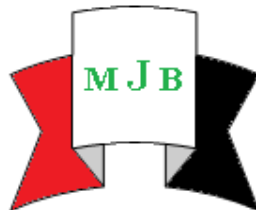


Serum Vascular Endothelial Growth Factor Levels in Iraqi Patients with Newly Diagnosed Acute Leukemia

Saba S. Mallouh* Najlaa B. Alawadi** Abass F. Hasson**

** College of Medicine , Babylon University



Received 20 November 2014

Accepted 23 November 2014

Abstract

Acute leukemia is an aggressive disorder which need a new therapeutic approaches to improve the response to treatment. Angiogenesis seems to be important both for leukemogenesis and susceptibility to intensive chemotherapy, and antiangiogenic strategies may therefore be considered for the treatment of acute leukemia. This study aim to assess serum VEGF levels in Iraqi patients with a newly diagnosed acute leukemias and to find the relation between VEGF levels and other hematological parameters.

This case-control study included 96 subjects who attended Marjan Teaching Hospital in Babil/Iraq during the period from Nov 2013 to Aug 2014. They divided into two groups; patient group included 48 patients newly diagnosed with acute leukemia (32 of them had AML and 16 had ALL) and control group included 48 normal volunteers. Complete blood count and serum VEGF (ELISA) were done for all while bone marrow examination was done for patients only.

Mean serum VEGF was significantly higher in patients than controls (643.8 ± 121.1 ng/dl vs. 203.4 ± 51.8 ng/dl respectively and P -value < 0.001) and a higher levels were found in patients with ALL compared to AML and in males compared to females.

No statistically significant age differences were found between patients and controls, while majority of patients (61.9%) and controls (63.4%) were males.

Mean PCV was significantly lower in patients than controls (P -value < 0.01), Mean WBC count was significantly higher in patients than controls (P -value < 0.01) and mean platelets count was significantly lower in patients than controls (P -value < 0.01).

This study concluded that serum VEGF in patients with acute leukemia was high compared to controls and a significant correlation was found between serum VEGF levels and both PCV and gender.

Key words: Vascular endothelial growth factor, Acute leukemia, AML, ALL.

الخلاصة

اللوكيميا الحادة هو اضطراب عدوانية التي تحتاج الى مناهج علاجية جديدة لتحسين الاستجابة للعلاج. يبدو أن تولد الأوعية هامة سواء بالنسبة ليوكيموجينيسيس وقابلية للعلاج الكيميائي المكثف ، والإستراتيجيات المضادة لتولد الأوعية ولذلك يمكن النظر لعلاج اللوكيميا الحادة. الهدف من الدراسة هو لتقييم مستويات عامل نمو بطانة الاوعية الدموية في المصل في المرضى العراقيين الذين يعانون اللوكيميا الحادة والتي تم تشخيصها حديثا، وإيجاد العلاقة بين مستويات عامل نمو بطانة الأوعية الدموية وغيرها من المعالم الدموية. دراسة شملت المواضيع ٩٦ الذين حضروا "مستشفى مرجان التعليمي" في بابل/العراق خلال الفترة من نوفمبر عام ٢٠١٣ إلى أغسطس ٢٠١٤. أنها تنقسم إلى مجموعتين؛ مجموعة المرضى شملت ٤٨ المرضى الذين شخصت حديثا بسرطان الدم الحاد (٣٢ منهم مصابين بسرطان الدم النقوي الحاد، وكان ١٦ مريض مصابين بسرطان الدم اللمفاوي الحاد) ومجموعة المراقبة شملت ٤٨ متطوعا. عد الدم الكامل ومستوى عامل نمو بطانة الاوعية الدموية في المصل تم القيام به للجميع بواسطة في حين تم فحص نخاع العظم للمرضى فقط. ان معدل عامل نمو بطانة الاوعية الدموية في المصل كان أعلى بكثير في المرضى من عناصر التحكم. وعثر مستويات أعلى في المرضى الذين يعانون من سرطان الدم اللمفاوي الحاد مقارنة بالمرضى المصابين بسرطان الدم النقوي الحاد، وفي الذكور مقارنة بالإناث . لم توجد فروق معتد بها إحصائيا في العمر عثر بين المرضى وعناصر التحكم ، بينما الغالبية العظمى من المرضى (٦١.٩%) وعناصر التحكم (٦٣.٤%) من الذكور . معدل حجم الخلية المضغوط في المرضى اقل منه في عناصر التحكم، كذلك معدل عدد كريات الدم البيضاء اكثر في المرضى منه في عناصر التحكم اما معدل الصفائح الدموية اقل في المرضى منه في عناصر التحكم.

