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Circulating Angiogenesis Factors in Patients with Non-Hodgkin's Lymphoma

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Authors' contributions

This work was carried out in collaboration between all authors. Conception and design and Provision of study materials: Authors RMT and RFS; Provision of study patients: Author NAB; Collection and assembly of data: Authors EAEM and RFS; Data analysis and interpretation: Authors RMT, EAEM and RFS. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of hematological malignancies. Several angiogenic factors are important in NHL. The objective of this study was to determine plasma levels of various proangiogenic [vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), tumor necrosis factor (TNF-α), transforming growth factor (TGF-β), interleukin (IL-6), IL-8] and antiangiogenic [IL-4, IL-12, interferon gamma (IFN-γ)] factors in NHL patients and implication of CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone) therapy on these mediators.
Study Design: This study was conducted on 100NHL patients recruited from the Oncology Hospital, Menofia University, Egypt. Fifty patients had different doses of CHOP chemotherapy and 50 patients were without treatment. Another 119 healthy blood donors were served as healthy controls.
Methodology: Enzyme-linked immunosorbent assay (ELISA) was used to detect the concentrations of these mediators in plasma of NHL patients and normal controls.
Results: Several proangiogenic (VEGF, PDGF, IL-8, and TNF-α) (P<.001, P<.01, P<.01,

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and *P*<.001) and antiangiogenic (IL-4,IL-12) (*P*=.05,*P*<.01) mediators were decreased in NHL patients. In contrast, IL-6, and IFN-γ) were increased (*P*<.001). After CHOP treatment, VEGF, and PDGF were significantly increased (*P*<.001) as compared to NHL without therapy Fig.1. Reduction in IL-8 (*P*<.01) and TNF-α (*P*<.001) in untreated NHL patients was continued after CHOP treatment. IL-6 was the only elevated cytokine in NHL patients with (*P*<.001) or without (*P*<.001) treatment compared to healthy individuals. Concerning the antiangiogenic mediators, treatment resulted in reduction in the secretion level of IFN-γ) (*P*<.001) and IL-12 (*P*<.001). Although CHOP treatment required more than 8 doses to be able to down regulate proangiogenic (VEGF,PDGF, IL-8,TNF-α and IL-6) and elevate antiangiogenic (IL-4,IL-12,IFN-γ) mediators, changes were statistically insignificant.

Conclusion: Collectively, our study stressed on the importance of having an angiogenic profile of NHL patients under treatment which could be used to monitor the efficacy of cancer therapy particularly in therapy with antiangiogenic drugs. Further studies are required to confirm these results.

Keywords: NHL; angiogenesis; anti angiogenesis; CHOP.

1. INTRODUCTION

Non-Hodgkin's lymphoma, the most common form of lymphoma, is a heterogeneous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment [1].It comprises many subtypes, each with distinct epidemiology, etiology, morphologic, immunophenotypic, and clinical features [2-3]. NHL originates from B-cells, T-cells, or natural killer (NK) cells. There are more than 30 different subtypes of NHL that are categorized by several factors, including rate of growth, location and certain histological characteristics of the tumor cells themselves [4]. The incidence of NHL that has occurred over recent decades is expected to continue [5]. Of the people diagnosed with NHL each year, 55% have aggressive or fast-growing NHL while 45% have indolent NHL [6].

Angiogenesis, the sprouting of new capillaries from preexisting ones, is an important component in many physiological and pathological processes [7]. In healthy adults extensive angiogenesis occurs only in the female reproductive system [8] and during tissue repair and wound healing [9]. It is a complex multistep process that requires several cytokines [10], including basic fibroblast growth factor (bFGF), interleukin (IL)-8, transforming growth factor (TGF)-α and -β, hepatocyte growth factor (HGF), tumor necrosis factor (TNF)-α, epidermal growth factor (EGF), angiogenin, angiopoietin-1, patelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). These angiogenesis factors are typically produced by several hematopoietic cells, including, monocytes, lymphocytes, dendritic cells, neutrophils, and mast cells, beside tumor cells. It is, therefore, not surprising to find these angiogenesis factors dysregulated in different types of hematologic malignancies such as lymphoma and leukemia [11-13]. Although potential pathophysiologic role of angiogenesis in solid tumors has been extensively studied, enhancement of angiogenesis in malignant hematological disorders has been recognized more recently. Several studies have shown enhanced angiogenesis in both Hodgkin's and non-Hodgkin's lymphoma [14-16]. In NHL, angiogenesis has been associated with adverse outcome or more aggressive clinical behavior [17].

As angiogenesis is regulated by the impact of competing influences between inhibitors and activators, balance between positive and negative angiogenic molecules is of great importance [18]. Increasing interest in evaluation of angiogenesis/antiangiogenesis mediators in NHLs arises from the availability of antiangiogenic therapy as a directed therapeutic tool [10,13]. Thus, this study was performed to evaluate the plasma levels of proangiogenic (VEGF,PDGF,IL-8,IL-6,TNF-α,TGF-β) and antiangiogenic (IFN-γ, IL-12, and IL-4) factors in NHL patients and correlate their levels with different stages, grades and international prognostic index (IPI) of the diseases and the implication of CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone) therapy on these mediators. To our knowledge, this work is the first study to examine circulating levels of proangiogenic/antiangiogenic mediators in NHL patients with and without CHOP chemotherapy.

2. MATERIALS AND METHOD

2.1 Patients and Controls

This study was conducted on 100NHL patients (50 patients without treatment and 50 patients with different doses of CHOP chemotherapy). Patients were recruited consecutively from the Oncology Hospital, Menofia University (Shebein El-Kom, Menofia governorate, Egypt). Another 119 healthy blood donors, free of any chronic diseases ans living in the same geographical area were served as healthy controls. Informed consent was obtained from all the study participants. All investigations were done in accordance with the Menofia University Health and Human Ethical Clearance Committee guidelines for Clinical Researches. Local ethics committee approved the study protocol.

Cases were categorized according to the WHO classification [19]. The initial medical evaluation consisted of a complete history and physical examination; chest radiographic examination; computed tomographic scan of the chest, abdomen, and pelvis; blood chemistry and bone marrow biopsy or aspirate performed at diagnosis. The extent of the disease was categorized according to the Ann Arbor classification and performance status was assessed using criteria of the Eastern Cooperative Oncology Group (ECOG) [20].

