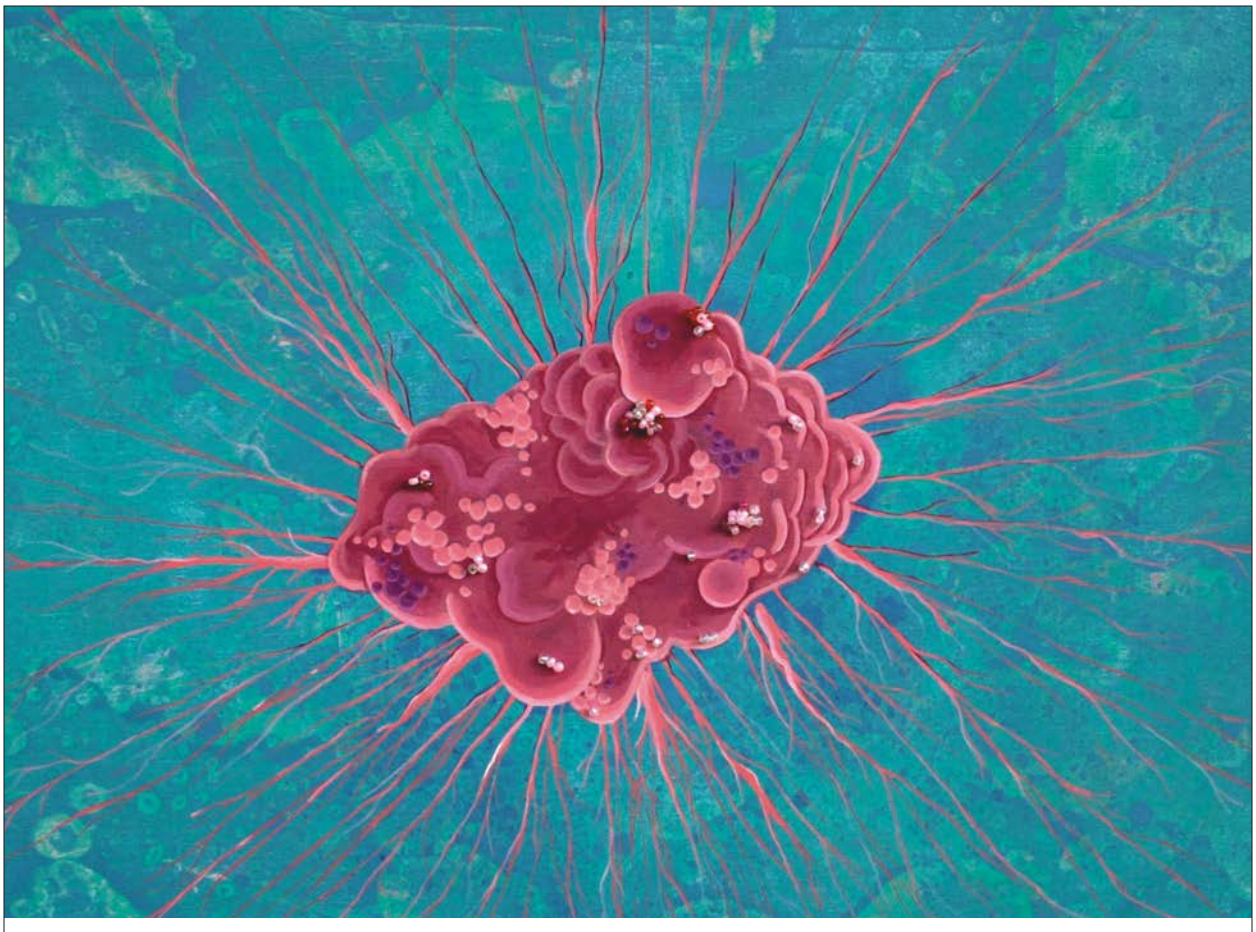

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Prognostic Utility of Angiogenic Growth Factors; Basic FGF, VEGF and PDGF-bb in Patients with Lymphoma