هذه الدراسة استنتجت ان مستوى عامل نمو بطانة الاوعية الدموية في المصل في المرضى الذين يعانون من سرطان الدم الحاد كان مرتفعاً بالمقارنة مع الضوابط وارتباط كبير بين مستويات عامل نمو بطانة الاوعية الدموية في المصل مع حجم الخلية المضغوط والجنس.

Introduction

Acute leukemias are aggressive disorders characterized by accumulation of immature malignant cells in the bone marrow[1]. They result from abnormal clonal proliferation of mutated progenitor cells. These mutations cause a block in the maturation process leading to expansion of an abnormal clone and subsequent suppression of the normal elements in the bone marrow[2].

There are two main types of leukemia: acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL). In AML, the five-year survival rate is 40%, except for APL (Acute Promyelocytic Leukemia) subtype, which is over 90%[3]. However, the survival rates in ALL vary by age to be 85% in children and 50% in adults[4]

The American Cancer Society estimates that 18,860 new cases of AML will occur in the United States in 2014, accounting for 32% of all leukemia cases in adults 20 years of age and older [5]. However, ALL is the most common cancer diagnosed in children and represents about 25% of all cancers diagnosed among children younger than 15 years [6].

Angiogenesis is the formation of new capillaries from established blood vessels, and is an essential process in growth and development [7]. However, it provides malignant cells with a survival advantage over their normal counterparts and confers potential of metastasis [8]. One of the major positive regulators of tumor-related angiogenesis is vascular endothelial growth factor (VEGF) [9, 10] which is a multifunctional cytokine that stimulates endothelial cells to proliferate, to migrate, and to increase their permeability to plasma proteins [11]. There are four types of VEGF named A, B, C and D acting on three types of receptors named VEGFR-1, 2 and 3. Both of VEGF and its receptors have a key role in the pathology of bone

marrow angiogenesis in leukemia [12]. Increased microvessels density in bone marrow samples from patients with acute leukemia was reported [13].

Some of young patients and most of elderly with acute leukemia usually receive less intensive chemotherapy or only supportive therapy due to the high treatment-related mortality when using intensive therapy for elderly individuals. Thus, there is a need for new therapeutic approaches to improve the treatment in younger patients and to make it with acceptable toxicity in elderly individuals. Angiogenesis seems to be important both for leukemogenesis and susceptibility to intensive chemotherapy, and anti-angiogenic strategies may therefore be considered for the treatment of acute leukemia [14].

This study aimed to assess serum VEGF levels in Iraqi patients with a newly diagnosed acute leukemias, to detect any significant differences between AML and ALL cases or according to age or gender, and to find the relation between VEGF levels and other hematological parameters.

Materials and Methods

This case-control study included 96 subjects who attended Marjan Teaching Hospital / Babil governorate / Iraq during the period from November 2013 to August 2014. Informed consent was obtained from all participants. The practical part of the study was performed at the Teaching Laboratories in the Departments of Biochemistry and Pathology / College of Medicine /Babylon University.

Forty eight patients diagnosed with acute leukemia in the laboratories of Marjan Teaching Hospital were included in the study. Complete blood count (CBC) was done for all patients, including PCV, WBC count and platelets count. The blood film and bone marrow examination were performed by a senior hematologist. The patients then divided into 2 groups:

1) Group (1): 32 patients with AML and mean age 44.6 ± 13.7 years (mean $\pm$ SD).

2) Group (2): 16 patients with ALL and mean age 26.3 ± 16.13 years (mean $\pm$ SD).

