Prognostic Relevance of VEGF-A and COX-2 in StageIV Non-Hodgkin's Lymphoma (NHL) Patients

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Abstract: Background: NHL comprises 85% of all lymphomas and 3-4% of all cancers around the world. Angiogenesis is required for tumor growth and metastasis, as an important component in the control of cancer progression. Inflammation is an important factor in the cancer phenomenon with COX-2 playing an important role in malignant cell proliferation. **Objective:** To observe the expression level of the angiogenetic factor VEGF-A, the inflammatory mediator COX-2 in NHL patients and their prognostic relevance. **Methods:** The patients were recruited from the Medical Oncology Department, National Cancer Institute, Cairo University over a period of 2 years. The methods we used were Immunohistochemical staining for VEGF-A and COX-2 in BMB samples of 40 adult patients with NHL stage IV. **Results:** The study revealed no significant difference between VEGF-A and COX2 markers expression (positive coexpression 10/40 (25%), negative coexpression 5/40 (12.5%) and single marker expression 25/40 (62.5%) (P=0.09). A statistically significant difference between males (37.5%) and females (6.3%) as regards positive coexpression was found (P=0.025). Although positive coexpression was higher with the lower age group and absence of B symptoms, yet showing statistically non-significant difference (P= 0.072and 0.09 respectively). There was a higher TLC mean and a higher lymphocyte count in the positive VEGF-Aexpressors versus negative ones, with a non-significant difference (P=0.065). A statistically significant difference between positive coexpressors group with the single positive and the double negative groups regarding TLC (P=0.036) was detected, also higher lymphocyte count among positive coexpressors compared to the other groups, with non-significant difference (P= 0.077). A near significant difference between patients with positive VEGF-A expressoion (71.4%) and negative ones (42.1%) as regards response to treatment (P=0.06). Finally, no statistical significant difference was found for positive and negative VEGF-A, COX-2 expression, positive and negative coexpression as regards overall survival (P=0.117, 0.84, 0.28 and 0.25 respectively). **Conclusion:** Our findings couldn't identify the association between VEGF-A and COX2 with prognosis of NHL, controversially, a better response to treatment in positive VEGF-Aexpressors than non expressors and in non coexpressors to VEGF-A and COX2. So, further studies with larger number of patients are required to clarify the association between the inflammatory mediator and the angiogenetic factor and to assess their role in the response to treatment for the possibility to use modifying drugs and improve the response in NHL patients.

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1. Introduction

Lymphoma is a solid tumor of lymphocyte. Its incidence varies according to age, geographical location and exposure to various viral factors [1]**.**NHL comprises 85% of all lymphomas & accounts for 3-4% of all cancers around the world. Its incidence and has shown to be on the rise. This increase is related to the increase in HIV incidence as well as the prevalent use of immune-suppressive drugs [2]. Angiogenesis is required for tumor growth and metastasis and is an important component in the control of cancer progression. Angiogenesis-associated parameters are important for prognosis in NHL [3].VEGF is the major angiogenic factor and together with its receptors regulates different aspects of vascular angiogenesis and lymphangiogenesis. Tumor cells produce VEGF-A with other angiogenic factors. VEGF-A also supports the survival, proliferation and migration of lymphoma cells that express VEGFR-1 and VEGFR-2 in an autocrine fashion. The tumor stroma made of fibroblasts, inflammatory and immune cells provides additional angiogenic factors [4].Inflammation is one of the important factors in the cancer phenomenon. COX-2 plays an important role in the tumor growth and malignant cell proliferation which is followed by an increase in angiogenesis, invasion and metastasis. COX-2 mRNA is not seen in the tissues normally, but it can increase following response to inflammation or mitogenic stimuli such as growth factor cytokines, oncogenes and several chemical factors. Stimulation for activating COX-2 gene may have an important role in the emergence of cancer. The increase in COX-2 expression can be seen in different cancers as pancreas, stomach, prostate, lung, colorectal, head and neck, breast and bladder. Specific inhibition of COX-2

can also be useful in some cancers by apoptosis stimulation [5].