2.2 Detection of Angiogenic and Antiangiogenic Molecules by Enzyme Linked Immunosorbent Assay (ELISA)

Blood samples were collected from all patients and controls. For CHOP [cyclophosphamide (750mg/m^2), hydroxydaunorubicin (50mg/m^2), oncovin (1.4mg/m^2), prednisone (40mg/m^2)] treated patients, samples were collected at the end of each cycle (every 21 days for more than 8 cycles). Plasma were separated by centrifugation at 2000xg for 3min at 4°C, aliquotted, and stored at -80°C until analysis of the expression of VEGF,PDGF,IL-6,IL-8, TNF-α and TGF-β (proangiogenic mediators) and IL-4,IL-12 and IFN-γ (antiangiogenic mediators) using a quantitative ELISA (R&D Systems, Minneapolis, MN) as previously described [21-23] with slight modifications.

Briefly, samples or standards (100µl/well) were added to a coated microtiter plate and incubated for 2h at room temperature. The plates were then rinsed, and 100µl/well biotinylated polyclonal antibodies were added for 2h. At the end of the incubation period, streptavidine conjugated to horseradish peroxidase was added to the wells. After additional 1h incubation, the wells were rinsed again and 100µl/well of hydrogen peroxide and

tetramethylbenzidine (TMB) substrate solution was added. The reaction was stopped by 50 µl/well of 1M HCl stopping buffer. The absorbance of each well was measured at 450nm using a microplate reader (SunriseTM, Tecan Group Ltd., Männedorf, Switzerland). Each plasma sample was analyzed in duplicate. The ELISA reader-controlling software (Softmax) readily processes the digital raw absorbance data into a standard curve from which cytokine concentrations of unknown samples can be derived directly and expressed as pg/ml plasma.

2.3 Statistical Analysis

All statistical analyses were performed using SPSS version 13 (SPSS, Inc., Chicago, IL). Data are presented as means with corresponding SE. For categorical variables, the statistical significances of case-control differences were tested by the Chi square test. The Kruskal Wallis nonparametric ANOVA test was used to examine the differences in angiogenic and antiangiogenic levels in patients with different clinical characteristics. The Mann-Whitney test was used to compare the levels of angiogenic and anti-angiogenic factors between control individuals and patients. Correlation among variables was determined using Pearson's correlation test. In all tests the level of significance was set at $P=.05$.

3. RESULT

3.1 Patient's Characteristics

The detailed clinical characteristics of the NHL patients enrolled in this study are presented in Table 1. Demographic and biochemical data of the NHL patients and normal controls are presented in Table 2. The study included 100 NHL patients, 42 women and 58 men. The mean age at diagnosis was 50.32 years (range 44–87 years). In controls, there were 49 male versus 70 female with the mean age group being 43.54.

3.2 Plasma Angiogenic/Anti angiogenic mediators in NHL patients

The data in Fig.1 indicated that a panel of proangiogenic mediators (VEGF,PDGF,IL-8, TNF-α) was significantly decreased ($P<.001$,$P<.01$,$P<.01$,$P<.001$, respectively) in NHL patients. On the other hand, a significant increase ($P<.001$) in IL-6 was observed in NHL patients in comparison to controls. No change was found in TGF-β secretion level in NHL patients. In contrast to a significant reduction ($P=.05$ and $P<.01$) in antiangiogenic mediators (IL-4 and IL-12, respectively), IFN-γ was significantly ($P<.001$) increased.

Classifying NHL patients based on Ann Arbor stage revealed that the plasma levels of some proangiogenic mediators were significantly increased (VEGF and PDGF) by increasing the stage of NHL. Stage (3) was characterized by a significant reduction in IL-8,TGF-β, and TNF-α ($P=.05$) as compared to stage (1) NHL. Coinciding with these data, the antiangiogenic mediators showed a significant reduction in IL-12 ($P<.01$) Fig. 2 secretion level. A slight elevation in IL-6 and IL-4 was observed; however, they are not statistically significant.

NHL was categorized into 3 different grades (low, intermediate and high grade). As illustrated in Fig. 3 gradual elevation of both VEGF and PDGF with grading was found. Significant increase ($P<.001$) in VEGF and PDGF in NHL patients at high grades in comparison with patients at low grade was demonstrated. A direct correlation between progress in grade of NHL and PDGF ($r=0.256$;$P=.05$) was demonstrated. On the other hand,

other proangiogenic (IL-8) and antiangiogenic (IL-4 and IL-12) factors are significantly reduced (*P*<.01, *P*=.05 and *P*<.001 for IL-8, IL-4, and IL-12, respectively) by progress of the grade with no change in TGF-β and IFN-γ levels. An inverse correlation was reported between IL-8 (r=-0.302; *P*<.01), TNF-α (r=-0.261; *P*=.05) and IL-12 (r=-0.414; *P*<.001) and advancement of the grade.

Table 1. Clinical data of non Hodgkin's lymphoma (NHL) patients

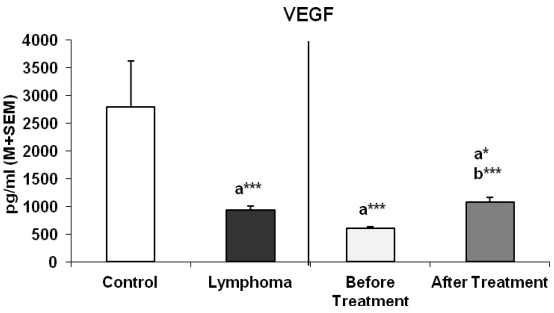
Parameter	NHL group
Grade	
Low	14 (14%)
Intermediate	13 (13%)
High	73 (73%)
Ann arbor stage	
Stage 1	11 (11%)
Stage 2	16 (16%)
Stage 3	25 (25%)
Stage4	48 (48%)
Bone marrow infiltration	
Infiltrated	72 (72%)
Non infiltrated	28 (28%)
HCV	
Positive	64 (64%)
Negative	36 (36%)
IPI	
Low	35 (35%)
Low-intermediate	32 (32%)
High-intermediate	27 (27%)
High	6 (6%)

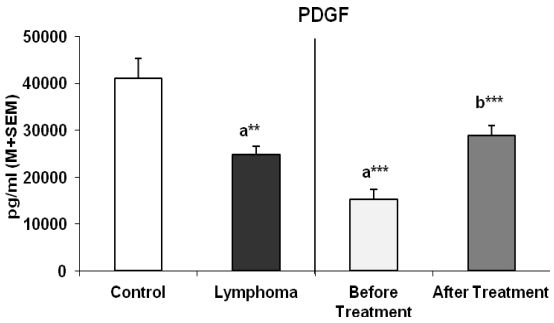
** Non Hodgkin's lymphoma (NHL), diffuse large B- cell lymphoma (DLBCL), follicular lymphoma (FL), international prognostic index (IPI)*