Forty eight sex and age matched healthy voluntaries were taken as a control group. It included persons attending Merjan Teaching Hospital for checking or for simple complaints. For all controls, blood film and complete blood count (CBC) were done, including packed cell volume (PCV), white blood cell (WBC) count, platelets count. The blood film was performed by a senior hematologist. Serum VEGF level was measured by ELISA assay in both patient and control groups.

For all patients and controls, EDTA (ethylenediaminetetra-acetic acid) tubes were used to collect blood samples for complete blood counts (CBC) done by auto-hematology analyzer from Diagon. Differential WBCs count was done

manually. Bone marrow aspiration was done only for the patients with Jamshidi needle from posterior superior iliac spine. Blood film smears and bone marrow aspiration smears were sent to a senior hematologist for analysis and report.

For all patients and controls, another blood samples were collected in a plane tubes without anticoagulant. Sera was taken from these blood samples to measure VEGF level using Boster's human VEGF-ELISA Kit that was based on a standard sandwich ELISA technology. Human VEGF-specific polyclonal antibodies were precoated onto 96-well plates and the human specific detection monoclonal antibodies were biotinylated.

By the end of the procedure, O.D. absorbance was read at 450nm in a microplate reader and VEGF standard curve was done.

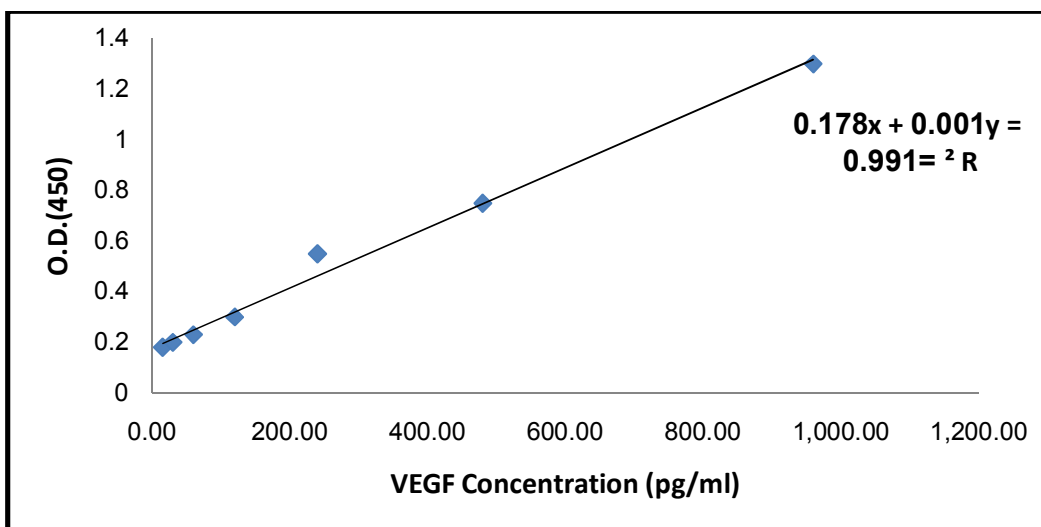


Figure (1) : VEGF standard curve.

The data were analyzed using computerized SPSS (Statistical Package of Social Science) program; Independent t-test was used to estimate differences between two groups in continuous variables while the analysis of variance (ANOVA) were used to determine the differences between the three age groups. A p-value < 0.05 is considered to be statistically significant (Daniel, 1999).

Results

The study included 48 Iraqi patients newly diagnosed with acute leukemia and 48 age and sex matched healthy controls. 32 (67%) of the patients had AML and 16 (33%) of them had ALL. The highest prevalence of AML was observed among patients aged > 40 years, while ALL was mostly diagnosed in the ages < 20 years.

No statistically significant age differences were found between patients and controls (39.2 ± 12.8 vs. 43.6 ± 15.9 years respectively and $P\text{-value} > 0.05$), however, a significantly higher ages were observed in patients with AML compared to those with ALL (44.6 ± 13.7 vs. 26.3 ± 16.13 years respectively and $P\text{-value} < 0.01$).

