Immunohistochemical Expression of Vascular Endothelial Growth Factor in Chronic Lymphocytic Leukemia and its Relation to Laboratory and Clinical Findings

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Abstract

The Aims of the study: To determine the immunohistochemical expression of VEGF in CLL patients and its relation to laboratory and clinical parameters and its prognostic role in those patients.

Patients and methods: This study was carried out during a period of 5 months from January 2011 to May 2011 including 40 cases of CLL (27 males and 13 females), randomly collected from the teaching laboratories in Medical City of Baghdad. Clinical information were collected with re-evaluation of complete blood counts, peripheral blood smear, bone marrow aspiration and bone marrow biopsy. BM biopsy paraffin embedded blocks subjected to the immunohistochemical method using the LSAB technique. Monoclonal Mouse Anti-Human VEGF was used as primary antibody for the detection of VEGF protein.

Results: The age of the patients ranged between 38-74 years with a mean of 57 years, including 27 males and 13 females of 2.1:1 M: F ratio. The most common clinical presentations of CLL patients were lymphadenopathy as seen in 70% of cases. The mean complete blood counts were PCV 34.5%; platelets count 134 x 10^9/L and WBC count 105 x 10^9/L. Bone marrow findings at diagnosis included mean marrow lymphocytes of 86.0 % of all nucleated cells with diffuse pattern 64%, mixed 19%, interstitial 11% and focal 6% of marrow involvement. According to modified Rai staging system; 60% of patients were in high risk group, 27.5% in intermediate group and 12.5% at low risk group.

VEGF was expressed in 45% of patient with CLL showing nuclear and/or cytoplasmic expression. The VEGF expression showed statistically significant correlation with PCV% and platelets counts, while no such correlation found with other complete blood counts and morphology of blood film. Bone marrow involvement pattern showied statistically significant correlation with VEGF expression, but not with other bone marrow findings. The VEGF expression showed no statistically significant correlation with any of clinical presentation of CLL patient, but significant correlation with Modefied Rai staging system.

Conclusions: VEGF was expressed in 45% of CLL patients with statistically significant association with laboratory findings of advance disease while had no association with clinical parameters of patients. VEGF expression might have role in determining advance stage of disease and prognostic significance in CLL patients and can be considered as an informative and useful tool for assessing disease activity.

التعبير المناعي النسيجي الكيمياوي المعلم (VEGF) في مرض ابيضاض الدم اللمفاوي المزمن وعلاقته بالعلامات المختبرية والسريرية.

الخلاصة

ألهدف من الدراسة: هو لتحديد وجود المعلم عامل نمو الخلايا المبطنة الوعائية VEGF في مرضى ابيضاض الدم اللمفاوي المزمن وعلاقته بالعلامات المختبرية والسريرية ومدى علاقته بمستقبل المرض.

المرضى والطرق: هذه الدراسة اجريت خلال فترة خمسة اشهر متضمنة اربعين حالة من ابيضاض الدم اللمفاوي المزمن (٢٧ ذكر و ١٣ انثى) جمعت بصورة عشوائية من المختبرات التعليمية في مدينة الطب/ بغداد. تم جمع جميع المعلومات السريرية ونتائج فحوصات صورة الدم الكاملة، مسحة الدم المحيطي، سحبة نخاع العظم، وخزعة نخاع العظم. خزعة نخاع العظم المخزونة في قوالب الشمع تم

معالجتها بطريقة الفحص الكيميائي النسيجي المناعي وباستخدام طريقة LSAB مع وجود عامل نمو الخلايا المبطنة الوعائية VEGF كمضاد مناعي اولي.