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ABSTRACT

Angiogenesis is essential for lymphoma growth, progression and metastasis which is stimulated by many pro-angiogenic factors as basic Fibroblast Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor (PDGF). This study aimed to delineate the role of angiogenic growth factors, basic FGF, VEGF and PDGF-bb in the patients with Non-Hodgkin Lymphoma (NHL) and Hodgkin Disease (HD) compared to healthy donors. The study included 94 patients, 54 of them diagnosed as NHL and 40 patients diagnosed as HD. The levels of basic FGF, VEGF and PDGF-bb in pre-treatment patients and in patients under chemo-radiotherapy (<8 cycles) were measured by Bio-Plex Pro assays. Twenty healthy donors were enrolled as controls. Our data show a significant increase in the levels of studied 3 factors in NHL pre-treatment patients compared to controls ($p = 0.025$, <0.001 and 0.02, respectively). These factors decreased significantly in whole patients under-treatment than pretreated ones ($p<0.001$ each). In HD, there was a significant increase of these factors in pretreated patients than controls ($p<0.001$ each). These factors significantly decreased in under-treatment patients than pretreatment ($p \leq 0.001$ each). VEGF was still significantly higher in under-treatment patients than controls ($p<0.001$) in NHL and HD. These factors were higher in patients with progressive course of lymphoma than those with complete or partial remission. FGF, VEGF and PDGF in lymphoma patients decreased significantly after chemo-radiotherapy but VEGF is still higher than controls. New anti-angiogenic strategies should be added to commonly used chemotherapy regimen in lymphoma.

Key words: Angiogenesis, lymphoma, Bio-Plex, FGF, VEGF, PDGF-bb

INTRODUCTION

Lymphomas are a heterogeneous group of lymphoid disorders that have in common clonal expansion of malignant lymphocytes. The current WHO classification takes into account clinical presentation, immunophenotype, cytogenetics and molecular markers to differentiate the Non-Hodgkin Lymphoma (NHL) from Hodgkin Disease (HD) (Wun and White, 2010).

Angiogenesis physiologically helps in regulation of reproduction and wound healing. The unregulated angiogenesis may result in tumor growth as the growing tumor needs an extensive network of capillaries to provide nutrients and oxygen (Liekens *et al*., 2001).

Naldini and Carraro (2005) explained that the angiogenesis process is regulated by a balance between angiogenic activators and inhibitors in tumorigenesis. In addition, the inflammatory cells, T lymphocytes and monocytes participate in the angiogenic process by secreting pro and anti-inflammatory cytokines that control Endothelial Cells (ECs) proliferation, their survival and apoptosis, as well as their migration and activation.

Lymphoma growth and progression is potentiated by angiogenic influences of proangiogenic tumor microenvironment on both local neovascular transformation and recruitment of circulating bone marrow-derived progenitors. Lymphoma-associated infiltrating host cells including hematopoietic monocytes, T cells and mesenchymal pericytes have increasingly been associated with the pathogenesis and prognosis of lymphoma, in part providing perivascular guidance and support to neoangiogenesis (Ruan *et al*., 2009).

VEGF expression by neoplastic cells has been demonstrated in aggressive subtypes of lymphoma including Peripheral T-cell Lymphoma (PTCL), Diffuse Large B-cell Lymphoma (DLBCL), Mantle Cell Lymphoma (MCL), primary effusion lymphoma and indolent histologies such as Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL) (Kay *et al*., 2002). Complementary to VEGF signaling pathways, several neoangiogenic pathways participate in the regulation of angiogenic switch. For instance, the PDGF family is essential for its role in vascular remodeling and maturation. PDGF-bb produced by endothelial cells promotes vascular stability and maturation (Abramsson *et al*., 2003).

