV, Dispenzieri A, et al. (2008) Improved survival in multiple myeloma and the impact of novel therapies. *Blood*.111 (5):2516-
- Pour L, Kovarova L, Büchler T, Penka M, Vorlicek J, Hajek R (2009) Evaluation of hepatocyte growth factor and endostatin in the bone marrow of patients with multiple myeloma and the effect of peripheral blood admixture. *Scripta medica* 82:123–126
- Barlogie B, Smith L, Alexanian R. Effective treatment of advanced multiple myeloma refractory to alkylating agents. *N Engl J Med*1984; 310: 1353–1358.
- Segeren CM, Sonneveld P, van der Holt B et al. Vincristine, doxorubicin and dexamethasone (VAD) administered as rapid intravenous infusion for first-line treatment in untreated multiple myeloma. *Br J Haematol*1999; 105: 127–130.
- Dimopoulos A ,A. Pouli A, Zervas K, Grigoraki V, Symeonidis A, Repoussis P, Mitsouli C, Papanastasiou C, Margaritis D, Tokmaktasis A, (2003) Prospective randomized comparison of vincristine, doxorubicin and dexamethasone (VAD) administered as intravenous bolus injection and VAD with liposomal doxorubicin as first-line treatment in multiple myeloma. *Annals of Oncology* 14: 1039–1044,

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