Majority of patients (61.9%) and controls (63.4%) were males. AML was the most common type seen in females (80% of females) and males (57.1% of males).

Mean PCV was significantly lower in patients than controls ($23.27 \pm 7.3\%$ vs. $38.55 \pm 8.6\%$ and $P\text{-value} < 0.01$). However, no significant difference was found in mean PCV between AML and ALL patients ($22.2 \pm 6.9\%$ vs. $23.8 \pm 7.7\%$ and $P\text{-value} > 0.05$).

Mean WBC count was significantly higher in patients than controls ($25179 \pm 5342/\text{cmm}$ vs. $7424 \pm 3314/\text{cmm}$ respectively and $P\text{-value} < 0.01$). However, a statistically insignificant higher WBC count was found in patients with AML compared to those with ALL ($27542 \pm 6321/\text{cmm}$ vs. $23896 \pm 5114/\text{cmm}$ respectively and $P\text{-value} > 0.05$).

Mean platelets count was significantly lower in patients than controls ($51072 \pm 16434/\text{cmm}$ vs. $233456 \pm 64984/\text{cmm}$ respectively and $P\text{-value} < 0.01$).

However, a statistically insignificant lower platelets count was found in patients with AML compared to those with ALL ($53788 \pm 15995/\text{cmm}$ vs. $49872 \pm 16896/\text{cmm}$ and $P\text{-value} > 0.05$).

Mean serum VEGF level was significantly higher in patients than controls (643.8 ± 121.1 ng/dl vs. 203.4 ± 51.8 ng/dl respectively and $P\text{-value} < 0.001$).

Higher levels of VEGF were found in patients with ALL as compared to those with AML (662.9 ± 136 ng/dl vs. 602.6 ± 113 ng/dl respectively). This difference was statistically insignificant ($P\text{-value} > 0.05$).

A statistically insignificant higher VEGF levels were found in males compared to females in patients with both AML and ALL (684.5 ± 156 ng/dl in male patients vs. 598.8 ± 142 ng/dl in female patients with $P\text{-value} > 0.05$). Another statistically insignificant higher levels of VEGF were found in patients aged older than 40 years compared to younger age group (702.3 ± 137 ng/dl vs. 614.6 ± 121 ng/dl respectively with $P\text{-value} > 0.05$).

There was a significant positive correlation between mean serum VEGF level and mean PCV ($P\text{-value} < 0.05$), while there was an insignificant negative correlation between VEGF and WBC count or platelets count ($P\text{-value} < 0.05$) Table (1).

Table 1: The correlation between serum VEGF level and PCV, WBC and platelets count.

		PCV	WBC	platelets
VEGF	r	.421*	-.163	-.241
	P value	.040	.446	.257
	N	48	48	48

r Correlation coefficient

* Correlation is significant if < 0.05 (2-tailed).

Discussion

Many studies confirmed that angiogenesis plays a key role in the growth of solid tumors. More recently, accumulating evidence has linked

angiogenesis to the pathophysiology of acute and chronic leukemias. Different investigators have shown evidence of increased angiogenesis in the bone marrows of patients with leukemia. A

potential role for VEGF as an autocrine growth factor in AML has been suggested but still no evidences in ALL [15-17]. However, Markovic et al found that the secretion of VEGF from blasts in childhood ALL can be induced via FLT-3 signaling pathway and concluded that inhibition of FLT-3/VEGF pathway may disrupt paracrine signaling between leukemia cells and bone marrow micro vessels [18].

In this study, serum VEGF level was significantly higher in patients than normal controls.

This result is consistent with the findings of FuatErdeml et al where they studied 45 patients with hematological malignancies along with 20 age and sex matched healthy individuals and found that serum VEGF level in the patients with hematological malignancies was significantly higher compared to controls [19].

This result was also consistent with the study done by Go`kselLeblebisatan et al where they studied 46 patients diagnosed with AML and ALL and 16 normal children were included as a control group. In that study; they found that serum VEGF level in patients with AML and ALL was higher than that in control group[20].

