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The Use of the Cytokines EMAP-II, IL-19 and IL-10 as Biomarkers to Determine Prognosis of Non – Hodgkin's Lymphoma

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Abstract

Objectives: Establishing diagnostic and prognostic factors are very important in the management of Non-Hodgkin Lymphoma (NHL). Our aim was to evaluate the clinical significance of serum EMAP-II, IL-19, and IL–10 in NHL patients for assessing treatment response and prognosis of patients.

Methods: Serum EMAP-II, IL-19, and IL-10 levels were measured in the serum of 64 NHL patients before and after treatment with CHOP-based chemotherapy by enzyme-linked immunosorbent assay. Correlations of marker levels to the laboratory, clinicopathological and immunophenotyping markers were performed.

Results: Serum levels of EMAP-II and IL-10 were higher before therapy and decreased significantly thereafter ($P<0.001$). High EMAP-II and IL-10 were correlated with serum ALT and blood urea respectively ($P=0.043$, $P=0.020$). Significantly higher levels of IL-19 were demonstrated in patients with relapse ($P<0.001$). A significant association was found between serum IL-19 and AST, CD23 and B-cl2 ($P=0.032$, $P=0.015$, $P=0.024$ respectively). There was a significant correlation between EMAP-II and IL-10 in NHL patients ($r=0.827$, $P<0.001$).

Conclusion: EMAP-II, IL-19, and IL-10 can all serve as useful diagnostic markers in NHL patients. They assessed response to therapy. IL-19 proved to be the most sensitive predictor of advanced disease and poor prognosis.

Keywords: EMAP-II; IL-19; IL-10; Non-Hodgkin's lymphoma

Introduction

Non-Hodgkin's lymphoma (NHL) is a group of lympho proliferative malignant disorders with heterogeneous histological and clinical features [1]. NHL is characterized by abnormal proliferation or accumulation of B or T lymphocytes [2]. Many clinical and laboratory parameters had been employed in the prognostic definition of NHL [3].

Cytokines are intercellular short-acting soluble mediators that are involved in the pathogenesis of cancer [4]. Serum concentration of the cytokines may be utilized as a marker of immunity status and prognosis and in cancer [5]. Some of these cytokines, e.g., Endothelial-monocyte activating polypeptide-II (EMAP-II), Interleukin-19 (IL-19) and Interleukin-10 (IL-10) allow tumor cells to evade immune surveillance through immune suppression mechanisms [6-12]. EMAP-II, IL-19, and IL-10 levels have been verified to be associated with inferior prognosis [13-18]. We therefore wished to determine whether EMAP-II, IL-19 and IL-10 have a role in the prognosis of NHL.

EMAP-II is a molecule with pleiotropic activities toward endothelial cells, monocytes/macrophages, and neutrophils [19,20]. It was initially identified in the supernatant of murine methylcholanthrene A–induced fibrosarcomas, which induced tissue factor procoagulant activity in endothelial cells [21]. Hypoxia was found to be an inducer of the release and processing of EMAP-II in prostate adenocarcinoma cells [22]. Endothelial cell–specific apoptosis was induced by EMAP-II [23]. Another function of EMAP-II has been described in tumors: Its expression induced apoptosis of lymphocytes in solid tumors. This suggests an immunosuppressive role of EMAP-II in growing tumors [24,25].

IL-19 is a member of the IL-10 family of cytokines. This cytokine is produced by many cells such as monocytes, macrophages, B cells, endothelial and epithelial cells [26]. High IL-19 expression in tumor tissues had been associated with advanced tumor stage, high tumor metastasis and poor clinical outcome [14,15]. IL-19 directly affected cancer cell proliferation and migration and indirectly affected tumor progression by inducing the expression of cytokines and chemokine [14].

IL-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 [27] but it is also produced by neoplastic B lymphocytes [10,27]. IL-10 suppresses antigen presenting cells thereby allowing tumor cells to escape immune system [8-10]. In addition, IL-10 may increase bcl-2 expression and protect malignant cells from apoptosis [28]. Elevated IL-10 levels have been found in patients with NHL and elevated levels were associated with poor prognosis [29-32].

This study assessed serum levels of EMAP–II, IL-19, and IL-10 and evaluated them as to their clinical value in NHL patients.