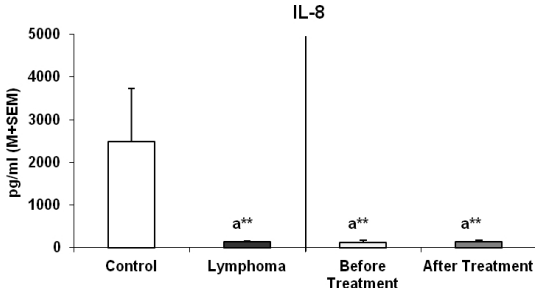
Table 2. Demographic and biochemical characteristics of control and NHL patients

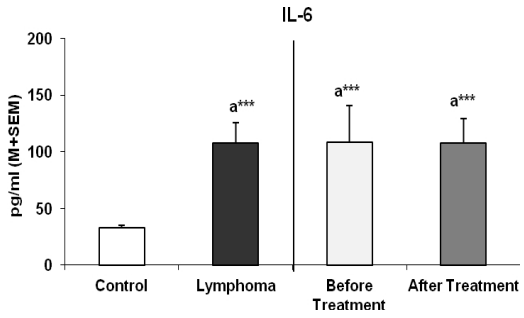
Laboratory investigations	Control group (N=119) (Mean±SD)	NHL group (N=100) (Mean±SD)	*P*	Correlation with NHL
WBCs (1000/mm3)	7.59±2.03	9.35±6.29	*P*<.01	r=0 .193 *P*<.01
Hemoglobin (mmol/L)	7.31±1.0	6.65±1.31	*P*<.001	r=-0.273 *P*<.01
PLT (1000/mm^3)	277.49±63.04	280.42±166.21	NS	-
AST (IU/L)	20.96±5.85	48.51±43.51	*P*<.001	r=0.421 *P*<.01
ALT (IU/L)	18.91±5.05	48.52±50.09	*P*<.001	r=0.399 *P*<.01
ALB (µmol/L)	615.82±57.09	507.15±80.12	*P*<.001	r=-0.621 *P*<.01
Bilirubin (µmol/L)	12.50±3.57	22.94±42.88	*P*<.01	r=0.178 *P*<.01
Creatinine (µmol/L)	78.70±13.69	108.64±107.76	*P*<.01	r=0.199 *P*<.01
LDH (IU/L)	336.71±43.27	538.79±477.67	*P*<.001	r=0.298 *P*<.01

All data are presented as mean ± SD. platlet (PLT), aspartate aminotransferase (AST), alanine transaminase (ALT), albumin (ALB), lactate dehydrogenase (LDH), non Hodgkin's lymphoma (NHL).


VEGF
pg/ml (M+SEM)
4000
3500
3000
2500
2000
1500
1000
500
0
a***
a***
a*
b***
Control
Lymphoma
Before Treatment
After Treatment


PDGF
pg/ml (M+SEM)
50000
40000
30000
20000
10000
0
a**
a***
b***
Control
Lymphoma
Before Treatment
After Treatment


IL-8
pg/ml (M+SEM)
5000
4000
3000
2000
1000
0
a**
a**
a**
Control
Lymphoma
Before Treatment
After Treatment


IL-6
pg/ml (M+SEM)
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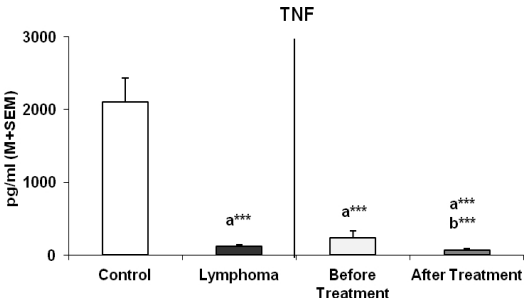
100
50
0
a***
a***
a***
Control
Lymphoma
Before Treatment
After Treatment


TNF
pg/ml (M+SEM)
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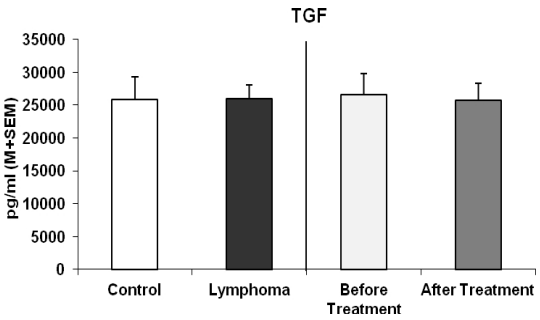
2000
1000
0
a***
a***
a***
b***
Control
Lymphoma
Before Treatment
After Treatment


TGF
pg/ml (M+SEM)
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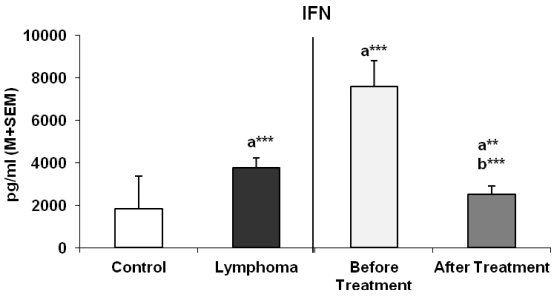
30000
25000
20000
15000
10000
5000
0
Control
Lymphoma
Before Treatment
After Treatment


IFN
pg/ml (M+SEM)
10000
8000
6000
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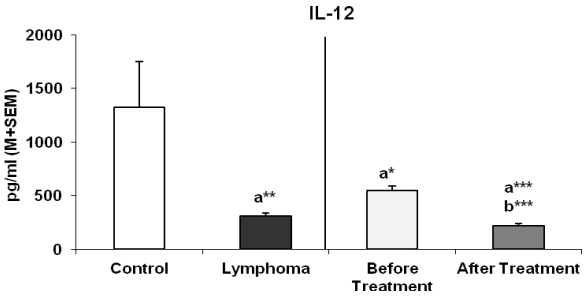
2000
0
a***
a***
a**
b***
Control
Lymphoma
Before Treatment
After Treatment


IL-12
pg/ml (M+SEM)
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1500
1000
500
0
a**
a*
a***
b***
Control
Lymphoma
Before Treatment
After Treatment

