The Correlation of Remission Induction Therapy with Plasma Vascular Endothelial Growth Factor Level in Acute Myeloid Leukemia Patients

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ABSTRACT:

BACKGROUND:

Angiogenesis seems to be important for leukemogenesis and susceptibility to intensive chemotherapy, and high levels of vascular endothelial growth factor (VEGF) is detected in acute myeloid leukemia (AML) patients

AIM OF THE STUDY:

To assess the correlation of plasma VEGF level with the response to remission induction therapy in adult AML PATIENTS.

PATIENTS AND METHODS:

An analytical cross-sectional study performed on thirty adult patients with newly diagnosed AML. Plasma VEGF level measured by ELISA in all patients twice, before and after chemotherapy.

RESULTS:

The VEGF level was significantly higher in AML patients, at presentation, than control group with median level of 88.13 pg/mL and 49.26 pg/mL, respectively (P=0.002). The median VEGF level decreased after chemotherapy in patients who achieved complete remission although not statistically significant (P=0.071). Whereas the level increased significantly after treatment in patients who failed to response to induction therapy, from 66.27 pg/mL to 165.37 pg/mL (P= 0.025).

CONCLUSION:

Plasma VEGF level was high in adult AML patients at presentation and increases significantly after induction therapy in AML patients who responded poorly to treatment. However, in patients who achieved complete remission the level normalize after therapy, although statistically insignificant.

KEY WORDS: Vascular endothelial growth factor, induction chemotherapy.

INTRODUCTION:

Acute myeloid leukemia (AML) is a cancer of hematopoietic stem and progenitor cells, characterized by series of molecular events that cause abnormal proliferation, differentiation and inhibition of normal hematopoiesis by the malignant cells.[1] Angiogenesis, a complex process of new microvessel formation from pre-existing vasculature, play a potential crucial role in the pathophysiology of hematological malignancies.[2] Vascular endothelial growth factor (VEGF) is one of the most important positive regulators of angiogenesis the expression of which is induced by hypoxia.[3] It promotes proliferation, migration, and differentiation of endothelial cells, and increases their permeability to plasma proteins.[4] In AML, blast cells in bone marrow and blood permanently produce and secrete VEGF, which binds to endothelial or leukemic cells, increases the phosphorylation and transmission of signals into the cells, and upgrades cell proliferation.[5-7] $VEGF^{165}$ is the principle isoform of VEGF-A, the prototype member of VEGF family. High VEGF levels in the bone marrow are associated with higher WBC counts, bone marrow blast percentage, lower remission rates and shorter survival.[8] This study aimed to correlate plasma VEGF level with the response to remission induction therapy, the clinical and laboratory parameters in adult AML patients.

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PATIENTS, MATERIAL AND METHODS:

This is an analytical cross sectional study performed on thirty adult patients (14 males and 16 females) with newly diagnosed AML, who attended Baghdad Teaching Hospital, Medical City, Baghdad, Iraq, from April to December 2017. Data were collected for each patient using questionnaires form including name, age, and sex, the presence of lymphadenopathy, splenomegaly and hepatomegaly. They were diagnosed according to the FAB classification based on cytomorphology, cytochemistry and flowcytometry (FC) immunophenotyping of the peripheral blood (PB) and/or bone marrow (BM) aspirate samples. Patients with acute promyelocytic leukemia, metastatic carcinoma, acute coronary syndrome, diabetic mellitus, relapsed or secondary AML, or those who died before assessing remission were excluded from the study. A total of 20 age- and sex-matched healthy individuals, 11 male and 9 females, were included in this study as a control group. For each patient and control, 2.5 ml venous blood sample was collected in K_3-EDTA tubes and analyzed for complete blood indices by hematology auto analyzer (ADVIA analyzer, Siemens, Germany). Plasma, obtained after centrifugation of the EDTA tubes at 1000 g for 15 minutes, separated into small aliquots and kept in deep freezer (below -40^{0}C) for 4 months, until plasma VEGF assay was done by sandwich enzyme-linked immunosorbent assay (ELISA) using the Human VEGF Immunoassay kit, R&D Quantikine, USA. Plasma VEGF level were assessed twice in patients, at diagnosis (pretreatment) and 21-35 days following induction chemotherapy (post treatment) and once in control group. All patients were treated with standard chemotherapy, three days of an anthracycline and 7 days of cytarabine (3+7), protocols. Remission status was evaluated after completion of cancer therapy according to Döhner et al definition (< 5% blasts in an aspirate bone marrow sample, no blasts with Auer rods or persistence of extramedullary disease, and have an absolute neutrophil count of > 1×10^9/L and platelet count of ≥ 100×10^9/L).[9]