Subjects and Methods

Patients and controls

Patients were chosen from the outpatient clinic of Minia Oncology Center. This group consists of 64 patients with NHL, their ages ranged from 13 to 75 years and this group included 38 males and 24 females. They were separated into four groups according to the response to treatment: Group I included patients newly diagnosed and not yet treated all the time of the study, group II with complete remission (CR), group III with partial remission (PR) and group IV with relapse.

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The diagnosis of NHL was confirmed by immunophenotyping and histopathology.

The controls were thirty, apparently healthy subjects, matched for age and sex with the patient group. Their ages ranged from 21 to 70 years and this group included 15 males and 15 females.

NHL treatment

Patients received treatment strategies as follows: patients with early-stage NHL received CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone) (6-8 cycles) [33] and patients with advanced stage NHL received CHOP in combination with rituximab [34]. Involved-field radiation therapy was considered consolidation treatment for patients in early and intermediate stages or for those presenting with bulky disease. CR was defined as the absence of clinical and radiographic evidence of disease, or disease-related symptoms, and appropriate laboratory results. Partial remission was defined as the decrease by at least 50% of tumor mass. Primary treatment failure was defined as progressive disease during initial treatment, failure to achieve complete remission or partial remission after initial therapy, or progressive disease within 5 months after complete remission [35].

Laboratory investigations: Complete blood count (CBC) was determined by automated cell counter Cell Dyn 1700 (USA). Lactate dehydrogenase (LDH), renal function tests (serum creatinine and blood urea), and liver enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT) were determined by using Vitros 350 dry chemistry (Ortho clinical diagnostics, Johnson-Johnson company; USA) and ACE automated quantitative multistation chemistry Analyzer (Schiapparelli Biosystems. INC; USA).

Measurement of serum EMAP-II, IL-19, IL-10

Serum IL-10, IL-19, and EMAP-II levels were measured by solid-phase enzyme-linked immunosorbent assay using 96 well microplates in accordance with the manufacturer's instructions (Sunred Biological Technology Co., Ltd, Shanghai). A monoclonal antibody specific for EMAP-II, IL-19 and IL-10 has been coated onto the wells of the microtiter strips provided. Optical densities at 450 nm were determined using a microtiter plate reader. Granting to the data supplied by the producer of the ELISA kits, the lower detection limits for EMAP-II, IL-19 and IL-10 were 0.073 pg/ml, 1.3 pg/ml, and 0.095 pg/ml, respectively.

Statistical method

The gathered data were coded, tabulated, and statistically analyzed using SPSS program (Statistical Package for Social Sciences) software version 20. Descriptive statistics were made out for numerical data by mean, standard deviation, and minimum and maximum of the orbit, while they were done for categorical data by number and percent. Non-parametric quantitative data are transformed into the logarithm before analysis (WBCs, Platelets, ALT, AST, IL-10, IL-19, EMAP-II). Analyses were done between more than two groups using one way ANOVA test for quantitative data between groups, followed by post HOC Tukey's correction between each two groups. Analyses were done between the two groups using the independent sample t test for quantitative data, Chi square test was employed for qualitative data between groups. The correlation between two quantitative variables was performed by using Pearson's correlation coefficient, and non-parametric Spearman's correlation coefficient for non-parametric ordinal data. Correlation coefficient ranges from (0-1): - weak (r=0-0.24), fair (r=0.25-0.49), moderate (r=0.5-0.74), strong (r=0.75-1).

Results

Patients' characteristics

Patients' characteristics are listed in Table 1. The average age of the group was 46 years (range 13-75). In total, 64 patients (38 male, 26 female) were included. For 23 patients (35.9%), blood samples were obtained at initial diagnosis of the lymphoma. Nine patients (14.1%) were in complete remission of the lymphoma. 10 patients (15.6%) experienced a partial remission and 22 (34.4%) were in relapse. Patients and controls were assessed for their main clinical and laboratory characteristic. There were significant differences in regard to haemoglobin (Hb), platelets, AST, ALT, LDH, splenomegaly, hepatomegaly, lymph node enlargement (P<0.001) and creatinine (P=0.010) (Supplementary Table 1).