Generally it has been found that lymphomas showing higher angiogenic potential are related with shorter disease free survival (DFS) and/or OS and poor prognostic indicators including aggressive histology and/or transformed morphology. Additionally, correlations between VEGF-A and Cox-2 were detected. These correlations show and confirm the strong prognostic value of VEGF-A and complex interaction with anti-apoptotic signals and also inflammatory signals [6].Taking into account the possible association between the angiogenesis and inflammatory signals, we aimed in the current study to assess the expression of COX2 and VEGF-A in disseminated bone marrow (stage IV) NHL patients and to clarify their association with the clinical parameters and the response to treatment of patients, to rule out their possible role as prognostic markers for NHL.

2. Materials and Method

2.1. Study setting

The study comprised 40 patients diagnosed as stage IVNHL, they were 24(60%) males and 16(40%) females with median age of 57 years. The patients were recruited from the Medical Oncology Department, National Cancer Institute, Cairo University over a period of 2 years.

2.1. Research ethics

A written informed consent was approved by the Institutional Review Board (IRB) Ethical Committee of the NCI, which follows the rules of Helsinki IRB, and was obtained from each patient before starting the data collection. For the sake of each patient's privacy, they were assigned code numbers.

2.3. Inclusion and exclusion criteria

Inclusion criteria for patients included the histologically confirmed NHL stage IV (BM infiltration), age above 18 years. Exclusion criteria included patients with Hodgkin's lymphoma, Burkitt's leukemia, LBL, age below 18 years and history of other malignancies.

2.4. Data collection

Our study patients were subjected to clinical**,** histopathological, radiological assessment and routine laboratory work-up for lymphoma patients. All patients received treatment with standard chemotherapy protocols (CHOP-R for DLBC, MALT, FL, MZL and unclassified lymphoma), (CHOP for T-NHL), (CVP for SLL) & Cladribine for HCL with complete remission in 23 (57.5%) patients. Complete remission (CR) was defined as normalization of clinical and radiological abnormalities, relevant laboratory data and bone marrow picture for four weeks after the last cycle of chemotherapy. Patients were classified as having partial remission (PR) if they have at least a 50% reduction in the sum of the product of the greatest cross-sectional diameters of measurable lesions. New lesions or more than 25% increase in an individual lesion over one treatment cycle was categorized as progressive disease (PD). Appearance of new lesions or the reappearance of old lesions in patients who achieved complete remission was categorized as relapse. Our study patients were subjected to collection of BM trephine biopsy specimen, processing, staining with Haematoxylin and Eosin [7], and finally VEGF-A (Dako, Monoclonal mouse IgG2a antihuman VEGF-A protein; clone c-1, code sc-7269) and COX-2 (Dako, polyclonal goat IgG anti-human COX2 protein; clone N-20, code sc-23983) immunohistochemical staining including positive and negative controls [8]. Positive controls for VEGF-A formalin -fixed, paraffin -embedded sections were from human lung cancer sections**,** and those forCOX-2 were from breast cancer sections [9].The negative controls for both VEGF-A and COX-2 were obtained by using phosphate buffer saline (PBS) instead of the primary antibody [9].

2.5. Interpretation of the Results

Microscopic examination of H and E stained BMB samples was performed using Olympus light microscope. Dissemination and lineage commitment was confirmed by appropriate markers immunohistochemically. For estimation of VEGF-A and COX2 positivity, the most cellular area of the tumor, with the minimum necrosis or inflammatory cell infiltration was selected and the number of positively stained cells was recorded in consecutive fields at x40 magnification.

2.6. Interpretation of VEGF-A

The percentage of tumor cells expressing VEGF-A was determined by counting 1000 cells per slide. VEGF-Aimmunostaining was scored according to the extent and severity of stained cells as follow:

1) Extent of staining was classified as 0 = 0-10% of tumor cells were stained, 1 = 11-25% of tumor cells were stained, 2 = 26-50% of tumor cells were stained, and 3= more than 50% of tumor cells were stained.

2) Severity of staining was classified as 1=light yellow, 2= dark yellow and 3= brown.

The sum of these two classifications was scored as negative if the score was 0-2, mild if the score was 3-4 and sever if the score was 5-6 [10]. Figure1 illustrate different patterns of VEGF positivity

2.7. Interpretation of COX-2

For estimation of COX-2 expression: A scoring method based on estimation of the percentage of immune-reactive cells in combination with an estimation of the severity of intensity was used as follows: 0= no staining, 1= weak diffuse cytoplasmic

labeling or may be if it was stronger labeling of <10% of the cancer cells, 2= moderate to strong granular cytoplasmic labeling of 10 to 90% of the cancer cells and 3= more than 90% of the tumor cells with strong intensity. Samples scoring 1 to 3 were considered positive for COX-2, while samples scoring 2 to 3 were considered to be overexpressing the enzyme [11].Figure 1 illustrates COX2 pattern of positivity

2.8. Statistical methods

Data was analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test or 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). Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. All tests were two-tailed. A p-value < 0.05 was considered significant.