Statistical analysis:

Microsoft excel 2016 and Statistical Package for Social Sciences (SPSS) version 25.0 were used. The quantitative variables is presented as mean ± SD, median, IQR (interquartile range) and range and the qualitative variables were expressed as number and percentage. The normality tests using SPSS show that data is irregularly distributed so we used nonparametric statistical tests (Kruskall-Wallis test, Mann Whitney U test and Wilcoxon test) to compare the median between multiple groups. Spearman's rho non-parametric correlation test was used to predict correlation between different variables. P <0.05 was considered statistically significant**.**

RESULTS:

The mean age for patients group was 35.27 ± 13.12 years (range, 15- 57 years) and the mean age for the control group was 35.15± 11.3 years (range, 19-60 years). Out of the 30 patients, 14 were males (46.66%) and 16 were females (53.33%) with a male to female ratio of 1:1.1.

According to AML FAB classification, 14 patients were M2 (46.66%); 5, M1 (16.66%); 4, M4 (13.3%); 4, M5a (13.33%) and 3 were M5b (10%) (Figure 1).

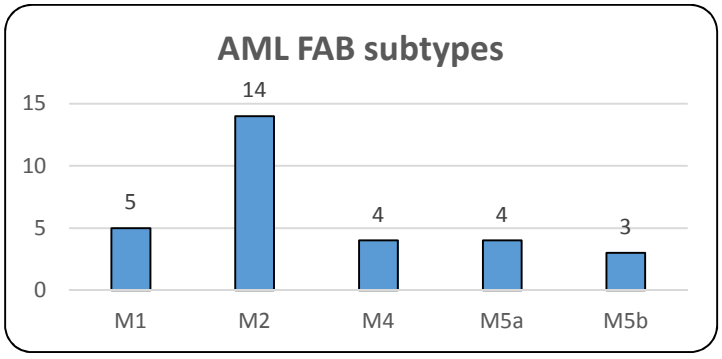

Figure 1: Distribution of patients according to AML FAB subtypes.

In AML patients at diagnosis, the median VEGF level in plasma was 88.13 pg/mL (range, 34.62-280.98 pg/mL). In normal control group, the median VEGF level was 49.26 pg/mL (range, 33.9-116.93 pg/mL). There is statistically significant difference between patients and control groups with P= 0.002, (Table 1).

Table 1: Comparison of VEGF level in control group and AML patients at diagnosis.

Parameter	Control N=20 Median (IQR)	Patients N=30 Median (IQR)	P*
VEGF level (pg/mL)	49.26 (44.92)	88.13 (97.92)	0.002

*Mann Whitney test

There was no statistically significant difference in the VEGF level at diagnosis between different AML FAB subtypes, (Table 2).

Table 2:Comparison of VEGF level in patients group according to AML FAB subtypes.

Parameter	M1 (N=5) Median(IQR)	M2(N=13) Median(IQR)	M4(N=4) Median(IOR)	M5 (N=7) Median(IQR)	P*
VEGF (pg/ml)	100.45(125.5)	77.31(100)	152.42(183.3)	81.0(69.3)	0.638

* Kruskal-Wallis test

No significant correlation was found between VEGF level and clinical and hematological parameters in AML patients, (Table 3).

Table 3: Correlation between VEGF level at diagnosis and other parameters in patients group .