In another study, Aguayo et al used an enzyme-linked immunosorbent assay (ELISA) to measure serum VEGF level in 99 patients [58 (58.5%) of them had AML and 41 (41.5%) of them had with previously untreated MDS and compared the results with that of normal control participants. They found a higher VEGF level in the patients group than in the control group[21].

On the other hand, another different results were reached by Kalra M et al where they found that serum VEGF level in an untreated children with ALL was significantly lower than controls and a more strange result was revealed in his study saying that at the end of induction therapy for these children, serum VEGF increased to reach levels similar to that of controls. They suggested a role for the ligand receptor interaction between serum

VEGF and VEGF receptors on leukemia cells [22].

VEGF is activated by hypoxia [23,24]. So when a cell is deficient in oxygen, it produces the hypoxia-inducible factor (HIF) which acts as a transcription factor. HIF stimulates the release of VEGF and may cause modulation of erythropoiesis [25].

VEGF exerts its effect by binding to its tyrosine kinase receptors named VEGFR-1,2 and 3 [26,27,28,29]. VEGF binding to these receptors promotes an increase in VEGF level and the signal is thus re-enforced[30].

VEGF is important in hematopoietic malignancies [31] as an increased microvessel density in bone marrow samples from patients with acute leukemia was reported with a positive correlation between VEGF expression and increased microvessel density in both AML and ALL [13].

VEGF is the main objective of this study and the presence of high serum levels in a statistically significant manner in untreated newly diagnosed patients with acute leukemia gives a clue that VEGF may be of a pathogenic importance in inducing leukemia or may be a result of the leukemia itself. Further studies are needed to clarify its role as a predictive factor of acute leukemia or to be used as a prognostic factor in follow up of leukemic patients under treatment and to assess the response to antiangiogenesis treatments.

Serum VEGF level showed an insignificant difference between AML and ALL patients. This is exactly what was revealed by FuatErdeml et al who took 15 AML and 10 ALL patients along with another cases of hematological malignancies and found that the difference between AML and ALL groups was statistically insignificant ($P > 0.05$) [19]. This finding may be explained by the fact that VEGF is increased in both types of acute leukemia and it may be produced by blast cells themselves or by another cells undergoing hypoxia.

From another point of view, Alvaro Aguayo et al studied serum VEGF level in 20 patients with ALL and 30 patients with AML and found that the mean level of VEGF in AML patients was higher than its level in ALL patients. That difference was statistically significant; however, there was no significant difference in the bone marrow vascularity between the two groups [24].

This study found a significant positive correlation between serum VEGF level and PCV; however, no significant negative correlation between VEGF level and WBC or platelets count was observed.

This finding was consistent with results of Mazure et al who found that there was a significant positive correlation between VEGF level and PCV[32]. Further studies are needed to find the prognostic significance of serum VEGF elevation at the time of diagnosis in acute leukemia.

Numerous studies showed a decreased overall survival and disease-free survival in those tumors overexpressing VEGFxxx e.g. breast cancer [33]. The overexpression of VEGFxxx may be an early step in the process of metastasis, a step that is involved in the "angiogenic" switch. Although VEGFxxx has been correlated with poor survival, its exact mechanism of action in the progression of tumors remains unclear [34].

In this study, a higher prevalence of AML was observed in the patients aged > 40 years; while ALL was commoner in the patients aged < 20 years. These results agree with the documented facts about acute leukemia that were mentioned by American Cancer Society [35] and Howlader et al who found that the patients with AML were older than those with ALL [6].

This study showed that majority of the patients were males and that AML is the most common type of acute leukemia in both sexes. This is consistent with the study done by Jackson N. et al where they took 188 patients with leukemia and 1019 control. They studied the blood group in each patient and found that 39.4% of males with acute leukemia had blood group O

whereas 24% of females with acute leukemia are group O. From these results, they suggested the presence of sex-responsive gene near ABO gene locus on chromosome 9, which relatively protect women against acute leukemia. The existence of such gene might also partly explain why acute leukemia, and possibly other childhood cancers are more common in males [36].