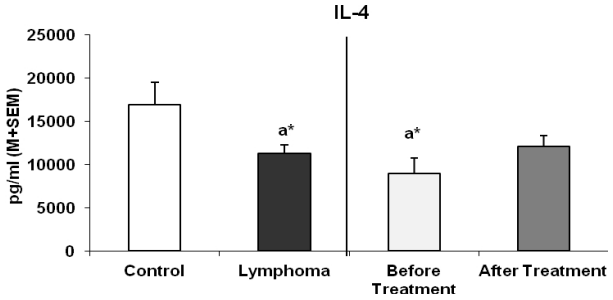
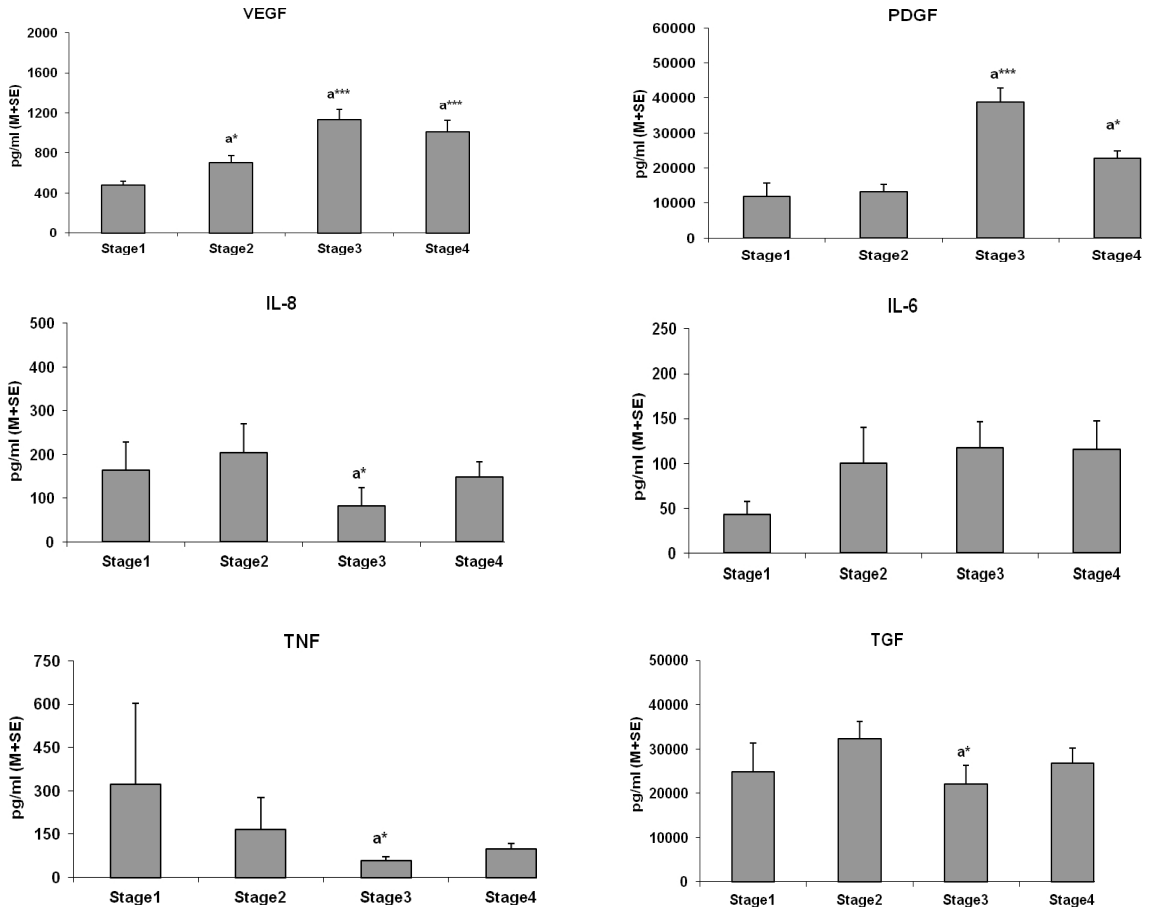


Fig. 1. Plasma levels of angiogenesis and antiangiogenesis factors in non-Hodgkin's lymphoma (NHL) patients before (left side) and after (right side) treatment. Results are expressed as mean ± standard error. VEGF: vascular endothelial growth factor; PDGF: platelet derived growth factor; TNF-α: tumor necrosis factor; TGF-β: transforming growth factor; and IFN-γ: interferon gamma. a: groups statistically significant different from controls; b: groups statistically significant different from NHL patients before treatment; **P*=.05, ***P*<.01; ****P*<.001



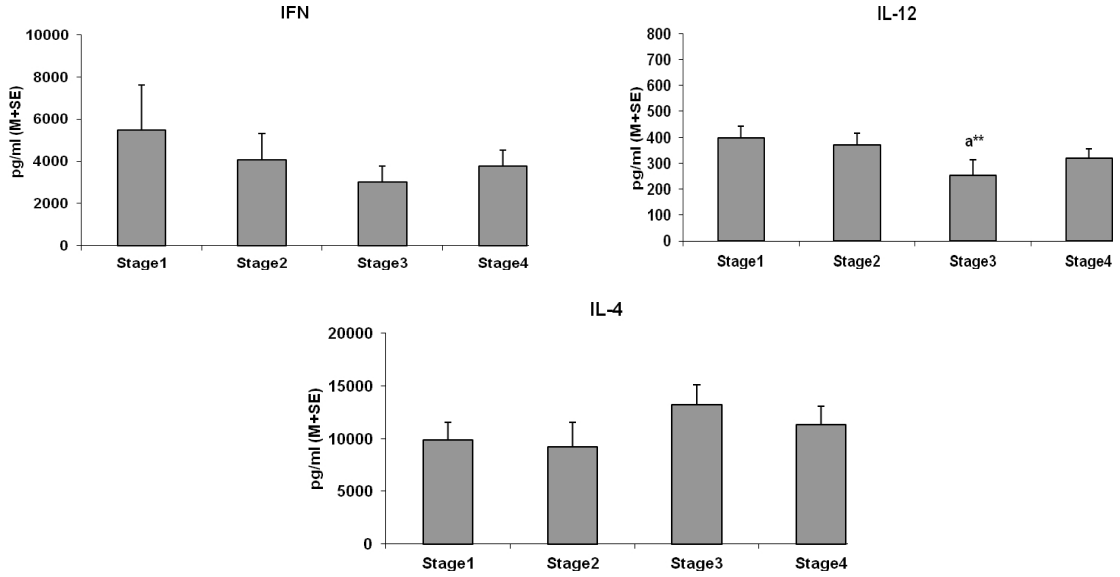
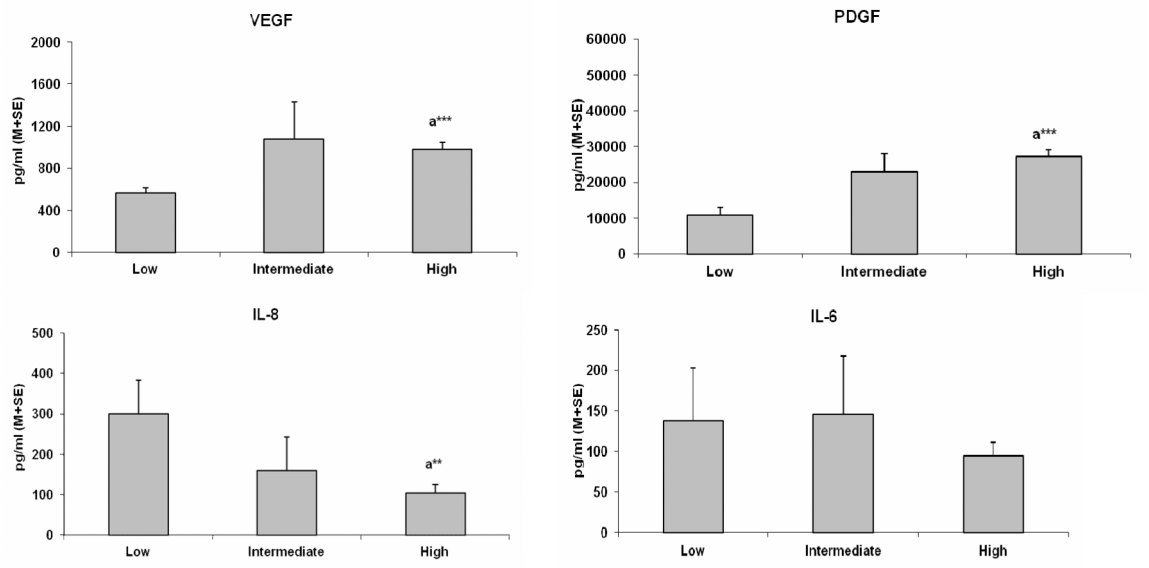