3. Results

The present study included 40 Egyptian adult patients with BM disseminated NHL (stage IV), they were 24 (60%) males and 16 (40%) females with median age of 57 years. Their demographic data is demonstrated in Table 1. As regards the hematological findings, hemoglobin level ranged from 2.4 to 14.9 gm/dl, with a mean value of 10.4±2.9 gm/dl. Total leucocytic count (TLC) ranged from 2.2×10^3 to $101\times10^3/cm^3$ with a median value $8\times10^3/cm^3$. Platelet count ranged from 18×10^6 to 569×10^6 $/cm^3$ with a median $194.5\times10^6/cm^3$ and finally, lymphocytes % which ranged from 6% to 96% with a median 32%. Bone marrow aspirate (BMA) was normocellular in (24/40 i.e. 60%), hypercellular in (11/40 i.e. 27.5%) and hypocellular in (5/40 i.e. 12.5%) with normal megakaryocytes in (26/40 i.e. 65%), increased megakaryocytes in (8/40 i.e. 20%) and decreased megakaryocytes in (6/40 i.e. 15%). BM lymphocytes % ranged from (6% to 88%) with a median of 16%.Cellularity in BM Biopsy revealed that (21/40 i.e. 52.5%) of cases were normocellular, (16/40 i.e. 40%) were hypercellular and (3/40 i.e. 7.5%) were hypocellular with fibrosis in (28/40 i.e. 70%). The pattern of lymphoid infiltration in BMB was; diffuse infiltration in (18/40 i.e. 45%), patchy infiltration in (15/40 i.e. 37%) and nodular infiltration in (7/40 i.e. 17.5%). Immunohistochemistry that was done on BMB revealed that: CD3 was positive in (4/40 i.e. 10%) while CD20 was positive in (36/40 i.e. 90%). There were other markers detected in (20/40 i.e. 50%) of the cases which were: Bcl2 in (8/20 i.e. 40%), CD23 in (2/20 i.e. 10%), CD5 in (4/20 i.e. 20%), CD19 in (4/20 i.e. 20%), CD22 in (2/20 i.e. 10%), IgMLA (3/20 i.e. 15%), CD75a in (6/20 i.e. 30%) and CD75b in (2/20 i.e. 10%) and each of CD45Ro, CD10, CD30, CD43 11C, CD103, CD25 and CD20 detected in (1/20 i.e. 5%).According to the response of NHL patients to treatment; (23/40 patients i.e. 57.5%) showed complete remission while (17/40 i.e. 42.5) did not show complete remission. Out of the responders; (5/23 i.e. 21.7%) showed relapse of the disease and (1/23 i.e. 4.3%) was dead. (21/40 i.e. 52.5%) of the patients were alive to the end of follow up while (19/40 i.e. 47.5%) were dead. Concerning Single marker expression: VEGF-A was expressed in (21/40 i.e. 52.5%) of the cases. (7/21 i.e. 33.3%) showed moderate expression and (14/21 i.e. 66.7%) showed strong expression, while it was not detected in (19/40 i.e. 47.5%). COX2 was expressed in (24/40 i.e. 60%) of the patients, (17/24 i.e. 70.8%) showed weak expression, (6/24 i.e. 25%) showed moderate expression and (1/24 only i.e. 4.2%) was strong expression, while it was not detected in (16/40 i.e. 40%)(Table 2). No significant difference was found between VEGF-A and COX2 expression (P=0.09) as both were positive in 10/40 (25%), both were negative in 5/40 (12.5%) and single marker (VEGF-A or COX-2) was positive in 25/40 (62.5%) (Table 3). Table 4 represents the descriptive study for cases with VEGF-A positive and negative expression (21/40 (52.5%), 19/40 (47.5%)), COX2 positive and negative expression (24/40 i.e. (60%), 16/40, (40%)) in relation to demographic data of patients showing no statistical difference with any of the studied parameters except with age which was higher in VEGF-A negative patients (P=0.041). Table 5 shows markers expression (positive and negative)in relation to pathological subtypes of the lymphoma.