The Patients' characteristics in the different groups are summarized in Table 2. There was a significant difference in white blood cells (WBCs) (P=0.016), but not with other features (P>0.05). There was a significant difference in CD 23 (P=0.027) when comparing patients with relapse versus those in complete remission, but not with other immunophenotyping markers (Supplementary Table 2).

Serum EMAP-II levels in patients with NHL

Patients with NHL had significantly higher serum EMAP-II levels than the control group (P<0.001) (Figure 1A). The mean serum EMAP-II level was 8379.37 ± 5223.75 pg/ml (470-27800) in the patient group and 102.66 ± 13.38 pg/ml (74-121) in the control group. EMAP-II showed significant negative correlation with WBCs (P=0.026), but not with other features (P>0.05) (Supplementary Table 3).

Next, serum EMAP-II levels were compared in different groups of

Clinico-pathological features of NHL patients	Descriptive statistics
Age	
Range	(13-75)
Mean ± SD	46.64 ± 15.11
Sex	
Male	38 (59.4%)
Female	26 (40.6%)
Response to Treatment	
No treatment	23 (35.9%)
Complete remission	9 (14.1%)
Partial remission	10 (15.6%)
Relapse	22 (34.4%)
NHL histologic subtype	
B	52 (81.3%)
T	12 (18.8%)
B-NHL histologic subtype	
FCL	10 (19.2%)
DLCL	23 (44.2%)
CLL	14 (26.9%)
SCL	1 (1.9%)
Others	4 (7.7%)
Hepatomegally	
No	28 (43.8%)
Yes	36 (56.2%)
Spleenomegally	
No	23 (35.9%)
Yes	41 (64.1%)
LN enlargement	
No	0 (0%)
Yes	64 (100%)

NHL: Non-Hodgkin's Lymphoma; FCL: Follicular Cell Lymphoma; DLCL: Diffuse Large B-cell Lymphoma; CLL: Chronic Lymphocytic Leukaemia; SCL: Small Lymphocytic Lymphoma; LN: Lymph Node.

Table 1: Characteristics of 64 patients with NHL.

	Group I No treatment (n=23)	Group II CR (n=9)	Group III PR (n=10)	Group IV Relapse (n=22)	P value					
Age					0.702					
Range	(14-75)	(20-63)	(37-65)	(13-70)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	45.04 ± 17.94	49.44 ± 13.93	50.7 ± 7.33	45.31 ± 15.32	0.884	0.764	1	0.998	0.904	0.793
Sex					0.365					
Male	12 (52.2%)	4 (44.4%)	8 (80%)	14 (63.6%)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Female	11 (47.8%)	5 (55.6%)	2 (20%)	6 (36.4%)	1	0.245	0.550	0.170	0.433	0.440
Hb					0.766					
Range	(7.4-14)	(9-13.5)	(7.4-14.1)	(8.5-15.8)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	11.39 ± 1.89	11.47 ± 1.83	11.65 ± 2.32	12 ± 1.92	1	0.986	0.733	0.998	0.908	0.966
WBCs					**0.016***					
Range	(2-10)	(6.1-20)	(1.5-18.9)	(2.1-13.9)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	5.25 ± 2.36	9.76 ± 5.06	8.45 ± 5.15	5.91 ± 3.11	**0.020***	0.242	0.946	0.744	0.059	0.473
Platelets					0.603					
Range	(50-455)	(60-275)	(150-512)	(62-651)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	231.43±114.41	181.22±83.36	251.2±118.81	240.54±148.09	0.755	0.915	1	0.532	0.770	0.922
AST					0.291					
Range	(14-566)	(17-163)	(13-77)	(14-241)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	69.86 ± 114.39	43.77 ± 45.19	33.3 ± 24.11	57.04 ± 48.83	0.885	0.393	0.991	0.902	0.781	0.281
ALT					0.781					
Range	(11-232)	(12-217)	(11-94)	(2.5-170)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	56.82 ± 51.53	47.44 ± 64.36	40.6 ± 31.17	53.65 ± 40.16	0.848	0.842	0.991	1	0.936	0.936
LDH					0.160					
Range	(274-1138)	(300-648)	(268-1416)	(277-940)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	677±245.96	479.44±118.89	606.7±374.11	555.27±158.36	0.174	0.486	0.440	0.933	0.792	0.995
Urea					0.186					
Range	(14-43.5)	(23-40)	(24-51)	(17.5-65)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	27.22 ± 9.47	31.88 ± 5.55	32.64 ± 7.93	34.1 ± 14.04	0.688	0.547	0.150	0.999	0.954	0.984
Creatinine					0.535					
Range	(0.5-1.3)	(0.6-1.1)	(0.5-1.4)	(0.5-2.7)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Mean ± SD	0.77 ± 0.24	0.76 ± 0.18	0.93 ± 0.31	0.87 ± 0.45	1	0.629	0.745	0.720	0.839	0.978
Hepatomegally					0.612					
No	8 (34.8%)	4 (44.4%)	6 (60%)	10 (45.5%)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Yes	15 (65.2%)	5 (55.6%)	4 (40%)	12 (54.5%)	0.696	0.257	0.550	0.656	1	0.704
Spleenomegally					0.930					
No	8 (34.8%)	4 (44.4%)	3 (30%)	8 (36.4%)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Yes	15 (65.2%)	5 (55.6%)	7 (70%)	14 (63.6%)	0.696	1	1	0.650	0.704	1
Enlarged LN					1					
No	0 (0%)	0 (0%)	0 (0%)	0 (0%)	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
Yes	23 (100%)	9 (100%)	10 (100%)	22 (100%)	1	1	1	1	1	1