النتائج: عمر المرضى في هذه الدراسة يتراوح بين ٣٨-٧٤ سنة وبمعدل ٥٧ سنة متضمنا ٢٧ ذكر و١٣ انثى بنسبة (١:٢,١). اشهر العلامات السريرية كانت تضخم الغدد اللمفاوية وجد في ٧٠% من الحالات، اما اشهر العلامات المختبرية كانت نسبة الدم ٣٤,٥% وعدد الصفيحات الدموية ١٣٤٠٠٠ وعدد كريات الدم البيضاء ١٠٥٠٠٠ . اما نتائج فحص نخاع العظم فكان معدل كريات الدم البيضاء اللمفاوية ٨٦% من جميع خلايا نخاع العظم مع غالبة لنمط الانتشار التام للخلايا اللمفاوية في خزعة نخاع العظم. حسب نظام Rai المعدل فان ٦٠% من المرضى وجدو ضمن مجموعة الخطر العليا. عامل نمو الخلايا المبطنة الوعائية VEGF وجد في ٤٥% من الحالات وكان ذو علاقة احصائية مهمة مع العلامات المختبرية المتعلقة بحالات المرض المتقدمة من نسبة الدم وعدد الصفيحات الدموية ونمط الانتشار التام للخلايا اللمفاوية في خزعة نخاع العظم و مجموعة الخطر العليا حسب نظام Rai المعدل، بينما لا توجد هكذا علاقة مع بقية العلامات المختبرية او السريرية.

الاستنتاج: عامل نمو الخلايا المبطنة الوعائية وجد في ٤٥% من حالات ابيضاض الدم اللمفاوي المزمن ومع علاقة احصائية مهمة مع العلامات المختبرية المتعلقة بحالات المرض المتقدمة وقد يكون وجود هذا العامل ذو علاقة مع انتشار او محدودية المرض ومستقبل الشفاء وهدف علاجي لهذه الحالات.

Introduction

CLL is a chronic lymphproliferative disorder characterize by the accumulation of long lived, mature neoplastic lymphocytes in blood and tissues, mainly of B-type. It is believed that prolonged in vivo survival of malignant cells contributes to their clonal expansion [1,2]. These clonal cells are largely non cycling, and their accumulation is secondary to reduce apoptosis/cell death rather than increase proliferation [3]. The fact that the malignant cells, despite progressively accumulating in vivo, rapidly undergo apoptosis when cultured in vitro implies that microenvironmental factors are likely to play a prominent role in prolonging the life span of CLL cells [2]. CLL is the most common form of leukemia in western countries and North America and mainly affects elderly individuals [1,4,5]. It follows an extremely variable course, with survival ranging from months to decades [5]. CLL is slightly more common in men than women and the median age at diagnosis is about 70 years [1].

The clinical diagnosis of CLL required an absolute lymphocytosis with a low threshold of greater than 5000 mature appearance lymphocytes /µL. Nevertheless, it is common to find small percentage of large or atypical cells, cleaved cells or prolymphocytes [5]. The routine availability of peripheral blood lymphocyte immunophenotyping has facilitated the diagnosis of CLL. Three main phenotypic features define B-CLL: the predominant population shares B-cell markers (CD19, CD2O, and CD23) with the CD5 antigen, in the absence of other pan-T-cell markers; the B cells are monoclonal with regard to expression of either κ or λ light chains and the B cells characteristically express surface immunoglobulin (slg), CD79b, CD20 and CD22 with low density. These characteristics are generally adequate for a precise diagnosis of CLL, and they also distinguish CLL from other disorders such as prolymphocytic leukemia, hairy cell leukemia, mantle-cell lymphoma, and other lymphomas that can mimic CLL [5,6]. The pathological features of the lymph nodes are those of a small lymphocytis lymphoma (SLL) [6].

In addition to traditional prognostic factors involving Rai and Binet clinical staging system, peripheral blood and bone marrow

findings, numbers of new biological prognostic markers have been identified. The most important are somatic hypermutation in the variable region of Ig heavy chain genes, membrane bound CD38 expression, intracellular ZAP-70 expression and cytogenetics aberration [1,6].

Staging system of CLL including the two widely accepted systems are those Rai et al [7] and Binet et al [8]. Modified Rai staging system classify the CLL patients into; Low-risk patients: patients with only lymphocytosis, intermediate-risk patients: patients with lymphocytosis and lymphadenopathy and/or hepatosplenomegaly, high-risk patients: patients with lymphocytosis and anemia and/or thrombocytopenia [9].