Fig. 2. Plasma levels of angiogenesis and antiangiogenesis factors in non-Hodgkin's lymphoma (NHL) patients at different stages. Results are expressed as mean ± standard error. VEGF: vascular endothelial growth factor; PDGF: platelet derived growth factor; TNF-α: tumor necrosis factor; TGF-β: transforming growth factor; and IFN-γ: interferon gamma. a: groups statistically significant different from stage (1); *P=.05, **P<.01; ***P<.001



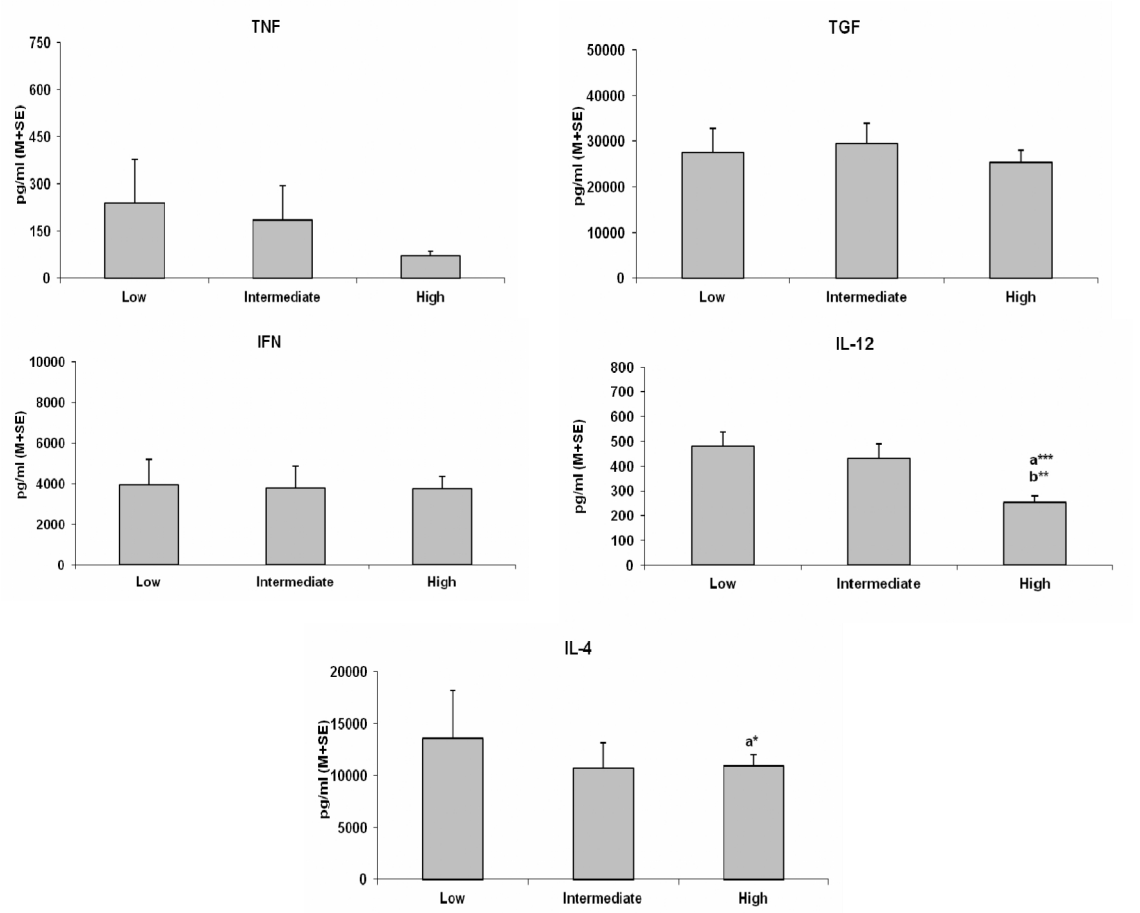
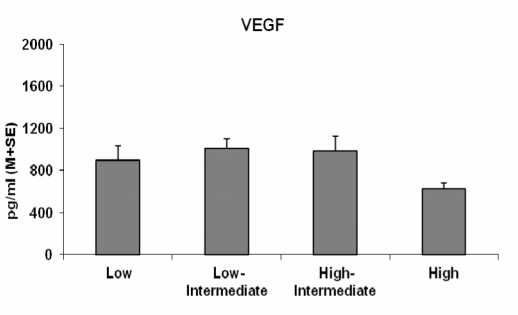
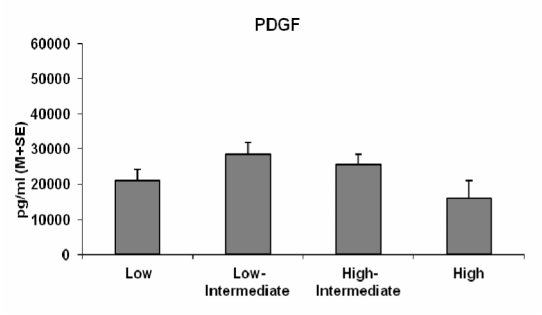


Fig. 3. Plasma levels of angiogenesis and antiangiogenesis factors in non-Hodgkin's lymphoma (NHL) patients at different grades. Results are expressed as mean ± standard error. VEGF: vascular endothelial growth factor; PDGF: platelet derived growth factor; TNF-α: tumor necrosis factor; TGF-β: transforming growth factor; and IFN-γ: interferon gamma. a: groups statistically significant different from low grade. b: groups statistically significant different from intermediate grade; *P<.05, **P<.01; ***P<.001

Classifying NHL patients depending on IPI score showed a slight insignificant reduction in most of proangiogenic/antiangiogenic mediators in NHL with high IPI (VEGF, PDGF, TNF-α,IL-6,TGF-β,IL-4, and IFN-γ). However, IL-8 and IL-12 were increased in NHL patients with high IPI score, data were not statistically significant Fig. 4.