FGFs are potent angiogenic inducers which promote ECs detachment and migration by regulating expression of cadherins and integrins and so disrupting cell-cell junctions. FGFs have also been shown to upregulate various Matrix Metallo-Proteinases (MMPs) in ECs facilitating degradation of the basement membrane. Endothelial cell proliferation is then induced by FGFs via., activation of the MAPK and Protein Kinase C (PKC) signaling pathways and in the later stages of angiogenesis, FGFs stabilize the cellular adhesions required for vessel maturation (Park and Dilda, 2010).

Initial treatment with chemotherapy is associated with a high rate of clinical response and the subsequent remissions can be obtained with further treatment. Chemotherapy aims at targeting the main peculiar characteristic of tumor cells, i.e., their proliferative derangement (MacDonald and Connors, 2007; D'Onofrio and Gandolfi, 2010). The four-drug combination CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) provides complete remission rate in patients with NHL (Pfreundschuh *et al*., 2006). Doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) chemotherapy currently is considered the gold standard for Hodgkin disease (Bonadonna *et al*., 2004).

The aim of this study is to evaluate the prognostic utility of some angiogenic growth factors in patients with NHL and HD before starting treatment and under chemo-radiotherapy then compared to those in healthy donors.

MATERIALS AND METHODS

This study was performed on 94 patients with ages (18-70 years), 57 males and 37 females. They included 54 patients diagnosed as NHL and 40 patients diagnosed as HD. The NHL patients were divided as 18 cases before starting treatment and 36 cases under treatment with chemo-radiotherapy in addition to 8 cases of those before treatment were assessed when they received treatment (<8 cycles), so total number of cases under treatment was 44 cases. Regards patients with HD, there were 12 cases before starting treatment and 28 cases under treatment with

Table 1: The pathological subtypes of NHL and HD of studied patients

Pre-treatment cases				Under treatment cases			
Disease/Subtype	Total No.	No.	%	Disease/Subtype	Total No.	No.	%
NHL	18			**NHL**	44		
DLBCL		16	88.8	DLBCL		30	68.2
				Small cell lymphoma		4	9.1
Small cell lymphoma		1	5.6	Follicular LCL		3	6.8
				Anaplastic LCL		2	4.5
Mantle cell lymphoma		1	5.6	Mantle cell lymphoma		1	2.3
				Mixed small and large cell		4	9.1
HD	12			**HD**	37		
Lymphocyte predominance		3	25	Lymphocyte predominance		4	10.8
Nodular sclerosis		3	25	Nodular sclerosis		5	13.5
Mixed cell		6	50	Mixed cell		28	75.7

chemo-radiotherapy in addition to 9 cases of those before treatment undergo the treatment (<8 cycles), so total number of cases under treatment was 37 cases. The pathological subtypes of lymphoma in studied patients shown in Table 1.

Patients of both NHL and HD under chemo-radiotherapy are subdivided according to their response to treatment into three groups (Group A: Complete Remission (CR), Group B: Partial Remission (PR) and Group C: Stable Disease (SD) and Progressive Disease [PD]).

Twenty apparently healthy persons aged from 18-50 years old were subjected as controls (10 males and 10 females).

The angiogenic growth factors; PDGF-BB, VEGF and FGF-b were assessed by Bio-Plex Pro assays that quantify multiple protein biomarkers for NHL, HD patients and for controls. The Bio-Plex® system is built around the three core elements of xMAP technology.

(1) Fluorescently dyed microspheres (beads), each with a distinct color code or spectral address. This allows simultaneous detection of different types of molecules in a single well of a 96-well microplate. (2) A dedicated flow-cytometer with two lasers and associated optics to measure the different molecules bound to the surface of the beads. (3) A high-speed digital signal processor.

Data was statistically analyzed using Sigma-Plot program version 10. The quantitative data were presented as mean ±standard deviation. The comparison of means was performed by t-test. Then significance was expressed as p-value. p-value was considered significant if it was ≤0.05 and considered highly significant if it was ≤0.001.