There was no significant difference between positive and negative VEGF-A patients regarding either their PB data (HB, TLC, PLTs count, P.B. lymphocyte relative count, LDH) (P= 0.361, 0.065, 0.117, 0.065, 0.229 respectively) or their BMA and BMB findings (BM cellularity, megakaryocytes, fibrosis, pattern of infiltration) (P= 0.227, 0.666, 0.836, 0.589 respectively).However, the TLC and the lymphocytes relative count were higher in the VEGF-A positive patients than in the negative ones. Also, no significant difference between COX2 positive and negative patients regarding P.B. data (HB, TLC, PLTs count, lymphocyte relative count, LDH) (P=0.452, 0.733, 0.576, 0.692, 0.497 respectively), and their bone marrow aspirate and biopsy findings (BM cellularity, fibrosis, pattern of infiltration) (P value=0.286, 0.888, 0.156 respectively). However, a statistically significant difference between the patients with positive and negative COX2 and the BM

megakaryocytes (P=0.008). Regarding the response to treatment, there was a border line significant difference between patients with positive VEGF-A responding to treatment (15/21 i.e. 71.4%) and those with negative VEGF-A who didn't respond to treatment (8/19 i.e. 42.1%) (P=0.06) (Figure2). No significant difference was found between the two patient groups regarding follow up of responders and survival status (P= 0.782 and 0,210 respectively) Regarding COX-2 expression, no significant difference was found between patients with COX-2 positive and negative expression as regards the response to treatment, follow up for responders and survival status (P=0.433, 0.796 and 0.433 respectively).

Considering the coeexpression of both markers as regards clinical and demographic data, VEGF-A and COX2 coexpression (10/40 i.e. 25%) was higher in males (9/24 i.e. 37.5%) than females (1/16 i.e. 6.3%) with a statistically significant difference (P=0.025). Despite being higher in the lower age group and with the absence of B-symptoms, no significant difference was found between markers coexpression and any of age, B-symptoms, hepatomegaly, splenomegaly, lymphadenopathy or extranodal involvement (P= 0.072, 0.09, 0.21, 0.196, 1.0 and 0.206 respectively). No significant difference was found between patients with double negative expression of VEGF-A and COX2 and the rest of the patients regarding their age, sex, and presence of B-symptoms, splenomegaly, hepatomegaly and lymphadenopathy (P=0.905, 0.329, 0.900, 0.397, 0.549, 0.738 respectively).

There was a statistically significant difference between the coexpression group (10/40 i.e. 25%) and the rest of the patients (double negative and single positive) (30/40 i.e. 75%) regarding TLC, where TLC mean was higher (23.2×10^3 /cm^3) in the coexpression group versus the others (10.6×10^3 /cm^3) (P=0.036). Despite a higher lymphocyte P.B. count in the coexpressor group compared to the other two groups, no significant difference was found regarding other PB data (HB, platelets count, lymphocyte %, LDH) (P value= 0.890, 0,292, 0.077 and 0.129 respectively) or BMA/BMB findings (BM cellularity, megakaryocytes, fibrosis and pattern of infiltration) (P=0.504, 0.139, 0.426, 0.627 respectively). Table 6 shows response to treatment and follow up of patients with positive VEGF-A and COX2 coexpression. The median overall survival (OS) for the whole group was 40 months, ranging between 0.5 and 66 months. Despite numerical increase in OS in VEGF-A positive group (64.7 months) versus only 15.2 months in VEGF-A negative group, but this was not statistically significant (p=0.117). Also, there was no significant difference in survival between COX-2 positive (64.7 months) and COX-2 negative (39.9 months) group (p=0.84) and finally no significant difference in OS weather both VEGF-A and COX-2 were both positive or both negative in comparison with the other group categories (p=0.28 and 0.25 respectively).

