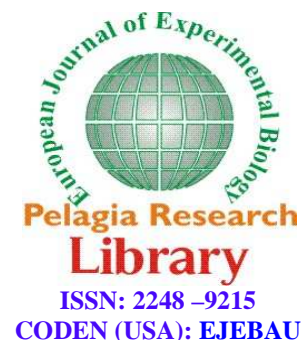




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Serum levels of interleukin-6 and interleukin-10 in adult newly diagnosed Iraqi Non Hodgkin's lymphoma patients

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ABSTRACT

Cytokines are believed to be involved in the pathogenesis of lymphomas. This study was conducted to investigate the serum levels of IL-6 and IL-10 in patients with non-Hodgkin's lymphoma and their relationships with clinicopathological characteristics. These serum factors were measured in 57 NHL patients in comparison with 30 healthy controls using enzyme-linked immunosorbent assays (ELISAs). There were significant differences in the serum IL-6 and IL-10 levels between NHL patients and controls ($p=0.0001$ and $p=0.001$, respectively). None of the analyzed parameters; age, sex, B symptoms, stage, grade and LDH was significantly related with the serum IL-6 concentrations except for CRP. Serum IL-6 levels were elevated in the group of patients with high level CRP compared with the group of patients with a normal level ($p=0.05$). Serum IL-10 levels also showed no significant relationships with analyzed parameters. In addition there was no significant correlation between the serum levels of IL-6 and IL-10 in patients with NHL ($r=0.002$, $P=0.99$).

Key words: Cytokines, IL-6, IL-10, Non Hodgkin's lymphoma, Iraqi patients.

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a group of lymphoproliferative malignant disorders with heterogeneous histological and clinical characteristics [1]. NHL is characterized by abnormal proliferation or accumulation of B or T lymphocytes [2].

Cytokines are intercellular short-acting soluble mediators that are involved in the pathogenesis of cancer [3] and might play important role in the pathogenesis of lymphomas [4]. Cytokines can either be produced by or exert effects on neoplastic or reactive cells [5]. Serum concentration of the cytokines may be utilized as a marker of immunity status and/or prognosis in cancer [6].

Interleukin-6 is a multifunctional cytokine involved in the regulation of various cellular functions, among them proliferation, apoptosis, angiogenesis, differentiation and regulation of immune response [7], being also implicated in the pathogenesis of several lymphoproliferative disorders [8][9], and the presence of blasts in B-cell lymphoma patients has been shown to correlate with IL-6 production [10].

Interleukin-10 is an immunosuppressive cytokine produced by many different cells of the immune system, including T and B lymphocytes, macrophages, monocytes, dendritic cells, and NK cells [11]. There are several lines of evidence that indicated IL-10 over expression in different malignancies might contribute to tumor development, in particular, by suppressing the antitumor immune response [12]. IL-10 may have some role in the development of lymphoma through two mechanisms: its proliferation-stimulating properties on B cells, and its immunosuppressive properties [13][14]. The high concentrations of IL-10 found in patients with NHL and possibly present in tumor sites could conceivably exert an inhibitory effect on macrophage and antigen-specific T cell response at the tumor site and thus contribute to lymphoma progression in vivo [15].

Non Hodgkin's lymphoma has been found to be associated with elevated levels of inflammatory and immune regulatory cytokines. This study was conducted to investigate the serum levels of IL-6 and IL-10 in newly diagnosed patients with NHL and their relationship with clinicopathological characteristics.

MATERIALS AND METHODS

Sample collection: The study was conducted during the period between September / 2012 and April /2013 at The Basrah Oncology and Haematology Center. Fifty seven adult newly diagnosed Iraqi patients with NHL were enrolled in the study with age range between (18-75) years. They were 40 males and 17 females (M: F ratio was 2.35:1). The blood samples were withdrawn from patients by vein-puncture at the time of diagnosis before taking therapy were allowed to clot before centrifugation then the serum was removed and stored frozen at -20 °C until assayed. Thirty from healthy individual whose ages ranged between (19-73) years were selectively included in this study as a control group.

Analytical methods: Assessment of IL-6 and IL-10 levels measured by using Enzyme-Linked Immunosorbent Assay kit (from Cusabio). Estimation of serum C-reactive protein (CRP) by using CRP ELISA detection kit (from Monobind). Estimation of serum LDH was performed by enzymatic methods on Cobas c 111Autoanalyzer (Roche /USA).

Statistical Analysis: All data were analyzed using the SPSS, version 19 software. Statistical analysis included descriptive statistics (frequency tables), Mann-Whitney-U test, Kruskal-Wallis test, and Spearman correlation (r). P value which is ≤ 0.05 was considered statistically significant [16].