In our study, it has been found that AML was more in females (80%) than males. This result is against the study conducted by Alaa F. Alwan et al in Baghdad Teaching Hospital where they studied 115 Iraqi patients and found that males were more frequently affected with AML than females.

This difference might be explained by small sample size and short study time[37].

In this study, PCV was significantly lower in patients than controls.

This finding was consistent with the results of Chessel et al [38].

The low PCV might result from many causes like bone marrow infiltration by malignant cells, autoimmune hemolytic anemia or hypersplenism. Other causes of low PCV in these patients might be the activity of inhibitory cytokines released by tumor cells on the red cells precursors, poor appetite and poor food intake which are common features among these patients⁽³⁹⁾.

It had been found in this study that PCV was lower in AML patients than ALL patients; however, this difference was statistically insignificant.

This low PCV in AML patients might be due to special characteristics of pathogenesis of AML as it is started by a clonal proliferation of myeloid precursors with a reduced capacity to differentiate into more mature cellular elements. As a result, there is an accumulation of leukemic blasts or immature forms in the bone marrow, peripheral blood, with a variable reduction in the production of normal red blood cells.

In this study, WBC count was significantly higher in patients than normal control volunteers. This can be explained by the fact that in leukemia there is a clonal

proliferation of malignant cells that may arise at any stage of maturation in the bone marrow including lymphoid, myeloid or pluripotent stages [40].

In this study, we found a higher WBC count in the patients with AML than those with ALL, despite that this difference was statistically insignificant. This result agreed with that of Wetzler et al who found that the percentage of hyper-leukocytosis (WBC more than 100000/cmm.) is seen in 25% of AML and in 12% of ALL patients [41].

A statistically significant lower platelets count was found in patients than controls in this study. This lower platelets count may be from the expansion of immature blast cell in the bone marrow resulting in bone marrow failure. Pancytopenia is eventual in acute leukemia partly from physical replacement of bone marrow cells by the immature blasts and the secretion of inhibitory factors by the immature blast that abort normal hematopoiesis [42,43].

A statistically insignificant lower platelets counts was observed in AML patients than ALL patients. This result was consistent with Tallman et al results [44] who found that the incidence of thrombocytopenia was more common in a specific subgroups of AML including M3 and M7. This may be explained by the fact that in AML, a malignant change begins in myeloid stem cells that normally forms, red cells, some types of white cells and platelets. While in ALL, the malignant change begins in lymphoid stem cells that normally forms lymphocytes [45].

Conclusions

This study concluded that serum VEGF levels in Iraqi patients with a newly diagnosed AML and ALL were high compared to normal Iraqi controls and a significant correlation was found between serum VEGF levels and both PCV and gender.

References

1. Norgaard JM, Langkjer ST, Palshof T, Clausen N, Pedersen B, Hokland P. Relation of blast cell survival and

proliferation to chemotherapy resistance in AML. *Br J Haematol* 1996;93:888-97.

2. Hoffbrand AV, Moss PAH, Pettit JE (ed). "Essential Haematology" 5th Edition. Blackwell Publishing, Oxford: 2006; 157.

3. Jameson JN StC; Dennis L Kasper Harrison, Tinsley Randolph; Braunwald, Eugene; Fauci Anthony S; Hauser, Stephen L; Longo Dan L (2005). Harrison's principles of internal medicine. New York: McGraw-Hill Medical Publishing Division. ISBN0-140235-7.

4. Colvin GA, Elfenbein GJ. The latest treatment advances for acute myelogenous leukemia. *Medicine and Health, Rhode Island* 2003; 86(8): 243-6. PMID 14582219.

5. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH et al and National Cancer Research Institute Adult Leukaemia Working Group. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* 2010;116: 354-65.

6. Childhood cancer. In: Howlader N, Noone AM, Krapcho M, et al, eds.: SEER Cancer Statistics Review, 1975-2010. Bethesda, Md: National Cancer Institute, based on November 2012 SEER data submission, posted to the SEER web site, April 2013, Section 28. Also available online. Last accessed April 04, 2014.