The plasma concentration of proangiogenic mediators was compared between the samples from NHL patients with and without CHOP treatment. After CHOP treatment, VEGF and PDGF were significantly increased (P<.001) as compared to NHL without therapy Fig. 1. Reduction in IL-8 (P<.01) and TNF-α (P<.001) in untreated NHL patients was continued after CHOP treatment. Among all tested proangiogenic mediators, IL-6 was the only elevated cytokine in NHL patients with (P<.001) or without (P<.001) treatment compared to healthy individuals. Concerning the antiangiogenic mediators, treatment resulted in a reduction in the secretion level of IFN-γ (P<.001) and IL-12 (P<.001) as compared with untreated NHL patients. A slight insignificant elevation in IL-4 level after CHOP treatment was recorded.


VEGF
pg/ml (M+SE)
2000
1600
1200
800
400
0
Low
Low-Intermediate
High-Intermediate
High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Low-Intermediate
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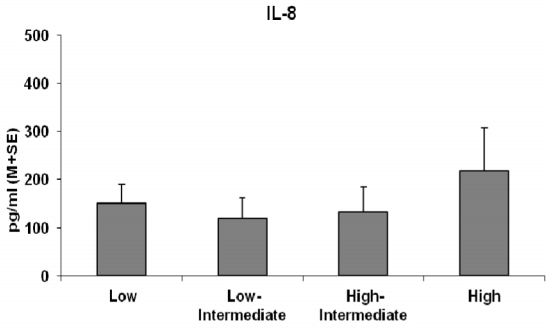
High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400
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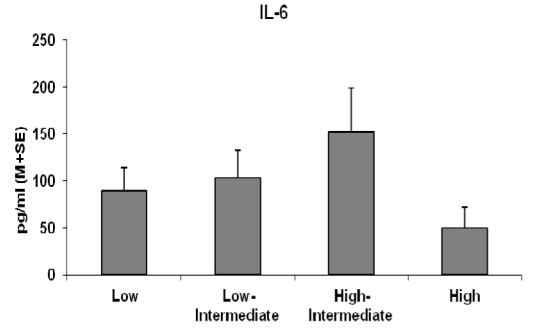
200
100
0
Low
Low-Intermediate
High-Intermediate
High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pg/ml (M+SE)
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200
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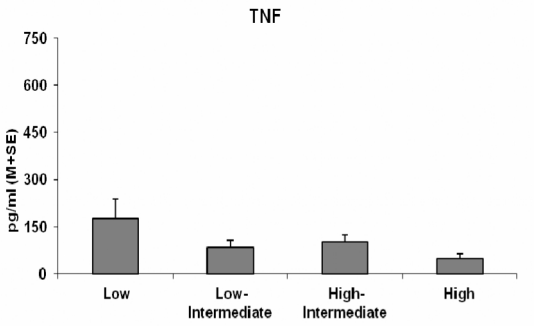
0
Low
Low-Intermediate
High-Intermediate
High


TNF
pg/ml (M+SE)
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600
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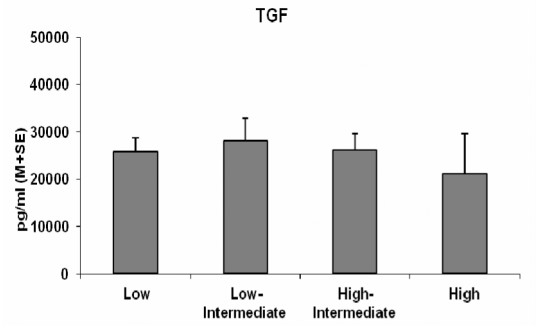
0
Low
Low-Intermediate
High-Intermediate
High


TGF
pg/ml (M+SE)
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40000
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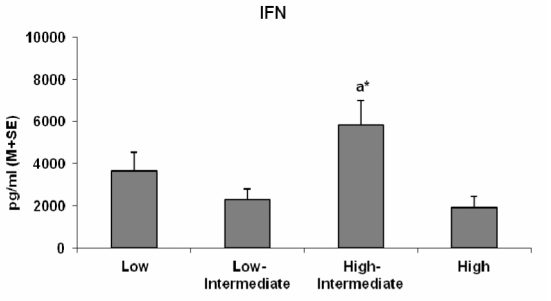
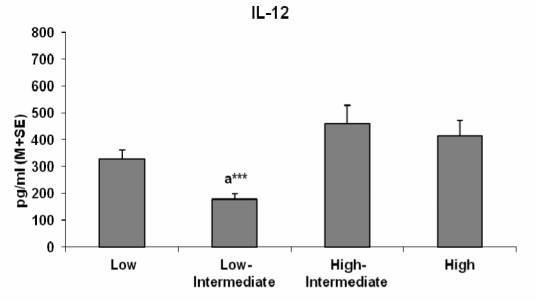
0
Low
Low-Intermediate
High-Intermediate
High