Group I : Newly diagnosed patients with no treatment; Group II : Patients with complete remission (CR); Group III : Patients with partial remission (PR); Group IV: Patients in relapse. P1 (Group I vs Group II); P2 (Group I vs Group III); P3 (Group I vs Group IV); P4 (Group II vs Group III); P5 (Group II vs Group IV); P6 (Group III vs Group IV). Statistically significant differences (P ≤ 0.05) are indicated with an asterisk (*).
NHL: Non-Hodgkin's Lymphoma; N: Number; CR: Complete Remission; PR: Partial Remission; Hb: Haemoglobin; WBCs: White Blood Cells; AST: Asparatate Aminotransferase; ALT: Alanine Aminotransferase; LDH: Lactate Dehydrogenase; LN: Lymph Node

Table 2: NHL Patients' characteristics in the different groups.

patients. The newly diagnosed patients showed serum EMAP-II levels ranging from 5900-27800 pg/ ml with a mean of 12475.65 ± 5164.58 pg/ ml. Patients with CR had EMAP-II values ranging from 470 to 4400 pg/ ml with a mean of 2096.66 ± 1354.81 pg/ml, and patients with partial remission had EMAP-II levels ranging from 4400 - 14800 pg/ml with a mean of 7730 ± 3533.34 pg/ml. Samples of patients who were in relapse were also analyzed. Their EMAP-II values ranged from 3900-16000 pg/ml with a mean of 6962.27 ± 3204.85 pg/ml (Figure 1B). P values were highly significant (P<0.001) in newly diagnosed patients when compared with CR, and relapse. In the same manner, p values were significant (P<0.001) in CR when compared with partial remission and relapse in NHL patients. There was a significant difference between EMAP-II levels in CR compared to PR (P=0.027).

The patients were split into high–EMAP-II groups (≥ 7000 pg/ ml vs. <7000 pg/ml). High serum EMAP-II levels were found in newly diagnosed patients not yet treated (P<0.001). There was a significant correlation between high serum EMAP-II levels and ALT (P=0.043) but not with other features (P>0.05) (Table 3). There was no correlation between high EMAP-II levels and immunophenotyping markers (Supplementary Table 4). Those with CR and in relapse had a significantly lower value of EMAP-II (P=0.002, P=0.017) respectively.