Parameter (median)		VEGF	
		*r	P
Age	36.5 years	0.147	0.438
WBC	11.58 X 10^9 /L	0.083	0.665
ANC	1.78 X 10^9 /L	0.203	0.282
Hb	8.35 g/dl	-0.085	0.655
Platelets	38 X 10^9 /L	0.02	0.915
PB blast %	45.5 %	-0.059	0.758
BM blast %	67.5 %	0.211	0.262

*Spearman rho correlation

After a follow up period of 5 months, patients were divided into two groups according to their response to treatment, those who achieved complete remission, 12/30 (40%), and those who responded poorly to treatment (no remission, NR), 18/30 (60%), whether this poor response was due to resistant disease, death or relapse. By comparing the median VEGF level before and after treatment, the level decreased after chemotherapy in patients who achieved complete remission although not statistically significant. Whereas the level increased significantly after treatment in patients who failed to response to induction therapy (Table 4 and Figure 2).

Table 4: Comparison of VEGF level before and after induction therapy according to their responses to treatment.

Parameter (pg/mL)	Remission (CR) n=12 Median (Range)	No Remission (NR) n=18 Median (Range)
VEGF (before treatment)	112.39 (34.62-280.98)	76.46 (43.53-171.97)
VEGF (after treatment)	66.27 (39.03-123.1)	165.37 (42.02-544.74)
P*	0.071	0.025

* Wilcoxon Rank test

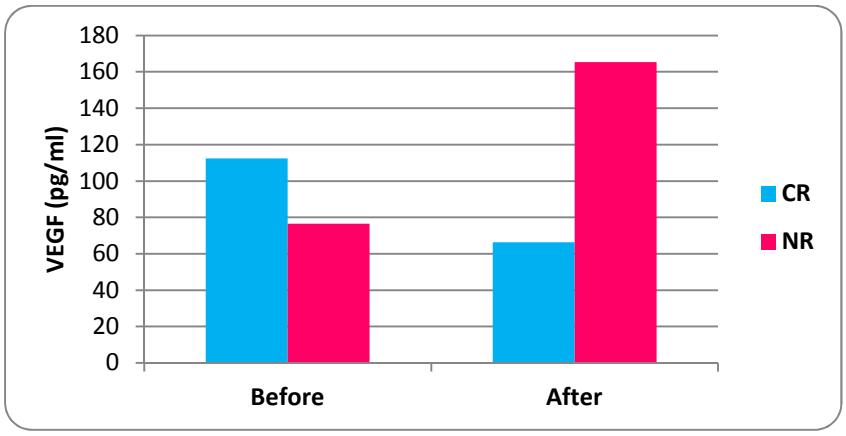

Figure 2: Comparison of VEGF level before and after induction therapy in patients groups according to their responses to treatment. (CR, complete remission. NR, no remission).

DISCUSSION:

Thirty newly diagnosed AML patients were enrolled in this study, their mean age was 35.27 ± 13.12 years. This result was comparable to other Iraqi studies done by Almohsen et al.[10] and Dhahir et al.[11] in 2014 and 2012, respectively. Our study showed that AML cases were observed slightly more in females (53.33%) than males (46.66%), this result disagree with that reported by Al-Maaroof et al.[12] and Udayakumar et al.[13]. This difference may be due to the exclusion of cases that received induction therapy other than "3 and 7" and when second blood samples could not be collected post induction. In the current study, VEGF levels, measured by ELISA, are significantly increased in the plasma of untreated AML patients compared to the control. This result is consistent with that of Aguayo et al.[14], who demonstrated that the median VEGF level in plasma of AML patients was 30.63 pg/mL (range, 21.47-389.82 pg/mL). This finding is also similar to the results obtained by Padro et al[6] who evaluated the expression VEGF and its receptors VEGFR-1 and VEGFR-2 by immunohistochemical staining in bone marrow core sections of 32 AML patients, his report showed a significant increase of VEGF and VEGFR-2 levels *in situ* in bone marrow of AML patients compared with controls. In agreement to the results obtained, it was postulated by Fiedler et al.[15] that VEGF is a possible paracrine growth factor in human AML. Supernatants of freshly leukemic blasts of 24 AML patients contained significantly more VEGF, measured by ELISA, compared with the supernatants of nine normal bone marrow donors or CD34-enriched cells of three normal volunteers. The significantly increased VEGF level in AML patients, as demonstrated by our study and others, suggests that VEGF-associated vasculogenesis and angiogenesis may support the proliferation of malignant progenitor cells and may be associated with the pathogenesis of AML. In addition, VEGF may be a target for the design of novel therapies for AML. No correlation was found between VEGF levels and gender, age, hematological parameters or