RESULTS

In NHL patients the basic FGF, VEGF and PDGF-bb were measured in the same 8 cases before starting treatment and their levels when they were under treatment (<8 cycles of treatment). From Table 2 the following results were found; A significant increase in the levels of three angiogenic factors in NHL pre-treatment patients compared to controls as basic FGF (p_1<0.001), VEGF (p_1<0.001), PDGF-bb (p_1 = 0.02). Those patients when received chemotherapy, the levels of some factors were significantly decreased compared to pretreatment as; basic FGF (p_2 = 0.02), VEGF (p_2 = 0.001) while insignificantly decreased in the level of PDGF-bb (p_2 = 0.1). By comparing the levels of these factors in patients under treatment to controls, it was found no significant difference except in VEGF which still significantly higher than controls (p_3<0.001).

Table 3 show the levels of angiogenic factors in the total cases of NHL before starting treatment (18 cases) compared to their levels in the whole cases under treatment (44 cases) and to

Table 2: Angiogenic growth factors in same cases of NHL before and under treatment compared to controls

	Cases mean (pg/mL) (±SD)						Significance p-value		
Factors	Control	(N: 20)	Pre-treatment	(N: 8)	Under treatment (<8 cycles)	(N: 8)	p_1	p_2	P_3
Basic FGF	33.2	(±12.8)	60.6	(±21.9)	33.3	(±17.1)	<0.001	0.02	0.98
VEGF	13.3	(±8.1)	73.0	(±12.8)	39.8	(±19.0)	<0.001	0.001	<0.001
PDGF-bb	1035.1	(±467.6)	1827.3	(±1176.6)	1000.1	(±680.7)	0.02	0.1	0.88

p_1: Pre-treatment versus controls, p_2: Pre-treatment versus under treatment, p_3: Under treatment versus controls

Table 3: Angiogenic growth factors in total cases of NHL pre-treatment and total cases under treatment in comparison to controls

	Cases mean (pg/mL) (±SD)						Significance p-value		
Factors	Control	(N: 20)	Pre-treatment	(N: 18)	Under treatment	(N: 44)	p_1	p_2	p_3
Basic FGF	33.2	(±12.8)	47.5	(±23.8)	26.1	(±14.4)	0.025	<0.001	0.06
VEGF	13.3	(±8.1)	62.0	(±43.1)	30.5	(±19.3)	< 0.001	<0.001	<0.001
PDGF-bb	1035.1	(±467.6)	2169.1	(±1995.2)	934.6	(±665.6)	0.02	<0.001	0.5

p_1: Pre-treatment versus controls, p_2: Pre-treatment versus under treatment, p_3: Under treatment versus controls

Table 4: Angiogenic growth factors in same cases of HD before and under treatment compared to controls

	Cases mean (pg/mL) (±SD)						Significance p-value		
Factors	Control	(N: 20)	Pre-treatment	(N: 9)	Under treatment (<8 cycles)	(N: 9)	p_1	p_2	p_3
Basic FGF	33.2	(±12.8)	71.9	(±25.2)	42.5	(±20.2)	<0.001	0.02	0.1
VEGF	13.3	(±8.1)	81.9	(±45.6)	39.3	(±25.2)	<0.001	0.03	<0.001
PDGF-bb	1035.1	(±467.6)	3565.8	(±2783.9)	1240.4	(±881.5)	<0.001	0.03	0.4

p_1: Pre-treatment versus controls, p_2: Pre-treatment versus under treatment, p_3: Under treatment versus controls

controls. There were significant increase in the levels of studied factors in pre-treatment patients compared to controls as following; basic FGF ($p_1 = 0.025$), VEGF ($p_1<0.001$) and PDGF-bb ($p_1 = 0.02$). These factors in whole patients under treatment were significantly lower than in pre treatment patients, basic FGF, VEGF and PDGF-bb ($p_2<0.001$ for each). The level of VEGF was still significantly higher than controls ($p_3<0.001$) while insignificant difference in levels of basic FGF and PDGF-bb ($p_3 = 0.06$ and 0.5, respectively).