VEGF is a member of the platelet-derived growth factor superfamily and an endothelial cell-specific growth factor. VEGF is a relatively small molecule (45 kDa) with diverse biologic activities including stimulates vasodilatation, cell proliferation, increases permeability and migration, generation of inflammatory cytokines and promotes endothelial cell survival [10]. The gene for human VEGF has been localized to chromosome 6p21 [13].VEGF exerts its effects by interacting with two high-affinity receptors, VEGF-R1 and VEGF-R2 [3]. VEGF is a stromal cell derived growth factor to which the hematopoietic cells in the bone marrow are exposed [11].

There is currently much interest in the role of vascular endothelial cell growth factor (VEGF) in tumor formation and development [12] Indeed, inhibitors of VEGF or of the function of its receptors are being tested for their clinical antitumor effects [13,14]. VEGF is known to have multiple roles in tumor formation. For example, it is a major stimulating factor for the endothelial-cell migration and proliferation required for tumor vascularization. Moreover, the cytokine also directly affects the migration, proliferation, and survival of certain tumor cells themselves [15]. VEGF is also a survival factor for endothelial cells. This latter feature is of interest since apoptosis resistance is a seminal feature of the chronic lymphocytic leukemia (CLL) B cell [10].

VEGF is a potent mitogen for endothelial cells and overexpression has been correlated with increased angiogenesis. Because of this characteristic, VEGF has been associated with tumor growth, invasion, and metastasis in solid tumors. Recently, however, angiogenesis has been found to play a role in hematological malignancies, e.g. CLL [16,17]. The capacity of CLL and other leukemia cells to secrete angiogenic factors as VEGF indicates that CLL cells play a proactive role in modulating their microenvironment. Secretion of these factors could foster marrow neoangiogenesis and could explain the increased microvessel density seen in marrows from CLL patients [18,19]. The malignant lymphocytes of CLL produce VEGF, both in vitro and in tissues, and that secreted cytokine is capable of stimulating endothelial-cell proliferation and new-vessel formation [20]. There is therefore the possibility that CLL-cell–derived VEGF might mediate important autocrine effects on CLL cells. Indeed, it has recently been reported that autocrine VEGF can enhance CLL-cell survival [21]. VEGF is involved in the motility of the malignant cells on and through endothelium—processes important in CLL cell homing to tissues, while the motility of normal B cells did not depend on VEGF [12,22].

Angiogenesis is influenced by a number of positive and negative regulatory factors. However, a key mediator of the abnormal angiogenesis in many hematological malignancies has been the cytokine designated as VEGF. Angiogenesis and tumor vascularity increases with increasing disease stage and assists in disease progression in B-CLL. Tumor progression in the form of growth, invasion, and metastasis depends on angiogenesis, whose increase is thus indicative of poor prognosis in solid tumors [4,10].

The Aims of the study

To determine the immunoh-istochemical expression of VEGF in CLL patients and its relation to laboratory and clinical parameters and its prognostic role.

Materials, Patients and Methods

This study was carried out during the period from January 2011 to May 2011 including 40 cases of CLL (27 male and 13 female) with 5 control cases of normal bone marrow, randomly collected from the teaching laboratories in Medical City of Baghdad. Clinical informations were collected including age, sex and presenting clinical features. Complete blood counts for each case were collected with re-evaluation of peripheral blood smear, bone marrow aspiration and bone marrow biopsy.

BM biopsy paraffin embedded blocks subjected to the immunohistochemical method using the LSAB technique. Monoclonal Mouse Anti-Human VEGF was used as primary antibody for the detection of VEGF protein (Dako cytomation, Copenhagen, Denmark).

The criteria for the positive reaction confirming the presence of VEGF protein is dark, brown, nuclear and/or cytoplasmic precipitation.

Control group: five cases of normal bone marrow biopsy were selected for the control group in parallel with study group in addition to positive and negative control slides processed with each set of immunostaining.

Statistical analysis: data were analyzed using the SPSS software and the chi-square was used. P value at level of significant less than 0.05.

Results

The age of the patients ranged between 38-74 years with a mean of 57 years, majority were within the 7th decade (42.5%). This study included 27 male and 13 female of 2.1:1 M: F ratio (Table 1).

The most common clinical presentations of CLL patients were lymphadenopathy in 70% of cases followed by splenomegaly, hepatomegaly, wt loss and fever (Table 1).