extramedullary manifestations of AML patients. These findings are in agreement with Lee et al.[16] who measured the plasma level of 7 angiogenesis related factors, among which was VEGF-A, in bone marrow aspirate of 52 AML patients by ELISA. These results were also consistent with that of Ismail et al.[17] who studied the expression of cellular VEGF in AML blasts by flowcytometry. In contrast, Brunner et al.[18] found strong correlation between serum VEGF level and platelet count only but not with WBC count, hemoglobin concentration or percentage of leukemic blasts in the peripheral blood and bone marrow. This positive correlation between serum VEGF with the peripheral blood platelet count is probably, because VEGF is stored in platelets and released during clotting due to platelets activation. There was no significant difference in the median VEGF levels between AML FAB subtypes. This is consistent with that of Lee et al.[16], Ismail et al.[17], and Zhang et al.[19] On subdividing the patients according to their response to treatment 12/30 (40%) of patients responded well to treatment. Their median VEGF level was 112.39 pg/mL at diagnosis and reduced to 66.27 pg/mL post induction therapy although not statistically significant. This result is similar to the report done by Lee et al.[16] Eighteen patients responded poorly to treatment (60%), whether this poor response was resistance "i.e., the presence of blast and the absence of bone marrow hypo-cellularity" or relapse "i.e., reappearance of blasts in PB or greater than 5% blasts in BM" or death. Their median VEGF level increased significantly after induction chemotherapy, the level was 76.46 pg/mL at diagnosis and 165.37 pg/mL after treatment (P=0.025). This result is comparable to report by Karp et al.[20] who conducted phase II clinical trial of bevacizumab (monoclonal anti-VEGF antibody) administered after chemotherapy to adults with refractory or relapsed AML. They found high serum VEGF level in those patients. This finding supports the role of VEGF as an autocrine factor (by acting on its receptor on leukemic blast) to promote blast proliferation, survival, and chemotherapy resistance, as well as its action in paracrine pathways between endothelial cells and leukemic blast to mediate vascular endothelial cell-controlled angiogenesis.

CONCLUSION:

Plasma VEGF levels was significantly higher in adult AML patients at presentation but no correlation found between its level and clinical, hematological parameters or FAB subtypes. The VEGF level increases significantly after induction therapy in AML patients who responded poorly to treatment, whereas in patients who achieved complete remission the level normalize after therapy, although statistically insignificant.

REFERENCES :