In HD same 9 cases, Table 4 show a highly significant increase of all studied factors compared to controls ($p_1<0.001$ for each). When those patients were under treatment the levels of these factors were significantly decreased as; basic FGF ($p_2 = 0.02$), VEGF ($p_2 = 0.03$) and PDGF-bb ($p_2 = 0.03$). By comparing the patients under treatment to controls, VEGF was still significantly higher than controls ($p_3<0.001$), while basic FGF and PDGF-bb were insignificantly different from controls ($p_3 = 0.1$ and 0.4, respectively).

The levels of studied angiogenic growth factors shown in Table 5 for the total cases of HD before treatment (12 cases) compared to their levels in the whole cases under treatment (37 cases) and to controls. It was found a highly significant increase of all factors in pre-treated patients compared to controls ($p_1<0.001$ for each). The levels of these factors in under treatment patients were significantly lower than pre-treatment ones; basic FGF ($p_2<0.001$), VEGF ($p_2<0.001$) and PDGF-bb ($p_2 = 0.001$). The VEGF was still significantly higher than controls ($p_3<0.001$), while others show insignificant difference; basic FGF and PDGF-bb ($p_3 = 0.8$ and 0.1, respectively).

On subdividing the whole cases of lymphoma under treatment into three groups according to response to treatment, Table 6 shows the levels of 3 angiogenic factors in NHL patients' groups under treatment compared to control.

Table 5: Angiogenic growth factors in total cases of HD pre-treatment cases and total cases under treatment in comparison to controls

	Cases mean (pg/mL) (±SD)						Significance p-value		
Factors	Control	(N: 20)	Pre-treatment	(N: 12)	Under treatment	(N: 37)	p_1	p_2	p_3
Basic FGF	33.2	(±12.8)	64.9	(±25.5)	32.0	(±17.2)	<0.001	<0.001	0.8
VEGF	13.3	(±8.1)	78.8	(±43.8)	36.2	(±25.2)	<0.001	<0.001	<0.001
PDGF-bb	1035.1	(±467.6)	3817.8	(±3063.5)	1582.6	(±1436.5)	<0.001	0.001	0.1

p_1: Pre-treatment versus controls, p_2: Pre-treatment versus under treatment, p_3: Under treatment versus controls

Table 6: Angiogenic growth factors in different groups of NHL patients and controls, a comparative study

	Cases mean (pg/mL) (±SD)								Significance p-value		
Factors	Control	(N: 20)	Group (A)	(N: 12)	Group (B)	(N: 14)	Group (C)	(N: 18)	p_1	p_2	p_3
Basic FGF	33.2	(±12.8)	18.6	(±8.1)	28.7	(±15.6)	30.6	(±15.5)	0.001	0.4	0.57
VEGF	13.3	(±8.1)	23.5	(±16.9)	33.4	(±17.2)	34.7	(±22.1)	0.03	< 0.001	< 0.001
PDGF-bb	1035.1	(±467.6)	602.4	(±322.5)	1000.6	(±664.5)	1104.7	(±776.8)	0.008	0.9	0.7

Group A: Complete remission, Group B: Partial remission, Group C: Stable disease and progressive disease, p_1: Group A versus controls, p_2: Group B versus controls and p_3: Group C versus controls

Table 7: Angiogenic growth factors in different groups of HD patients and controls; a comparative study