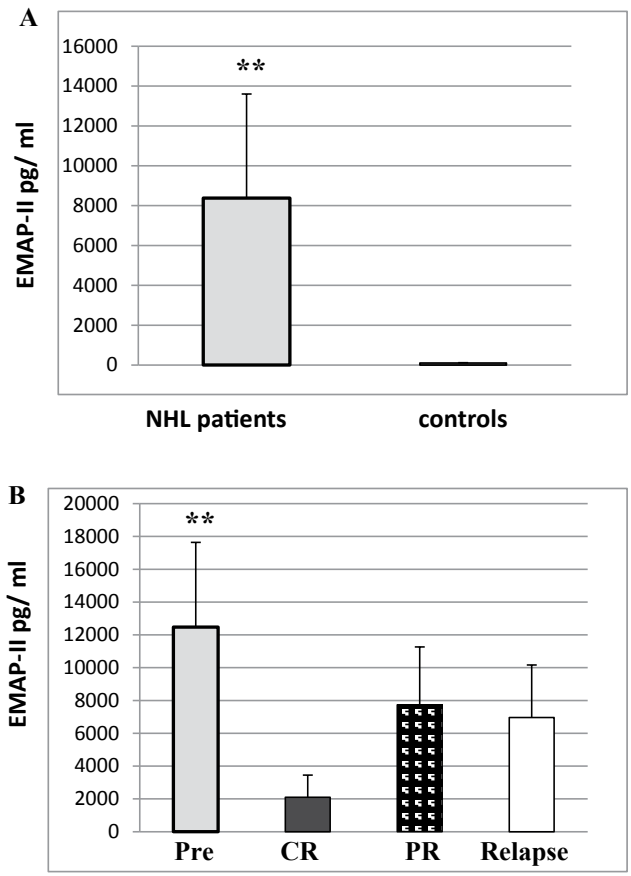

Figure 1: EMAP-II levels in NHL patients. **(A)** Serum EMAP-II level was measured and compared in NHL patients (n=64) and controls (n=30). Serum level of EMAP-II was significantly higher in NHL patients than in healthy controls (¨ P<0.001). **(B)** Serum EMAP-II levels in different groups of NHL patients. EMAP-II levels were significantly higher in pretreatment group compared to other groups (¨ P<0.001). NHL: Non-Hodgkin's Lymphoma; EMAP-II: Endothelial Monocyte Activating-Polypeptide-II; Pre: Pretreatment; CR: Complete Remission; PR: Partial Remission.

In 32 patients, we found levels of EMAP-II ≥ 7000 pg / ml in 22 patients (68.8%) in group I, 4 patients (12.5%) in group III, and 6 patients (18.8%) in group IV. In 32 NHL patients, we found levels of EMAP-II <7000 pg/ml in 1 (3.1%) patient in group I, in 9 (28.1%) patients in group II, in 6 (18.8%) patients in group III and 16 (50%) patients in group IV (Table 3).

Serum IL-19 levels in NHL

We compared the level of IL-19 in sera of patients and controls. There was a significantly higher mean serum IL-19 level in NHL patients (mean: 151.25 ± 140.41 pg/ml; range: 14-693 pg/ml) compared to the control group (mean: 7.99 ± 2.87 pg/ml; range: 2-13 pg/ml; P<0.001; Figure 2A). Interestingly, our results showed IL-19 in NHL was positively associated with AST (P=0.032), Bcl-2 (P=0.024), and negatively correlated with CD23 (P=0.015) (Supplementary Table 5).

Serum IL-19 levels were compared in the different groups. The newly diagnosed patients showed serum IL-19 levels ranging from 28-693 pg/ml with a mean of 99.73 ± 145.92 pg/ml. Patients with CR had IL-19 values ranging from 14 to 229 pg/ml with a mean of 75.22 ± 76.55 pg/ml. Patients with partial remission had IL-19 levels ranging from 40-271 pg/ml with a mean of 118.2 ± 84.03 pg/ml. IL-19 values in relapse ranged from 151-693 pg/ml with a mean of 251.22 ± 123.33 pg/ml (Figure 2B). P values were highly significant (P<0.001) in patients with relapse when compared with CR, and patients with no treatment. There was a significant difference between IL-19 levels in relapse compared to PR (P=0.006).