The mean complete blood counts were PCV 34.5%; platelets count 134 x 10^9/L and WBC count 105 x 10^9/L. Bone marrow findings at diagnosis including mean marrow lymphocytes percent of 86.0 % of all nucleated cells with diffuse pattern 64%, mixed 19%, interstitial 11% and focal 6% of marrow involvement (Table 2).

According to modified Rai staging system; 60% of patients were in high risk group, 27.5% in intermediate group and 12.5% at low risk group (Table 3).

VEGF express it was found in 45% of patient as showing in (table 4) with nuclear and/or cytoplasmic expression (figure 1).

The VEGF expression showed statistically significant correlation with PCV% and platelets counts, while no such correlation found with other complete blood counts and morphology of blood film. The VEGF expression showed statistically

significant correlation with bone marrow involvement pattern, but not with other marrow findings (Table 2).
The VEGF expression showed no statistically significant correlation with any of clinical presentation of CLL, but with statistically significant correlation with Modefied Rai staging system (Tables 1,3).

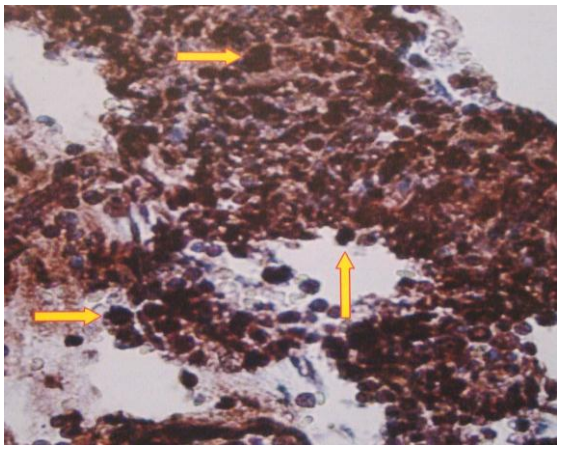

Figure 1: bone marrow tissue of CLL stained with VEGF most of cells show brown membrane and cytoplasmic stain (VEGF positive) (X40).

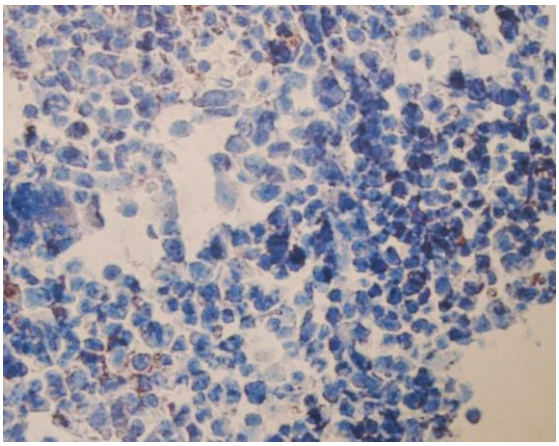

Figure 2: bone marrow tissue of CLL stained with VEGF stain (VEGF negative) (X40).

Table 1 The correlation between VEGF expressions and clinical parameters in CLL patients.

Clinical parameters				VEGF expression		P .value
Parameters		No	Percent	Negative	Positive	
Gender	Male	27/40	67.5%	14/27	13/27	Not Significant (0.09)
	Female	13/40	32.5%	7/13	6/13	
Age	< 60 yr	8/40	20 %	4/8	4/8	Not Significant (0.06)
	60-70 yr	15/40	37.5%	8/15	7/15	
	> 70 yr	17/40	42.5%	7/17	10/17	
LAP	Positive	28/40	70%	15/28	13/28	Not Significant (0.08)
	Negative	12/40	30%	7/12	5/12	
SM	Positive	19/40	47.5%	7/19	12/19	Not Significant (0.05)
	Negative	21/40	52.5%	17/21	4/21	
HM	Positive	10/40	25%	4/10	6/10	Not Significant (0.05)
	Negative	30/40	75%	18/30	12/30	
Wt loss	Positive	6/40	15%	4/6	2/6	Not Significant (0.06)
	Negative	34/40	85%	28/34	6/34	
Fever	Positive	4/40	10%	3/4	1/4	Not Significant (0.07)
	Negative	36/40	90%	25/36	11/36	

Table 2 The correlation between VEGF expressions and laboratory parameters in CLL patients.