	Cases mean (pg/mL) (±SD)								Significance p-value		
Factors	Control	(N: 20)	Group (A)	(N: 12)	Group (B)	(N: 14)	Group (C)	(N: 11)	p_1	p_2	p_3
Basic FGF	33.2	(±12.8)	27.3	(±22.2)	33.4	(±14.6)	35.4	(±14.6)	0.35	0.97	0.67
VEGF	13.3	(±8.1)	20.5	(±17.9)	42.8	(±30.8)	44.9	(±17.8)	0.1	<0.001	<0.001
PDGF-bb	1035.1	(±467.6)	1001.5	(±857.9)	1217.7	(±749)	2680.9	(±1992.9)	0.9	0.4	0.001

Group A: Complete remission, Group B: Partial remission, Group C: Stable disease and progressive disease, p_1: Group A versus controls, p_2: Group B versus controls and p_3: Group C versus controls

As regard group (A) the following factors were significantly lower than controls, basic FGF ($p_1 = 0.001$), PDGF-bb ($p_1 = 0.008$). VEGF was still significantly higher than control ($p_1 = 0.03$). In group (B) the VEGF was significantly higher than controls as ($p_2<0.001$), while others show insignificant differences than controls as; basic FGF ($p_2 = 0.4$), PDGF-bb ($p_2 = 0.9$). Group C, show significantly higher level of VEGF than controls ($p_3<0.001$) and insignificant differences of other 2 factors.

Regarding HD, Table 7 shows the comparison between the levels of angiogenic growth factors in patients of group A, B, C and controls. It was found that there were no significant differences in the levels of all studied angiogenic factors in group (A) patients compared to controls ($p_1>0.05$). In group (B), there was significant higher level of VEGF ($p_2<0.001$) while other 2 factors show insignificant differences; basic FGF ($p_2 = 0.97$), PDGF-bb ($p_2 = 0.4$). As regard group (C), there were significant higher levels VEGF ($p_3<0.001$) and PDGF-bb ($p_3 = 0.001$), while insignificant increase in basic FGF ($p_3 = 0.67$).

DISCUSSION

Angiogenic growth factors, basic FGF, VEGF and PDGF-bb were studied in lymphoma patients before starting treatment and patients under chemo-radiotherapy by multiplexed assays.

Our study revealed that the levels of these factors in same patients of NHL were significantly increased in pre-treatment patients compared to controls (p_1<0.001, <0.001 and 0.02, respectively). The basic FGF and VEGF were significantly decreased after treatment, but statistically insignificant decrease of PDGF-bb (p_2 = 0.1). Also, it was found that the levels of growth factors in whole NHL patients before treatment were significantly higher than controls as and significantly decreased in total cases under treatment (p_2 = <0.001 for each).

In HD same patients, it was found that angiogenic growth factors; basic-FGF, VEGF, PDGF-BB were significantly higher in pre-treatment patients compared to controls (p_1<0.001 for each) and significantly decreased with treatment. The total cases of HD before treatment and the whole patients under treatment in different cycles were expressed, where the levels of the growth factors; basic-FGF, VEGF, PDGF-BB were highly significantly increased in pretreatment patients compared to controls (p_1<0.001 for each) and also significantly decreased in patients under treatment.

It was observed that after multiple cycles of chemotherapy in patients of both NHL and HD, the levels of FGF and PDGF were insignificantly differs from the controls (p_3>0.05) while the VEGF inspite of decreasing significantly than pre-treatment but still significantly higher than controls in both diseases (p_3<0.001 in both NHL and HD).

Salven *et al.* (2000) reported that there were highly significant increase of basic FGF and VEGF (measured by ELISA) in 200 NHL patients and significantly decreased with multiple cycles of treatment either chemotherapy or radiotherapy and this is agreed with the results of the present study where basic FGF and VEGF were significantly higher than controls (p = 0.025 and <0.001, respectively) and after multiple cycles of chemotherapy were highly significantly lower than pretreatment patients (p<0.001).