Table 1. Demographic data of NHL patients at Diagnosis

Age (range, mean±SD, median) 33-75 y		55±10.7 y	57 y
		Frequency (n=40)	%
Sex	Male	24	60.0
	Female	16	40.0
B –Symptoms: Fever, night sweat, weight loss		25	62.5
Splenomegaly		23	57.5
Hepatomegaly		21	52.5
Lymphadenopathy		34	85.0
Extra nodal involvement		10	25.0
Pathological data of NHL patients			
DLBCL		15	37.5
CLL/SLL		9	22.5
Follicular NHL		8	20.0
T-NHL		3	7.5
MALT-lymphoma		2	5.0
Marginal zone NHL		1	2.5
HCL		1	2.5
B-NHL Unclassified		1	2.5

DLBCL (Diffuse Large B-cell Lymphoma), Cll/Sll (chronic lymphocytic leukemia/small lymphocytic leukemia/lymphoma), HCL (hairy cell leukemia)

Table 2. Immunohistochemistry for VEGF-A and COX-2 among NHL patients

Variables	n	%		n	%
VEGF positive	21/40	52.5	Moderate	7/21	33.3
			Strong	14/21	66.7
COX2 positive	24/40	60.0	Weak	17/24	70.8
			Moderate	6/24	25.0
			Strong	1/24	4.2
Both positive	10/40	25%			
Both negative	5/40	12.5%			
Either one is positive	25/40	62.5%			

Table 3. The relation between VEGF-A and COX-2 expression in NHL patients

		VEGF-A		P-value
		Positive n=21/40	Negative n=19/40	
COX2	Positive n=24/40	10 (41.7%)	14(58.3%)	0.093
	Negative n=16/40	11 (68.8%)	5 (31.3%)	

Table 4.VEGF-A and COX2 expression in relation to the demoghraphic data

		VEGF-A			COX2		
		Positive n= 21/40	negative n= 19/40	P-value	Positive n= 24/40	negative n=16/40	P-value
Age	Range	33-74	33-72	0.041*	33-72	33-74	0.713
	mean±SD	52±9.9	58±10.8		55±10.4	54±11.4	
Sex	Male n= 24/40	15(62.5%)	9(37.5%)	0.121	16(66.7%)	8(33.3%)	0.292
	Female n= 16/40	6 (37.5%)	10(62.5%)		8(50.0%)	8(50.0%)	
B-symptoms	Yes n= 25/40	12(48%)	13 (52%)	0.462	14(56.0%)	11(44.0%)	0.505
	No n=15/40	9 (60%)	6 (40%)		10(66.7%)	5(33.3%)	
Hepatomegaly	Yes n= 21/40	13(61.9%)	8(38.1%)	0.210	13(61.9%)	8(38.1%)	0.796
	No n= 19/40	8(61.9%)	11(39.1%)		11(57.9%)	8(42.1%)	
Splenomegaly	Yes n= 23/40	14(60.9%)	9(39.1%)	0.218	11(47.8%)	12(52.2%)	0.068
	No n= 17/40	7(41.2%)	10(58.8%)		13(76.5%)	4 (23.5%)	
Lymphadenopathy	Yes n= 34/40	19(55.9%)	15 (44.1%)	0.308	20(58.8%)	14 (41.2%)	1.000
	No n= 6/40	2(33.3%)	4(66.7%)		4(66.7%)	2 (33.3%)	
Extra-nodal involvement	Yes n= 10/40	3(30.0%)	7(70.0%)	0.100	6(60.0%)	4 (40.0%)	1.000
	No n= 30/40	18 (60.0%)	12(40.0%)		18(60.0%)	12 (40.0%)	