7. Risau W, Drexler H, Mironov V et al. Platelet-derived growth factor is angiogenic *in vivo*. *Growth Factors* 1992; 7:261-266.

8. Cristofaro and C Emanuelli. Possible novel targets for therapeutic angiogenesis. *Current Opinion in Pharmacology* 2009, vol. 9, no. 2, pp. 102-108.

9. Leung DW, Cachianes G, Kuang WJ et al. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; 246:1306-9.

10. Roskoski Jr. "Vascular endothelial growth factor (VEGF) signaling in tumor

progression," Critical Reviews in Oncology/Hematology 2007, vol. 62, no. 3, pp. 179–213.

11. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol* 1995; 146:1029.

12. Kessler T, Fehrmann F, Bieker R, Berdel WEMesters RM. vascular endothelial growth factor and its receptor as drug targets in hematological malignancies. *Current Drug Targets* 2007; 8(2):257-68.

13. Matuszewski L, Persigehl T, Wall A, Meier N, Bieker R, Kooijman H, et al. assessment of bone marrow angiogenesis in patients with acute myeloid leukemia by using contrast enhanced MR imaging with clinically approved iron oxides initial experience. *Radiology* 2007; 242 (1):217-24.

14. Hatfield KJ, Olsnes AM, Gjertsen BT, Bruserud O. Antiangiogenic therapy in acute myelogenous leukemia: targeting of VEGF and interleukin 8 as possible antileukemic strategies. *Curr Cancer Drug Targets* 2000 5(4): 229-48.

15. Mesters RM, Padro T, Steins M, Bieker R, Retzlaff S, Kessler T, et al. Angiogenesis in patients with hematological malignancies. *Onkologie* 2001 24 Suppl 5: 75-80.

16. Dickson DJ, Shami PJ. Angiogenesis in acute and chronic leukemias. *Leuk Lymphoma* 2001 42(5):847-53.

17. Kessler T, Fehrmann F, Bieker R, Berdel WE, Mesters RM. VEGF and its receptors as drug target in hematological malignancies. *Curr Drug Targets* 2007 8(2):257-68.

18. Markovic A, Karen LM, Richard BL. Induction of VEGF secretion by childhood acute lymphoblastic leukemia cells via the FLT-3 signaling pathway. *Mol Cancer Ther* 2011; 11(1): 183-93.

19. Fuat Erdeml, Mehmet Gündoğdu1, Ahmet Kızıltunç. Serum vascular endothelial growth factor level in patients with hematological malignancies. Ataturk University, Faculty of Medicine, Division

of Hematology of the Department of Internal Medicine1, Department of Biochemistry2, Erzurum, Turkey. *Eur J Gen Med* 2006; 3[3]:116-120.

20. Go'ksel Leblebisatan, Bu' lent Antmen, I' lgen S as maz, Yurdanur Kilinc. Vascular Endothelial Growth Factor Levels in Childhood Acute Lymphoblastic and Myeloblastic Leukemia; Indian Society of Haematology & Transfusion Medicine 2011. *Indian J Hematol Blood Transfus* (2012); 28(1):24–28.

21. Aguayo A, Kantarjian HM, Estey EH, 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.

22. Kalra M, Dinand V, Choudhary S, Sachdeva A, Yadav SP. Serum vascular endothelial growth factor-a levels during induction therapy in children with acute lymphoblastic leukemia. *Indian Pediatr.* 2013 50(7):659-62. *Epub* 2012 Dec 5.

23. Mukhopadhyay D, Tsiokas L, Zhou X-M, Foster D, Brugge JS, Sukhatme VP. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. *Nature (Lond.)* 1995, 375: 577-81.

24. Alvaro Aguayo, Hagop Kantarjian, Taghi Manshouri, Cristi Gidel, Elihu Estey, Deborah Thomas, et al. From the Departments of Leukemia, Hematopathology, and Bioimmunotherapy. Angiogenesis in acute and chronic leukemias and myelodysplastic syndromes. University of Texas, M. D. Anderson Cancer Center, Houston, TX *Blood*, 15 2000 volume 96, number 6.