	EMAP-II		P value
	Low EMAP-II (n= 32)	High EMAP-II (n=32)	
Age Range Mean ± SD	(13-68) 45.96 ± 14.18	(14-75) 47.31 ± 16.19	0.725
Sex Male Female	20(62.5%) 12(37.5%)	18(56.2%) 14(43.8%)	0.611
Response to treatment No treatment Complete remission Partial remission Relapse	1(3.1%) 9(28.1%) 6(18.8%) 16(50%)	22(68.8%) 0(0%) 4(12.5%) 6(18.8%)	**<0.001**** **0.002*** **0.732** **0.017***
B-NHL histologic subtype FCL DLCL CLL SCL Others	4(17.4%) 9(39.1%) 7(30.4%) 1(4.3%) 2(8.7%)	6(20.7%) 14(48.3%) 7(24.1%) 0(0%) 2(6.9%)	0.769
NHL histologic subtype B T	23(71.9%) 9(28.1%)	29(90.6%) 3(9.4%)	0.055
Hb Range Mean ± SD	(7.4-14.9) 11.67 ± 1.87	(7.4-15.8) 11.63 ± 22	0.934
WBCs Range Mean ± SD	(1.5-20) 7.41 ± 4.5	(2-15.2) 5.81 ± 2.99	0.157
Platelets Range Mean ± SD	(60-651) 233.81 ± 124.63	(50-512) 227.37 ± 124.8	0.683
AST Range Mean ± SD	(13-163) 41.65 ± 32.96	(14-566) 70.5 ± 102.15	**0.102**
ALT Range Mean ± SD	(2.5-217) 42.23 ± 41.35	(11-232) 61.53 ± 49.93	0.043*
LDH Range Mean ± SD	(268-1416) 568.93 ± 237.38	(274-1138) 623.84 ± 237.33	0.358
Urea Range Mean ± SD	(17-65) 33.6 ± 11.88	(14-50) 28.58 ± 9.37	0.065
Creatinine Range Mean ± SD	(0.5-2.7) 0.87 ± 0.4	(0.5-1.3) 0.8 ± 0.23	0.400
Hepatomegally No Yes	16(50%) 16(50%)	12(37.5%) 20(62.5%)	0.313
Spleenomegally No Yes	14(43.8%) 18(56.2%)	9(28.1%) 23(71.9%)	0.193
LN enlargement No Yes	0 (0%) 32 (100%)	0 (0%) 32 (100%)	1

Low EMAP-II was < 7000 pg/ml; high, ≥ 7000 pg/ml. Statistically significant differences (P ≤ 0.05) are indicated with an asterisk (*). Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**).
NHL, Non-Hodgkin's lymphoma; N, number; EMAP-II, endothelial monocyte activating-polypeptide-II; FCL, Follicular cell lymphoma; DLCL, diffuse large B - cell lymphoma; CLL, chronic lymphocytic leukaemia; SCL, small lymphocytic lymphoma; Hb, haemoglobin; WBCs, white blood cells; AST, asparatate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; LN, lymph node.

Table 3: Correlation of low versus high EMAP-II levels and NHL patient disease characteristics.

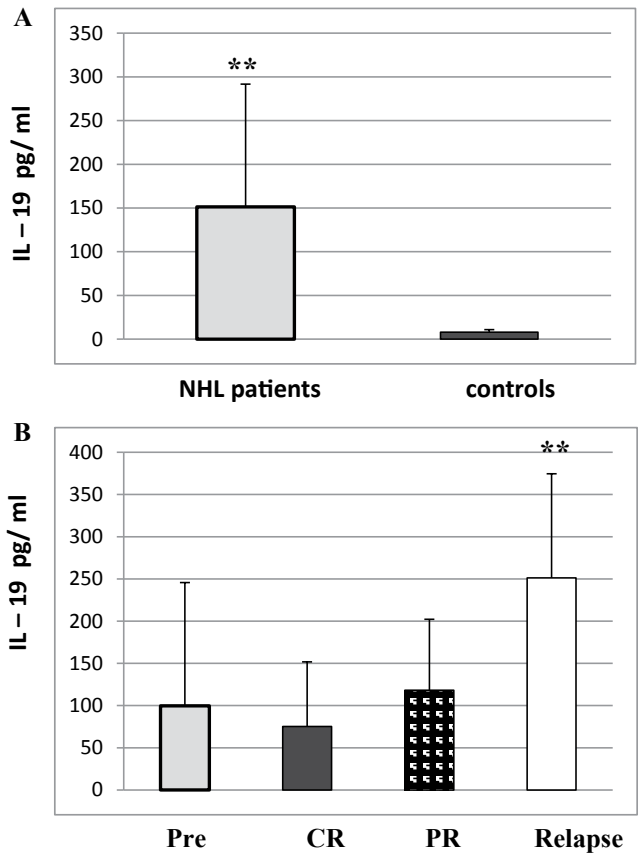

Figure 2: IL-19 levels in NHL patients. **(A)** SerumIL-19 level was measured and compared in NHL patients (n=64) and controls (n=30). Serum level of IL-19 was significantly higher in NHL patients than in healthy controls (** P<0.001). **(B)** Serum IL-19 levels in different groups of NHL patients. IL-19 levels were significantly higher in patients with relapse compared to other groups (**P<0.001). NHL: Non-Hodgkin's Lymphoma; IL-19: Interleukin-19; Pre: Pretreatment; CR: Complete Remission; PR: partial remission.