RESULTS

Fifty seven newly diagnosed patients with NHL, with an age range of 18-75 years and age mean 53.42 years were enrolled in this study. The descriptions of patient characteristics are listed in (Table1).

Table 1: Non- Hodgkin's lymphoma patients' clinicopathological characteristics

Parameters	NHL patients (n=57)		
	NO.	%	
Age	≤60	34	59.6
	>60	23	40.4
Sex	Male	40	70.2
	Female	17	29.8
B-symptoms [□]	Present	22	38.6
	Absent	35	61.4
Stage #	I & II	19	33.4
	III & IV	34	59.6
Grade ‡	Low grade	24	42.1
	Intermediate grade	13	22.8
	High grade	18	31.6
Serum LDH • (U/L)	Normal	32	56.1
	High	25	43.9
Serum CRP Δ (µg/ml)	Normal	17	29.8
	High	40	70.2

□: B symptoms: fever, night sweat and weight loss, #: Four cases are missed, ‡: Two cases are missed, •: LDH: Lactate Dehydrogenase, Δ: CRP: C-reactive protein.

Serum levels of Interleukin-6 in NHL patients and controls: Estimation of IL6 was significantly higher in NHL patients than its levels in control group ($p=0.0001$) (Table 2).

Serum levels of Interleukin-10 in NHL patients and controls: Estimation of IL-10 was significantly higher in NHL patients than its levels in control group ($p=0.001$) (Table 3).

Table 2: Serum levels of IL-6 (pg/ml) in NHL patients and controls

Groups	NO.	Range	Mean \pm S.D	Median	S. E	P value*
Patients	57	(0.1- 282.0)	10.17 \pm 44.80	1.20	5.9349	0.0001
Control	30	(0.1- 2.2)	0.77 \pm 4.96	0.75	0.0854	

*: Mann Whitney-U test

Table 3: Serum levels of IL-10 (pg/ml) in NHL patients and controls

Groups	NO.	Range	Mean \pm S.D	Median	S. E	P value*
Patients	57	(1.1- 97.0)	8.46 \pm 12.42	6.00	1.6456	0.001
Control	30	(0.03-28.0)	4.96 \pm 5.52	3.40	1.0084	

*: Mann Whitney-U test

Relationship of serum IL-6 levels in NHL patients with clinicopathological studied characters: Interleukin-6 was not significantly related with any of the age, sex, B symptoms, stage, grade and LDH ($p=0.181, 0.355, 0.530, 0.853, 0.458$ and 0.658 , respectively). But it was significantly higher in patients with high levels of CRP than the patients with normal CRP ($p=0.050$), as shown in (Table 4).

Relationship of serum IL-10 levels in NHL patients with clinicopathological studied characters: Statistical analysis demonstrated that there was no significant relation between IL-10 levels in patients and age, sex, B symptoms, stage, grade, LDH and CRP ($p=0.304, 0.586, 0.453, 0.889, 0.291, 0.334$ and 0.631 respectively) (Table 5).

Relationship between the serum IL-6 and IL-10 levels in NHL patients sera: There was no correlation between IL-6 and IL-10 levels in NHL patients ($r=0.002, P=0.99$).

Table 4: Relationship between serum IL-6 levels in NHL patients and clinicopathological characteristics

Parameters		NHL patients (n=57)	Mean \pm S.D (IL-6 pg/ml)	S.E	P value
Age	≤ 60	34	15.63 \pm 57.67	9.8910	0.181*
	>60	23	2.09 \pm 2.58	0.5392	
Sex	Male	40	13.78 \pm 53.26	8.4213	0.355*
	Female	17	1.67 \pm 1.78	0.4340	
B-symptoms	Present	22	14.93 \pm 59.70	12.7292	0.530*
	Absent	35	7.17 \pm 32.88	5.5581	
Stage	I & II	19	1.98 \pm 1.73	0.3978	0.853*
	III & IV	34	15.81 \pm 57.65	9.8877	
Grade	Low	24	2.26 \pm 2.49	0.5087	0.458¥
	Intermediate	13	16.82 \pm 53.85	14.93	
	High	18	16.82 \pm 66.19	15.6011	
Serum LDH (U/L)	Normal	32	10.55 \pm 49.55	8.7601	0.658*
	High	25	9.67 \pm 38.88	7.7777	
Serum CRP (μ g/ml)	Normal	17	2.61 \pm 2.54	0.6163	0.050*
	High	40	13.38 \pm 53.33	8.4332	

*: using Mann Whitney-U test, ¥: Kruskal-Wallis test

Table 5: Relationship between serum IL-10 levels in NHL patients and clinicopathological characteristics