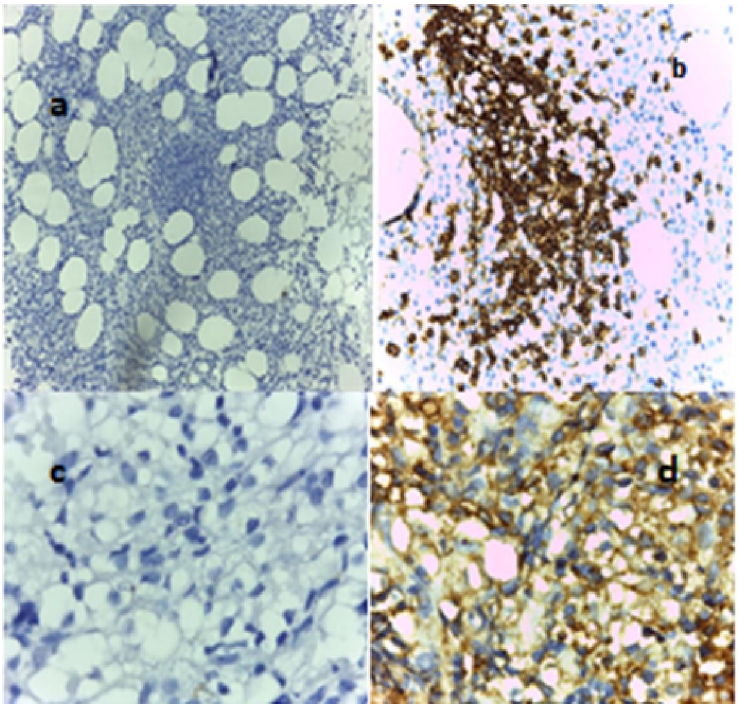
* Significant

Table 5. VEGF-A and COX2 expression regarding pathological subtypes of NHL

Variables	VEGF-A		COX2	
	Positive n=21/40	Negative n=19/40	Positive n= 24/40	Negative n=16/40
DLBCL n= 15/40	5 (36.5%)	10 (63.5%)	5 (33.3%)	10 (66.7%)
CLL/SLL n= 9/40	6 (66.7%)	3 (33.3%)	5 (55.6%)	4 (44.4%)
Follicular NHL n= 8/40	4 (50%)	4 (50%)	4(50.0%)	4(50%)
T-NHL n= 3/40	2(66.7%)	1 (33.3%)	2 (66.7%)	1(33.3%)
MALT-lymphoma n= 2/40	1 (50%)	1 (50%)	1 (50.0%)	1(50.0%)
Marginal zone NHL n= 1/40	1 (100%)	0	1(100.0%)	0
HCL n= 1/40	1 (100%)	0	0	1 (100.0%)
B-NHL Unclassified n= 1/40	1 (100%)	0	1(100.0%)	0
No p-value because of small no of cases within subgroups.				

Table 6. Comparison between NHL patients with both (VEGF-A and COX-2) coexpression and the others groups regarding response to treatment and follow up

Variables		Both (VEGF&COX) Positive, n=10/40	0thers, n=30/40	P-value
Response to treatment	CR n=23	8(80%)	15(50%)	0.097
	Not CR n=17	2(20%)	15(50%)	
Survival status	Dead	4/10 (40%)	15/30 (50%)	0.58
	Alive	6/10 (60%)	15/30 (50%)	
Relapse	Yes n=5/23	3/8 (37.5%)	2/15 (3.3%)	0.181
	No n=18/23	5/8 (62.5%)	13/15 (86.7%)	



Figures 1.(a) VEGF-A negative expression with power magnification (10x), (b)BMB section of VEGF-A strong positive expression with power (40x) in BM focal infiltration, (c) COX-2 negative expression with power magnification (100x), (d) BMB section of COX-2 moderate positive expression with power magnification (100x).

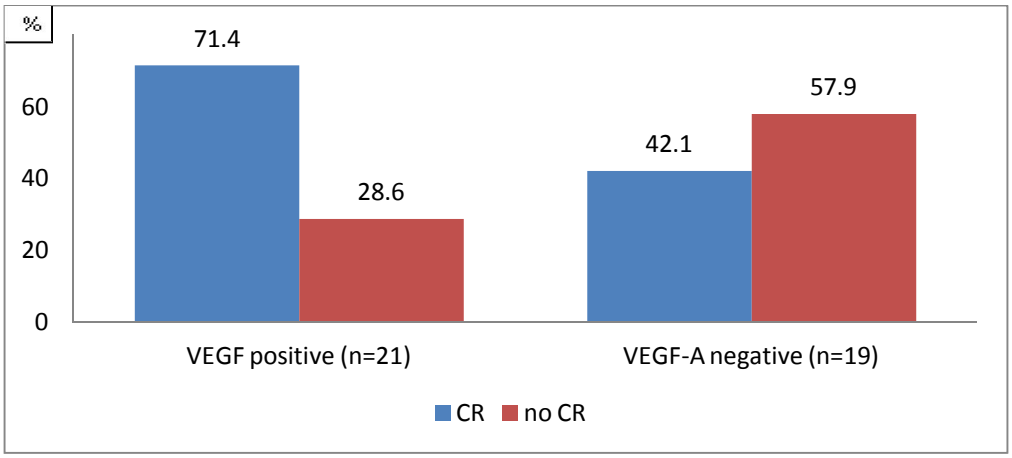


Figure 2. VEGF-A expression regarding response to treatment.