25. Ali Ibne et al. (2013) "Expression and localization of locally produced growth factors regulating lymphangiogenesis during different stages of the estrous cycle in corpus luteum of buffalo" (Bubalus bubalis). *Theriogenology*.

26. Wiesmann C, Fuh G, Christinger HW, Eigenbrot C, Wells JA, de Vos AM. A resolution of VEGF in complex with

- domain 2 of the Flt-1 receptor. *Cell* 1997; 91: 695-704.
27. Fuh G, Li B, Crowley C, Cunningham B, Wells JA. Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor. *J Biol Chem* 1998; 273: 11197-204.
 28. Brozzo MS, Bjelic S, Kisko K, Schleier T, Leppanen VM, Alitalo K, et al. Thermodynamic and structural description of allosterically regulated VEGF receptor 2 dimerization. *Blood* 2012b; 119: 1781-8.
 29. Kaipainen A. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. *Proc Natl Acad Sci USA* 1995; 92[8]: 3566-70.
 30. Santos SCR, Dias S. Internal and external autocrine VEGF/KDR loops regulate survival of subsets of acute leukemia through distinct signaling pathways. *Blood*.2004;103[10]:3883-9.
 31. Podar K, Anderson KC. The pathophysiologic role of VEGF in hematological malignancies,therapeutic implication. *Blood* 2005; 105(4):1383-95.
 32. Mazure NM, Chen EY, Laderoute KR, Giaccia AJ. Induction of vascular endothelial cell growth factor by hypoxia is modulated by a phosphatidylinositol 3-kinase/Akt signaling pathway in Ha-ras-transformed cells through a hypoxia-inducible factor-1 transcriptional element. *Blood* 1997; 90: 3322-31.
 33. Dabrosin C. Variability of vascular endothelial growth factor in normal human breast tissue in vivo during the menstrual cycle. *J Clin Endocrinol Metab* 2003;88:2695-8.
 34. Karkkainen MJ; Petrova TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. *Oncogene* 2000; 19 [49]: 5598-605.
 35. American Cancer Society: Cancer Facts and Figures. Atlanta, Ga: American Cancer Society. Available online. Last accessed. May 21, 2014.
 36. Jackson N, Menon BS,Zarina W,Zawawi N et al. Why is acute leukemia more common in males? A possible sex-determined risk linked to the ABO blood group genes. *Ann Hematol* 1999; 78: 233-6.
 37. Alaa FA, Zedan JZ, Omar SS. Acute myeloid leukemia: clinical features and follow-up of 115 Iraqi patients admitted to Baghdad Teaching Hospital. *Tikrit Med. J.* 2009; 15 (1):1-8.
 38. Chessel JM, O'Calloghan U, and Hardisty RM. Acute Myeloid Leukaemia in Childhood: Clinical Features and Prognosis. *Br J Haematol* 1986; 63:555-62.
 39. Burns CP, Armitage JO, and Frey AL. Analysis of Presenting Features of Adult Leukaemia. *Can* 1981; 47:2460-6.
 40. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med.* 29 2011;365 (26):2497-506.
 41. Wetzler M, Byrd JC, Bloomfield CD. Acute and chronic myeloid leukemia. In Harrison's principles of internal medicine 18thed. McGraw-Hill.2012; vol.1:631-637.
 42. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *Cancer J Clin.* 2009;59(4):225-49.
 43. Karesh JW, Goldman EJ, Reck K, Kelman SE, Lee EJ, Schiffer CA. A prospective ophthalmic evaluation of patients with acute myeloid leukemia: correlation of ocular and hematologic findings. *J Clin Oncol.* 1989; 7[10]:1528.
 44. Tallman MS, Neuberg D, Bennett JM, et al. Acute megakaryocytic leukemia: the Eastern Cooperative Oncology Group experience. *Blood* 2000; 96:2405.
 45. Dastugue N, Lafage-Pochitaloff M, Pagès MP, et al. Cytogenetic profile of childhood and adult megakaryoblastic leukemia (M7): a study of the GroupeFrançais de Cytogénétique Hématologique (GFCH). *Blood* 2002; 100:618.