	IL-19		P value
	Low IL-19 (n=33)	High IL-19 (n=31)	
Age Range Mean ± SD	(14-75) 46.78 ± 16.19	(13-70) 46.48 ± 14.14	0.937
Sex Male Female	18(54.5%) 15(45.5%)	20(64.5%) 11(35.5%)	0.417
Response to treatment No treatment Complete remission Partial remission Relapse	20(60.6%) 7(21.2%) 6(18.2%) 0(0%)	3(9.7%) 2(6.5%) 4(12.9%) 22(71%)	**<0.001**** **0.150** **0.734** **<0.001****
B-NHL histologic subtype FCL DLCL CLL SCL Others	3(10.3%) 14(48.3%) 9(31%) 1(3.4%) 2(6.9%)	7(30.4%) 9(39.1%) 5(21.7%) 0(0%) 2(8.7%)	0.380
NHL histologic subtype B T	29(87.9%) 4(12.1%)	23(74.2%) 8(25.8%)	0.161
Hb Range Mean ± SD	(7.4-14.1) 11.76 ± 1.94	(8.5-15.8) 11.53 ± 1.95	0.646
WBCs Range Mean ± SD	(1.5-15.8) 6.41 ± 2.91	(2-20) 6.82 ± 4.73	0.803
Platelets Range Mean ± SD	(50-455) 237.39 ± 100.32	(60-651) 223.35 ± 145.41	0.287
AST Range Mean ± SD	(13-163) 36.3 ± 29.57	(14-566) 77.12 ± 102.61	**0.002***
ALT Range Mean ± SD	(11-217) 42.66 ± 41.47	(2.5-232) 61.69 ± 50.15	0.131
LDH Range Mean ± SD	(268-1416) 619.36 ± 272.66	(277-1138) 571.93 ± 193.75	0.428
Urea Range Mean ± SD	(14-51) 29.39 ± 9.51	(17.5-65) 32.89 ± 12.12	**0.203**
Creatinine Range Mean ± SD	(0.5-1.4) 0.78 ± 0.24	(0.5-2.7) 0.89 ± 0.39	0.186
Hepatomegally No Yes	14(42.4%) 19(57.6%)	14(45.2%) 17(54.8%)	0.825
Spleenomegally No Yes	14(42.4%) 19(57.6%)	9(29%) 22(71%)	0.264
LN enlargement No Yes	0 (0%) 33 (100%)	0 (0%) 31 (100%)	1

Low IL-19 was < 100 pg/ml; high, ≥ 100 pg/ml; Statistically significant differences (P ≤ 0.05) are indicated with an asterisk (*). Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**). NHL, Non-Hodgkin's lymphoma; IL-19, interleukin-19; N; number; FCL, Follicular cell lymphoma; DLCL, diffuse large B - cell lymphoma; CLL, chronic lymphocytic leukaemia; SCL, small lymphocytic lymphoma; Hb, haemoglobin; WBCs, white blood cells; AST, asparatate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; LN, lymph node

Table 4: Correlation of low versus high IL-19 levels and NHL patient disease characteristics.

The patients were split into high IL-19 groups (≥ 100 pg/ml vs. <100 pg/ml). High serum IL-19 levels were found in patients in relapse (P<0.001). There was a significant correlation between high serum IL-19 levels and AST (P=0.002) but not with other features (P>0.05) (Table 4). Newly diagnosed patients not yet treated had a significantly lower value of IL-19 (P<0.001).

In 31 patients, we found levels of IL-19 ≥ 100 pg /ml in 22 patients (71%) in group IV, 3 patients (9.7%) in group I, 2 patients (6.5%) in group II, and 4 patients (12.9%) in group III. In 33 NHL patients, we found levels of IL-19 <100 pg /ml in 20 (60.6%) patients in group I, in 7 (21.2%) patients in group II, in 6 (18.2%) patients in group III (Table 4).