Also, Pazgal *et al.* (2002) measured FGF-2 serum concentration in 58 patients with NHL before starting treatment and 19 of them after treatment, using ELISA technique; they demonstrated that FGF-2 expression was correlated with poor survival and progression-free survival. On other hand, they did not detect a significant change in serum bFGF levels after 2-3 cycles of chemotherapy (p>0.05). After 6 months of treatment completion, Etto *et al.* (2008) reported that VEGF and FGF serum levels measured by ELISA in 87 NHL pretreatment levels were higher than 10 controls and decreased significantly (p<0.05).

As regard patients with HD, Marri *et al.* (2013) documented that some angiogenic growth factors as; FGF-b and VEGF (measured by ELISA) were significantly (p<0.05) higher in 140 patients with classical Hodgkin lymphoma (cHL) before start treatment than 50 controls and these markers could be used as significant prognostic factors for cHL disease. These results are similar to our results where serum levels of these markers in pretreatment patients were significantly higher than controls (p_1<0.001).

Shih *et al.* (2006) stated that monocytes are recruited into tumors and differentiated into Tumor-Associated Macrophages (TAMs) and then accumulate in the hypoxic areas. The increased levels of some growth factors, including; VEGF, basic FGF, Epidermal Growth Factor (EGF) and Transforming Growth Factor-α (TGF-α) is explained by Naldini and Carraro (2005), who documented that these factors are produced by TAMs accumulated in lymphoma. They are not only growth factors for tumor cells, but also potent mitogens to promote endothelial cell proliferation. The basic FGF stimulates VEGF expression in endothelial cells and stromal cells which is required for the FGF's angiogenic response. FGF signaling, furthermore, controls VEGFR2 signaling responsiveness (Tsunoda *et al.*, 2007).

Murakami and Simons (2008) postulated that the FGF system is capable of regulating other growth factor signaling, it is, therefore, reasonable to hypothesize that the FGF system is positioned upstream of more specialized growth factor systems such as VEGF for endothelial cells and PDGF for smooth muscle cells, thus regulating the entire angiogenic process in an indirect manner.

The most important mediator of the angiogenic switch is VEGF which produced by a variety of tumor cells as well as certain tumor-associated stromal cells binds to two related receptor tyrosine kinases, namely, VEGFR-1 and VEGFR-2 (Ferrara *et al*., 2003). In contrast, Gougelet *et al*. (2009) documented that the resistance to cytotoxics is associated with the overproduction of several cytokines, in particular VEGF.

PDGF-bb displays a potent biological activity on PDGFR-expressing Vascular Smooth Muscle Cells (VSMCs), it usually lacks biological effects on ECs that do not express detectable levels of PDGFRs. Thus PDGF-bb is considered as a mitogenic and chemotactic factor for VSMCs/pericytes, but not for ECs (Nissen *et al*., 2007).

In the study of Guler *et al*. (2005), the PDGF was measured by ELISA method in 9 HD patients and 12 NHL patients. The PDGF values in these patients before starting treatment were significantly raised ($p<0.01$) compared to 20 controls. The observation of a 5-fold increase in PDGF values in the disease group when compared to the controls group suggests that PDGF could itself be considered as a possible factor in the pathogenesis of HD and NHL. This agreed with the results of the present study where the PDGF-bb was significantly higher than controls in NHL ($p = 0.02$) and in HD ($p<0.001$).

Basic FGF, soluble VEGF and PDGF-bb levels decline after radiotherapy in NHL, suggesting that may have predictive significance for response to treatment and recurrence (Ria *et al*., 2008).

The CHOP-regimen decrease of VEGF, PDGF-BB and b-FGF significantly. Cyclophosphamide-based metronomic chemotherapy beside it has cytotoxic effects, it helps in enhancement of antiangiogenic factors expression or induction of pro-apoptotic signal (NF-κB) (Stempak *et al*., 2006; Calleri *et al*., 2009).

Engert *et al*. (2007) reported that the chemotherapy for HD, consisting of two cycles of ABVD plus Radiotherapy (RT) is superior in disease controls and has similar toxicity compared with RT alone. Thus, chemotherapy is being regarded as standard of care for early favorable HD patients by most groups.