IFN
pg/ml (M+SE)
10000
8000
6000
4000
2000
0
a*
Low
Low-Intermediate
High-Intermediate
High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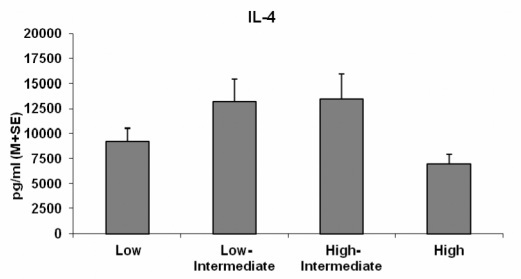
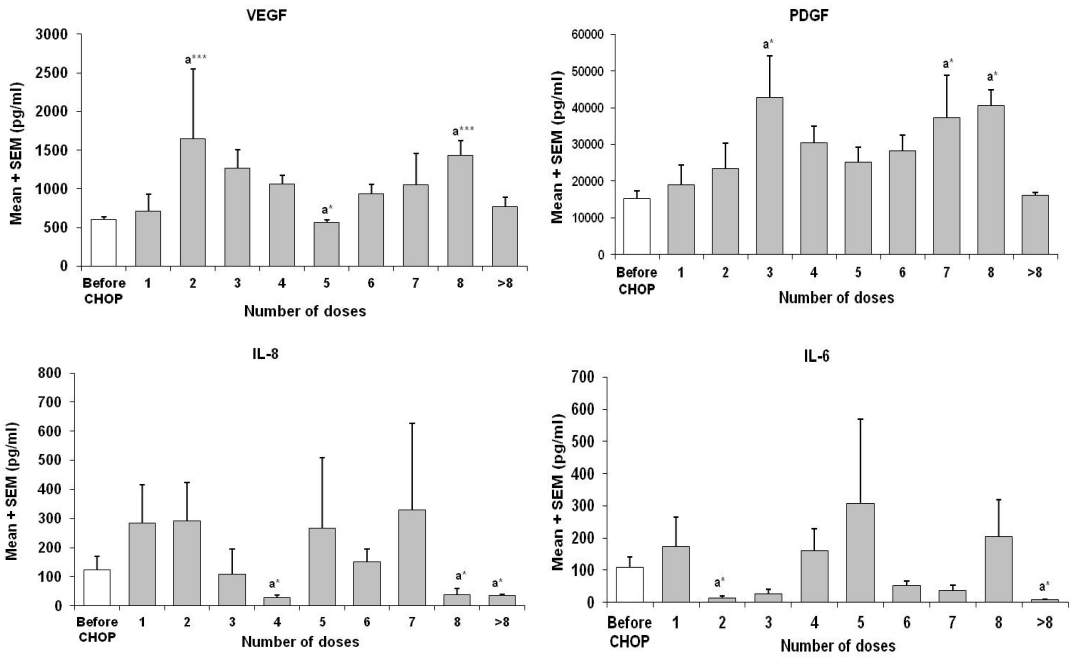


Fig. 4. Plasma levels of angiogenesis and anti angiogenesis factors in Non-Hodgkin's lymphoma (NHL) patients at different international prognostic index (IPI). Results are expressed as mean ± standard error. VEGF: vascular endothelial growth factor; PDGF: platelet derived growth factor; TNF-α: tumor necrosis factor; TGF-β: transforming growth factor; and IFN-γ: interferon gamma. a: groups statistically significant different from low IPI; *$P=.05$,; ***$P<.001$

A close insight into proangiogenic/antiangiogenic factors in treated patients with different doses of chemotherapy revealed that a fluctuation in secretion level of all measured parameters was found during treatment. Although CHOP treatment required more than 8 cycles to be able to down regulate proangiogenic (VEGF, PDGF, IL-8, TNF-α, and IL-6) and elevate antiangiogenic (IL-4, IL-12 and IFN-γ) mediators, changes were statistically insignificant Fig. 5.



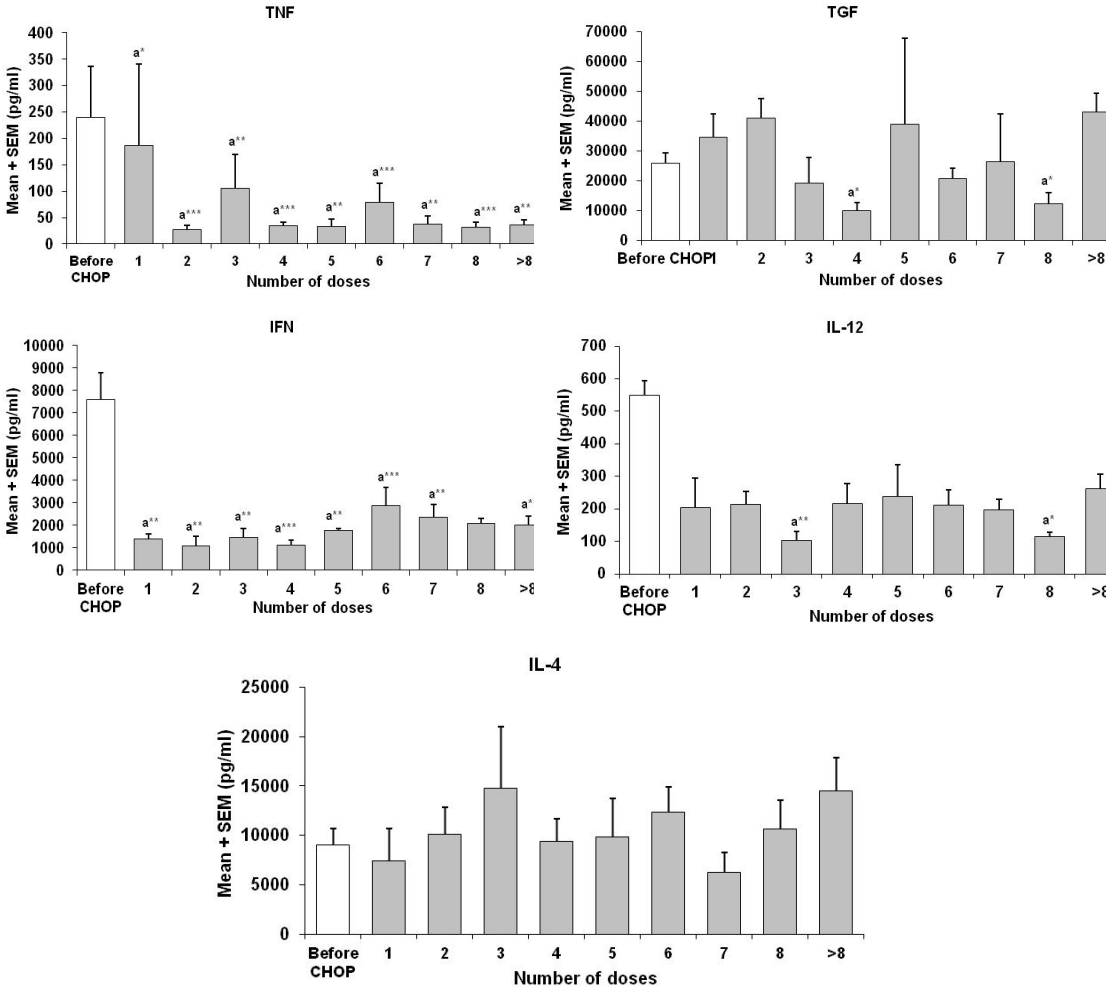


Fig. 5. Plasma levels of angiogenesis and antiangiogenesis factors in non-Hodgkin's lymphoma (NHL) patients after different doses of CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone). Results are expressed as mean±standard error. VEGF: vascular endothelial growth factor; PDGF: platelet derived growth factor; TNF-α: tumor necrosis factor; TGF-β: transforming growth factor; and IFN-γ: interferon gamma. a: groups statistically significant different from controls; *$P=.05$, $P<.01$; ***$P<.001$**