1. Brunner AM, Graubert TA. Pathobioloby of acute myeloid leukemia, In: Hoffman R, Benz EJ, Silberstein LE, et al. editors. Hematology Basic Principles and Practice. 7th ed. Philadelphia: Elsevier, Inc. 2018: 913-16.
2. Petrovic, N. Targeting Angiogenesis in Cancer Treatments: Where do we stand? Journal of Pharmacy & Pharmaceutical Sciences. 2016; 19:226-38.
3. Lee SH, Jeong D, Han YS, Baek MJ. Pivotal role of vascular endothelial growth factor pathway in tumor angiogenesis. Annual Surgical Treat and Research. 2015;89:1-8.
4. Salajegheh A. Vascular Endothelial Growth Factor (VEGF). In: Angiogenesis in Health, Disease and Malignancy. Springer, Cham. 2016: 363-74.
5. List AF, Glinsmann-Gibson B, Stadheim C, Meuillet EJ, Bellamy W, Powis G. Vascular endothelial growth factor receptor-1 and receptor-2 initiate a phosphatidylinositide 3-kinase-dependent clonogenic response in acute myeloid leukemia cells. Experimental Hematology 2004; 32: 526-35.
6. Padro T, Bieker R, Ruiz S, et al. Overexpression of vascular endothelial growth factor (VEGF) and its cellular receptor KDR (VEGFR-2) in the bone marrow of patients with acute myeloid leukemia. Leukemia 2002; 16:1302-10.
7. Kalra M, Dinand V, Choudhary S, Sachdeva A, et al. Serum vascular endothelial growth factor-A levels during induction therapy in children with acute lymphoblastic leukemia. Indian Pediatrics 2013; 50: 659-62.
8. Najafabadi MM, K. Shamsasenjan K, Akbarzadehalaleh P. Angiogenesis Status in Patients with Acute Myeloid Leukemia: From Diagnosis to Post-hematopoietic Stem Cell Transplantation. International Journal of Organ Transplant Medicine 2017; 8: 57-67.

9. Döhner H, Estey E, Grimwade D, Amadori S, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017; 129:424.
10. Almohsen FS, Al-Mudallal SS. Relationship between the expression of CD34, CD123 and myeloperoxidase markers by flow cytometry and response to induction therapy in acute myeloid leukemia. Iraqi Journal of Medical Sciences 2014; 12: 161-67.
11. Dhahir EK, Al-Mudallel SS, Dhahi MA. The frequency of FLT3 mutation in fifty five Iraqi adult patients with acute myeloid leukemia. Iraqi Journal of Medical Sciences 2012; 10: 140-47.
12. Al- Maaroof ZW, Yahya DhJ, Hassoon AF. Evaluation of leukemia inhibitory factor, interleukin 6 and leptin in acute and chronic myeloid leukemia in Babylon Province. Medical Journal of Babylon 2016; 2:513-21.
13. UdayaKumar AM, Pathare AV, Al-Kindi S, Khan H, Rehmen JU, Zia F, et al. Cytogenetic, morphological, and immunophenotypic patterns in Omani patients with de novo acute myeloid leukemia. Cancer Genetics and Cytogenetics 2007; 177: 89-94.
14. Aguayo A, Kantarijian HM, Estey EH, Giles FJ, et al. Plasma vascular endothelial growth factor levels have prognostic significance in patients with acute myeloid leukemia but not in patients with myelodysplastic syndromes. Cancer 2002; 95:1923-30.
15. Fiedler W, Graeven U, Ergun S, et al. Vascular endothelial growth factor, a possible paracrine growth factor in human acute myeloid leukemia. Blood 1997; 89:1870-75.
16. Lee CY, Tien HF, Hu CY, Chou WC, Lin LI. Marrow angiogenesis- associated factors as prognostic biomarkers in patients with acute myeloid leukemia. British Journal of Cancer 2007; 97: 877-82.
17. Ismail MA, Eisaa DS, Heiba NM. Celullar vascular endothelial growth factor and serum angiogenin in acute myeloid leukemia: clinical and prognostic significance. Egyptian Journal of Haematology 2013; 38:41-46.
18. Brunner B, Gunsilius E, Schumacher P, Zwierzina H, et al. Blood levels of angiogenin and vascular endothleial growth factor are elevated in myelodysplastic syndromes and in acute myeloid leukemia. Journal of Hematotherapy & Stem Cell Research 2002; 11:119-25.
19. Zhang J, Ma D, Ye J, Zang S, Lu F, et al. Prognostic impact of d-like ligand 4 and notch 1 in acute myeloid leukemia. Oncology Reports 2012;28:1503-11.
20. Karp JE, Gogo I, Pili R, Gocke CD, et al. Targeting vascular endothelial growth factor for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential 1-β-D-arabinofuranosylcytosine, mitoxantrone, and bevacizumab. Clinical Cancer Research. 2004;10: 3577-85.