4. Discussion

Among our 40 BM disseminated NHL patients, VEGF-A expression was detected in 52.5% of the patients, which was close to others who reported VEGF in 50% of NHL group [12], but slightly differing from other studies [13,14], reporting VEGF in 61% of NHL group, in64.8% of DLBCL group and 62% of NHL group, 41.2% of low-grade B-cell NHL, and 100% of intermediate-grade B-cell NHL and T-cell lymphoma). We detected COX2in 60% of our patients, similar to our results, reports in 57% among NHL group [15] and (56% among NHL cases) [16], but slightly higher than a study reporting COX2 in 53.3% of NHL group [17] and lower than another reporting it in 79% among NHL group [18]. We report no difference between VEGF-A and COX2 expression, as both were positive in 25%, both were negative in 12.5% and a single one was positive in 62.5% of cases. As mentioned before [13] found that among DLBCL group; COX2was positive in 71.6%, and VEGF-A was positive in 64.8% with a (P=0.038). Also, different authors, found that VEGF expression was positive in 70% and was strong positive in 8% among B-cell lymphoma group, also COX2 was expressed in 8% and so strong VEGF expression correlated with COX2 expression (P=0.012) [19]. Several in situ studies have been performed on small paraffin-embedded B cell lymphoma series to assess tumor angiogenesis and expression of VEGF and COX2, with somewhat controversial results [19]. Generally, correlations between VEGF-A and Cox-2 were detected. These correlations show and confirm the strong prognostic value of VEGF-A and complex interaction with inflammatory signals of COX2 [13].However this correlation wasn't approved in our study, may be due to geographical and ethnic variation among the studied groups or may be due to technical variation of the studied method and the sample size, that need to be verified in a larger group, as the association between angiogenic factors and COX2 may lead to development of combination therapeutic strategy for modifying treatment in NHL.

The results of our study demonstrated lack of a significant association between VEGF-A expression and the clinico-pathologic features of B-NHL, as well as the studied standard prognostic factors including age, sex, organomegaly, lymphadenopathy, B symptoms, pathological subtypes, WBC count, Hb level, PLTs count and LDH level. Different studies were in consistent with our findings, [20] reporting that VEGF overexpression did not correlate with performance status, LDH level, IPI score, tumor staging, B symptoms, or NHL relapse, also no association between serum VEGF and clinical features at diagnosis [21], and the clinical prognostic indicators were not significantly different between VEGF-A (+) and (-) cases [13]. In addition, others found no significant relationship between VEGF and age, gender, stage, histological grade, IPI, and overall survival in 71 patients with NHL [15], and that among 60 cases with LGL and 117 cases with AL, PS, stage of the disease, age, extra-nodal involvement, sex, relapse, the presence of B symptom and IPI were not different in VEGF-A (+) and (−) cases, with also an association between VEGF-A with aggressive histology (P=0.031) [22]. In the same line, others reported that serum VEGF level was significantly higher in patients with aggressive lymphoma or adult T-cell leukemia/lymphoma [23], and was evaluated in 27 patients with NHL and concluded that VEGF was associated with higher tumor grading of NHL and high-grade transformation of low-grade lymphoma [14]. Similarly, among 58 NHL patients, serum VEGF level of patients at stage II, III and IV increased significantly (P< 0.05) as compared with patients at stage I [12]. However another study, in 24 NHL patients, concluded the serum level of VEGF in NHL patients was higher than controls. The high serum level of VEGF has a tendency to drop to the normal standard after reaching CR, but, a high serum level was not found to be associated with stage, gender, PS. score, IPI score, serum LDH and "B" symptoms [24].

As regards the response to therapy, the frequency of VEGF positive patients who responded to treatment in our study was border line statistically higher than VEGF negative patients (71.4% versus 42.1%, P=0.06). Regarding follow up and survival status, there was no statistically significant difference between the two patient groups (P= 0.782, 0.210 respectively).In consistent with our findings, others found that the VEGF expression was related to a favorable response (P=0.002) among advanced gastrointestinal stromal tumor group [25]. It was reported also that the higher levels of serum VEGF was not associated with a poorer complete remission (CR) rate [21], and the serum VEGF was not predictive of survival among NHL patients [26]. On the other hand; Yang et al, 2015 [20] found a significant correlation between VEGF overexpression and overall survival (P=0.001). Also, they found that VEGF overexpression in surgically resected tissue, was associated with poorer prognosis (P<0.001). Wang et al, 2011 [27] also found that VEGF expressions on CD14+ monocytes by flow cytometry in NHL patients in non-remission group before chemotherapy (n=11) was obviously higher than that in remission group (P <0. 001). Several studies by Jørgensen et al found that, among follicular B-cell lymphoma, patients with diffuse VEGF expression in lymphoma cells had poorer overall survival than those