The use of more intensive regimens for treatment of HD patients, such as doxorubicin, vinblastine, nitrogen mustard, vincristine, bleomycin, etoposide, prednisone (BEACOPP) or Stanford V administered as initial therapy has suggested improved Complete Remission (CR) rates over standard regimens (Diehl *et al*., 2003).

Subdividing patients under treatment of both NHL and HD into three groups according to response to treatment group (A): Complete remission, group (B): Partial remission and group (C): Stable disease and progressive disease) then comparing the serum levels of studied growth factors in patient groups to controls. It was observed that the VEGF of group (A) NHL patients was still significantly higher than controls ($p_1 = 0.03$) while significant lower levels than controls of FGF and PDGF-bb ($p_1 = 0.001$ and 0.008, respectively). In groups (B) and (C) patients, the VEGF was significantly higher than controls (p_2, $p_3<0.001$, in each) while other factors show insignificant differences (p_2, $p_3>0.05$).

As regard groups of HD patients under treatment, there were insignificant differences in the levels of all studied factors in group (A) patients than controls ($p_1>0.05$). In group (B) patients,

there was significant increase in VEGF level than controls (p_2<0.001). The levels of VEGF and PDGF-bb in group C were significantly higher than controls while basic FGF show insignificant difference than controls (p_3 = 0.67). This agreed with Gougelet *et al.* (2009) who reported that resistance to cytotoxics is associated with the overproduction of several cytokines, in particular VEGF.

Bertolini *et al.* (1999) used ELISA technique to assess the levels of VEGF and FGF in the plasma collected from NHL patients before treatment and patients received different chemotherapy courses according to their diagnosis. VEGF levels of patients in (CR) were significantly lower than those of patients with (PD) (p = 0.016). Serum VEGF level is highly predictive of a poor outcome in NHL. Regarding b-FGF, there was a trend indicating lower baseline plasma levels in CR compared to PD patients but this difference did not reach statistical significance (p = 0.19). Baseline b-FGF levels were not significantly different in CR compared to PD patients the prognostic significance of b-FGF was less clinically relevant than that of VEGF. This agreed with our results, the level of VEGF was significantly higher in PR, SD and PD compared to CR in both NHL and HD patients (p<0.05). Also, the FGF level in PR, SD and PD compared to CR in NHL patients (p<0.05) while in HD there was an insignificant increase in FGF of PR ,SD and PD patients compared to CR. Dirix *et al.* (1997) reported the positive association of a short tumor volume-doubling time with elevated basic FGF and VEGF serum levels in advanced cancer patients is largely independent from the metastatic pattern and the extent of the disease.

The serum levels of angiogenic factors have prognostic significance in human cancers of epithelial or hematological origin. VEGF may be a better reflection of ongoing angiogenesis and accordingly, a better prognostic marker for patients with cancer (Chen *et al.*, 2012).

Induced hypoxia leading to the up-regulation of many growth factors such as PDGF-bb, FGF, TGF-β and EGF also it allows a paradoxal increase in cell chemo-resistance and increase angiogenesis and cell proliferation (Rohwer and Cramer, 2011).

In conclusion, there is a significant increase in the levels of FGF, VEGF and PDGF in both NHL and HD. After treatment, FGF and PDGF are markedly decreased while level of VEGF inspite of decreasing but still higher than controls. Also, resistance to cytotoxics is linked with the overproduction of VEGF. These studied factors were higher in patients with progressive course of disease both NHL and HD than those with complete or partial remission. Understanding the pathways of angiogenesis in lymphoma provide much needed insights for the rational design of future effective antiangiogenic therapy and schedules that are customized to appropriate clinical settings. Antiangiogenic strategies have become an important therapeutic modality for solid tumors including lymphoma. More efforts should be directed to VEGF pathway which is considered a potential additional target in the treatment of lymphoma.

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