4. DISCUSSION

Cytokines play an important role in the pathogenesis of lymphomas and may contribute to the clinical manifestations in NHL [24-25]. Although the role of neovascularization in hematologic malignancies has been extensively explored, few studies have been conducted on the role of angiogenesis in lymphoma [14-18]. In addition, the predictive value of angiogenesis markers in lymphoma is still controversial due to disease heterogeneity and various detection methods [26]. Many patients with NHL are cured by therapy; however, the remainders are not cured and ultimately die of their disease [27]. Angiogenic profile might be

used to monitor cancer therapy [28]. Hence, it might be possible to obtain an angiogenic profile of a cancer patient's blood sample by measuring the concentrations of several circulating angiogenic and antiangiogenic molecules; this study was undertaken to assess the clinical significance of the plasma proangiogenic (VEGF,PDGF,IL-6,IL-8,TGF-β and TNF-α) and antiangiogenic (IL-4,IL-12, and IFN-γ) molecules in Egyptian patients with NHL with and without CHOP treatment.

Our biochemical data showed a significant increase in lactate dehydrogenase enzyme (LDH) in NHL patients, a finding which agrees with that reported by other investigators [29-31]. Plasma albumin was significantly decreased in NHL patients. This albumin reduction might be attributed to increased catabolism induced by tumor proliferation. Hypoalbuminemia was found to be an important clinical feature of intravascular large B-cell lymphoma [32]. Reduction in hemoglobin level observed in our NHL patients was previously demonstrated by [31-33]. LDH and HB were reported to be independent prognostic factors for survival in high grade NHL [34]. Moreover, high LDH level, HB <12gm/dl and albumin <3.5gm/dl were demonstrated to be among the factors which were associated with poor response in DLBCL [33].

In the present study, proangiogenic mediators (VEGF,PDGF,IL-8, and TNF-α) in most of NHL patients seemed to be lower than in normal controls. This result is in agreement with the study of Saber-Hosnijeh et al. [35] who reported a reduction in most of cytokines in NHL cases in relation to control subjects. Our data showed a remarkable elevation of IL-6 in treated NHL patients in agreement with Mellgren et al. [36]. Jones et al. [37] have detected high levels of IFN-γ and IL-6 expression in NHL.

Our study showed elevated plasma level of VEGF and PDGF in NHL with a higher level in stage (3) patients when compared with stages (1) and (2). In patients with NHL, higher levels of VEGF and PDGF correlated with more advanced disease stage. Elevated levels of angiogenic growth factors are also associated with an adverse prognosis in patients with NHL [38]. In contrast, TNF-α, and IL-12 were decreased by staging especially in stage (3). A slight elevation in both IL-6, and IL-4 was observed. This data proved the Th1 and Th2 unbalance (in favor of Th2 cells) in NHL patients as represented by elevation in IL-4 and IL-6 and reduction in TNF-α. In agreement with this data, Nicolaides et al. [33] and Mori et al. [39] concluded that the Th1/Th2 balance was polarized to Th2 in NHL patients. In accordance with the presented data, Zhu et al. [40] found that TNF-α did not increase by clinical staging of the disease. In contradiction to our data, Khalifa et al. [25] reported an elevation in TNF-α by Ann Arbor staging. This difference could be returned to the difference in patient group as they did not mention whether all NHL patients are belonging to one or different subtypes.

Our study noticed elevation of both VEGF and PDGF in high grade of NHL compared to low grade. An increased level of VEGF has been reported more frequently in high grade NHL [41]. The study of Foss et al. [42] showed that VEGF was minimally expressed in low grade lymphoma. Supporting to our results, Hazar et al. [43] and Aguayo et al. [44] studies revealed a significantly higher microvessel counts in NHLs with high-grade lymphomas than in those with low-grade lymphomas, implying that angiogenesis occurring in NHLs increases with tumor progression. On the other hand, our results showed a significant reduction in IL-4 levels by the progress of the grade. A reduction of IL-4 in the high grade tumor group was previously reported by Jones et al. [37].

In this study, a high post treatment level of plasma VEGF and PDGF was detected. Studies of Seymour et al. [45-46] showing changes in VEGF and PDGF levels during chemotherapy

have also been shown to predict the effect of chemotherapy in terms of tumor response and survival in patients with solid tumors. In our study, CHOP treatment resulted in elevation in plasma levels of VEGF and PDGF (proangiogenic factors). The elevation of proangiogenic mediators after therapy might be attributed to the therapy-induced cell damage and increased release of angiogenic factors to the circulation. Ugurel et al. [47] reported that angiogenic factors may be influenced by various therapeutic regimens as the plasma level of angiogenin, bFGF, was found to be significantly higher in melanoma patients under treatment with chemotherapy. A high VEGF, PDGF content may reflect active angiogenesis and lymphoma growth, and it is possible that similar associations with unfavorable survival can be found in other types of human cancer as well [48].

Decreasing the secretion level of both TNF-α and IFN-γ approved that CHOP treatment might reduce the Th1 response in NHL patients. It has been demonstrated that increased endogenous TNF-α production by tumor cells could be contributed to the chemotherapeutic drug resistance [49]. TNF-α was also shown in some models to stimulate the growth of malignant B-cells [50]; thus reduction of circulating TNF-α might represent a good monitor for therapy. Higher plasma levels of TNF-α are associated with poor disease outcome [51-53]. In this study, CHOP treatment resulted in severe reduction in the secretion level of IL-12 as compared with untreated NHL patients. Serum IL-12p40 and IL-12mix (p35 and p40) levels were decreased after CHOP chemotherapy [54].

5. CONCLUSION

Taken together, our data stressed on the importance of pro- and antiangiogenic mediators in monitoring CHOP therapy. In the future, it might be possible to obtain an angiogenic profile by measuring the concentrations of several circulating stimulators and inhibitors of angiogenesis which could be used as a monitor of cancer therapy or as a predictor of outcome after cancer has been diagnosed. Thus, our data might be helpful specially in assessing the utilization of antiangiogenic therapy in patients with NHL. However, further studies on larger population and on different NHL subtypes are required to confirm these results.

CONSENT

All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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