with focal expression [28], and in FL, diffuse intratumoral VEGF staining correlated with shorter overall survival (OS) (P= 0.008) and found in peripheral T-cell lymphomas (PTCL) that, diffuse tissue distribution of VEGF mRNA correlated with an unfavorable 5-year OS (p=0.004) [29]. Others as Paydas et al, 2009 [13] found that, the overall survival (OS) rate was shorter in cases with VEGF-A (+) cases than with negative cases (P=0.03) and that among DLBCL, the mean survival rates were significantly shorter in cases expressing VEGF-A than cases not expressing it. Hazar et al, 2003[15] found that in 71 patients with NHL, complete and partial response rates to therapy were significantly higher in VEGF-negative patients than in the VEGF-positive patients (P=0.003) and Paydas et al, 2008 [22] also found that among 60 cases with LGL and 117 cases with AL, the overall survival times were shorter in VEGF (+) cases as compared with (−) cases. In addition, a previous old study, documented upon, baseline VEGF levels of NHL patients in CR after a median follow-up of 21 months were significantly lower than those of patients with progressive disease (P 0.016) and the event-free survival (EFS) rate was significantly higher in patients who had baseline VEGF level below the median values of 147 and 19.5 pg/ml (P=0.018) [30]. Several other authors reported a positive correlation between increased VEGF serum level and serum lactate dehydrogenase level, and that the levels of VEGF were not significantly different in aggressive or indolent NHL patients [31]. It was documented also that upon sequential analyses of VEGF serum level in NHL, its level decreased significantly after 6 months of treatment completion [32]. Moreover, a trend towards significant correlation between high initial levels of sVEGF and high tumor burden (p=0.077) was detected by others [33].

The results of our study demonstrated lack of significant association between COX2 expression and the clinico-pathologic features of B-NHL as well as the studied standard prognostic factors including age, sex, organomegaly, lymphadenopathy, B symptoms, staging, WBC count, hemoglobin, Platelet count. Also there was no statistically significant difference between positive and negative COX-2 expression as regards response to treatment, follow up and survival status (P=0.433, 0.796, 0.433). Consistent with our results; Ma et al, 2012 [17] found that,COX-2 expression was not correlated with the gender, age, LDH levels, β2M levels, extranodal involvement, disease stage, B symptoms or IPI of the patients. Also COX-2 expression showed no difference between the indolent and aggressive subtypes of NHL. On the same line, other studies also did not find any statistically significant correlation between expression of COX2, MRP1 and MRP2n and clinicopathological data from patients (P>0.05) and no correlation was found between expression of the investigated proteins and total observation time or progression-free survival time (P>0.05) [18]. Similarly, reports that, there was no correlation between Cox-2 expression and age, IPI score, extranodal involvement, tumor grade, and finally B symptoms were documented [34]. However in contrast to our results; some found an important association between aggressive histology and COX2 expression (p=0.036) and although the overall survival times were longer in cases with lower or no COX2 expression as compared with higher COX2, the difference was not significant [16], and others reported a positive correlation between COX2 and stage of the disease (P=0.037) in NHL patients [34]. Also, they found that, the complete response rate to therapy was significantly higher in COX2 negative patients than the COX2 positive ones (70.6% vs. 20.8%, respectively, P=0.001), and the overall survival of COX2 positive patients was less than that for those without COX2 expression, but the difference was not significant statistically (16.4+/-11.4 vs. 14.7+/-8.2 months, respectively, P=0.552). Besides, they demonstrated that there is a clinical correlation between the COX2 expression and prognostic factors in lymphoma patients and the combination of COX2 inhibitors with standard chemotherapeutics may enhance the potential of treatment options for malignant lymphomas [34].

5. Conclusions

In conclusion, the results obtained by the current study couldn't identify the association between VEGF-A and COX2. Controversially, we found a better response to treatment in patients expressing VEGF-A than not expressing with borderline statistically significant difference (p=0.06). Further studies with larger number of patients are required to clarify the association between the inflammatory mediator and the angiogenetic factor and to assess their role in the response to treatment for the possibility to use modifying drugs and improve the response of NHL patients.

Conflict Of Interest

None of the authors of this paper has any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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