Laboratory parameters	VEGF expression	Mean	P .value
PCV (Mean 34.5 %)	Negative	39.2	**Significant (0.03)**
	Positive	30.5	
Platelets count (Mean 135 x 109/L)	Negative	160.0	**Significant (0.04)**
	Positive	114.2	
WBC count (Mean 34.5 %)	Negative	102.4	Not Significant (0.06)
	Positive	118.9	
Peripheral blood lymphocytes %	Negative	87.0%	Not Significant (0.05)
	Positive	91.3%	
Typical Lymphocytes morphology %	Negative	96%	Not Significant (0.05)
	Positive	93%	
Lc % in BM aspiration (Mean 86.0% of ANC)	Negative	84%	Not Significant (0.06)
	Positive	87%	
Myeloid and Erythroid % in BM aspiration	Negative	6%	Not Significant (0.07)
	Positive	5%	
BM biopsy pattern:			
Interstitial 11%	Negative	66%	**Significant (0.04)**
	Positive	44%	
Focal 6 %	Negative	57%	
	Positive	43%	
Mixed 19%	Negative	61%	
	Positive	39%	
Diffuse 64%	Negative	15%	
	Positive	85%	

Table 3 The correlation between VEGF expression in CLL patients and modified Rai system.

Modified Rai system		VEGF expression		
		Negative	Positive	P value
Low risk	5 (12.5 %)	4	1	**Significant (0.03)**
Inter. Risk	11 (27.5 %)	10	3	
High risk	24 (60.0 %)	8	15	
Total	40 (100 %)	22	18	

Table 4 Scoring of VEGF expression in CLL patients.

	VEGF expression		Total
	Negative	Positive	
No of cases	22	18	40
Percent	55%	45%	100 %

Discussion

CLL is a low-grade B-lineage lymphoid malignancy with variable clinical course [2]. There are several prognostic factors has been used for evaluation of the clinical course. Current study was conducted to study the expression of VEGF in bone marrow of CLL patients and its correlations to main laboratory and clinical parameters of such patients.

The mean age of the patients in this study was 57 years and slightly predominant in male than female, which is similar to most studies in our country [23,24] while the mean age of CLL patient in most studies in western counties [25,26] was younger than our patient which is most likely due to the difference in life expectancy between the countries.

The most common clinical presentations of CLL patients were lymphadenopathy in 70% of cases which is similar to most other studies in our country, while asymptomatic CLL found more in western countries due to close follow up in clinical care and routine checkup of their population.

In this study, the mean PCV and platelets count were lower than normal range due to marrow replacement by malignant lymphoid cells with diffuse bone marrow pattern of involvement, which is compatible with most local studies while most of western studies showed early interstitial marrow involvement of the marrow as predominant pattern [27].

According to modified Rai staging system; 60% of patients in this study were in high risk group, while early clinical stage of disease is the most finding in western studies.

The VEGF expression showed statistically significant correlation with PCV% and platelets counts, while no such correlation found with other complete blood counts and morphology of blood film. The VEGF expression showed statistically significant correlation with bone marrow involvement pattern, but not with other bone marrow findings.

The VEGF expression showed statistically non significant correlation with any of clinical presentations of CLL patient, but showed such correlation with Modefied Rai staging system. These findings reveal the association of VEGF expression and advance disease of CLL patient with significant replacement of bone marrow haemopoietic elements by lymphoid cells. Accordingly the VEGF expression could be considered as bad prognostic factor in patients with CLL, as considered in many other studies [28, 29,16, 30] and if validated, the measurement of serum VEGF could help discriminate patients with high-risk early stage CLL and encourage therapeutical trial of the effects of VEGF blocking in patients with CLL.

Conclusions

In this study, VEGF express in 45% of CLL patients with significant association with laboratory findings of advance disease while had no association with clinical parameters of patients. VEGF expression had role in determine advance stage of disease and prognostic significance of CLL patients and can be considered as informative and useful tool for assessing disease activity.

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