Parameters	NHL patients (n=57)	Mean±S.D (IL-10 pg/ml)	S.E	P value	
Age	≤60	34	6.71±3.28	0.5626	0.304*
	>60	23	10.94±19.12	3.9868	
Sex	Male	40	9.01±14.65	2.3178	0.586*
	Female	17	7.02±3.68	0.8927	
B-symptoms	Present	22	6.84±3.79	0.8083	0.453*
	Absent	35	9.41±15.58	2.6336	
Stage	I & II	19	6.75±3.59	0.8248	0.889*
	III & IV	34	8.95±15.74	2.7008	
Grade	Low	24	11.596±18.52	3.7821	0.291¥
	Intermediate	13	6.192±4.42	1.2260	
	High	18	6.133±2.325	0.5480	
Serum LDH (U/L)	Normal	32	10.15±16.33	2.8881	0.334*
	High	25	6.14±2.40	0.4813	
Serum CRP (µg/ml)	Normal	17	6.53±2.37	0.5765	0.631*
	High	40	9.22±14.73	2.3298	

*: using Mann Whitney-U test, ¥: Kruskal-Wallis test

DISCUSSION

There exists strong evidence that tumor growth can be actively controlled by the host immune system and cytokines are known to play a significant role in immune response regulation [17]. The endogenous cytokines are aberrantly produced in many cancers, and serve as autocrine growth factors or indicators in immune response to the tumors. Hence, cytokine deregulation is likely to participate in the development or evolution of the malignant process. Over the last few years, endogenous cytokine levels have been correlated with phenotypic manifestations of cancer and with prognosis. Cytokines play important role in the pathogenesis of lymphomas [18].

The present study demonstrated that the serum level of IL-6 was significantly higher in NHL patients than in healthy control; a finding which agreed with that reported by other investigators [19][20][18][21][22].

There evidence of literature suggests that the level of IL-6 from both the lymphoma cells themselves and reactive lymphocytes in lymphomatous nodes can produce IL-6[23][24][25]. Furthermore, IL-6 has an autocrine role in two lymphoma cell lines, one of B-cell origin and one of T-cell origin [8].

In this study IL-6 showed no significant relation with the analyzed parameters including age, sex, B symptoms, stage and LDH level. This finding was supported by Guney and his colleagues [22]. In contrast to other studies that found the presence of B symptoms, advance stage and elevated LDH levels were all significantly associated with an increased IL-6 level and suggested that the release of cytokines by lymphoma cells may modulate the general status of NHL patients[26][27].

Current data analysis revealed that there was no significant relation between IL-6 level and malignant grade while in another study, IL-6 level was found to be increased significantly in patients with high-grade than in those with low-grade malignancy [28].

The present study showed that IL-6 was significantly related with CRP. Similar finding was reported by Legouffe et al., who concluded that CRP may be considered as a valuable and easy prognostic biomarker of NHL [29].

Interleukin-6 is the major determinant of CRP synthesis rate. CRP may be considered as an indicator of the malignant potential of the tumor [30]. In lymphomas, the high serum CRP levels reflect the elevated serum inflammatory cytokines levels that are associated with the malignant process and in particularly of IL-6[31].

Present results also revealed a significant elevation in IL-10 serum levels of NHL patients in comparison to healthy control group, similar results were reported by other investigators [20][21][22]. The most acceptable explanation for such increase in the level of IL-10 might be due to its production by malignant cells or by different cells of the immune system, including T and B lymphocytes, macrophages and monocytes [13][25][11]. IL-10 can act as a

growth factor for malignant B cells such as the plasma cell clone of myeloma and other B cell lymphoproliferative diseases [32]. An alternative interpretation could rely on the immunosuppressive properties of IL-10[33].

Current study revealed that IL-10 levels was not significant related with the age, sex, B symptoms, stage and LDH. A similar finding was reported previously by other investigators [34][35][36][22].

There was also no significant relation between IL-10 and malignant grade; A similar finding was reported previously by some investigators [36].

Furthermore, no relation was found between IL-10 and CRP. In contrast to other studies which found that B symptoms, advanced stage, elevated LDH and CRP levels were all significantly associated with an increased IL-10 level [37][27].

Finally this study revealed no significant correlation between the serum levels of IL-6 and IL-10 in patients with NHL while in other studies showed significant correlation between the serum levels of IL-6 and IL-10 in patients with NHL [22][27].

CONCLUSION

Patients with NHL have significantly increased serum levels of IL-6 and IL-10 compared with healthy controls. The results of the present study suggest that higher serum levels of these immunological parameters can be useful in diagnostic work up of NHL. However, the sample size is small, and performed in a single center. Larger scale research is needed in this field to provide new knowledge.

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