Serum IL-10 levels in NHL

The mean serum values for IL-10 in patients and controls, respectively, were: 957.79 ± 630.58 pg/ml (range, 98-2870) compared with 10.04 ± 2.97 pg/ml (range, 0.5–14) (P<0.001) (Figure 3A). Increased levels of IL-10 expression were significantly negatively correlated with WBCs (P=0.02), but not with other parameters (Supplementary Table 6).

Serum IL-10 was detected in patients with no treatment, and the mean concentration was 1524 ± 651.29 pg/ml (range 716-2870 pg/ml), which was significantly higher than that of CR patients with a mean of 335.77 ± 137.71 pg/ml (range 98 – 523) pg/ml. Patients with partial remission had IL-10 levels ranging from 435 to 1520 pg/ml with a mean of 740.2 ± 331.3 pg/ml. Patients in relapse had IL-10 levels ranging from 439-1650 pg/ml with a mean of 719.22 ± 308.48 (Figure 3B). P values

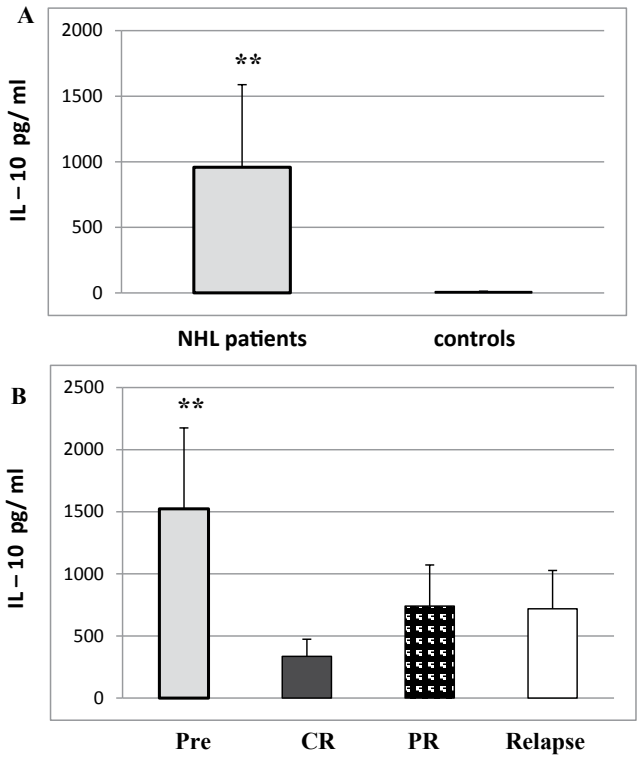

Figure 3: IL-10 levels in NHL patients. **(A)** Serum IL-10 level was measured and compared in NHL patients (n=64) and controls (n=30). Serum level of IL-10 was significantly higher in NHL patients than in healthy controls (** P<0.001). **(B)** Serum IL-10 levels in different groups of NHL patients. IL-10 levels were significantly higher in pretreatment group compared to other groups (** P<0.001). NHL: Non-Hodgkin's Lymphoma; IL-19: Interleukin-19; Pre: Pretreatment; CR: Complete Remission; PR: Partial Remission.

were highly significant (P<0.001) in patients with no treatment when compared with CR, partial remission and patients with relapse.

The patients were split into high IL-10 groups (≥ 700 pg/ml vs. <700 pg/ml). High serum IL-10 levels were found in newly diagnosed patients not yet treated (P<0.001). There was a significant correlation between high serum IL-10 levels and blood urea (P=0.020) but not with other features (P>0.05) (Table 5). There was no correlation between high EMAP-II levels and immunophenotyping markers (Supplementary Table 7). Those with CR and in relapse had a significantly lower value of IL-10 (P=0.002, P=0.003) respectively.

We found levels of IL-10 ≥ 7000 pg / ml in 23 patients (71.9%) in group I, 4 patients (12.5%) in group III, and 5 patients (15.6 %) in group IV. In 32 NHL patients, we found levels of EMAP-II <7000 pg/ml in 9 (28.1%) patient in group II, in 6 (18.8%) patients in group III, in 17 (53.1%) patients in the group with IV (Table 5).

Association between EMAP-II, IL-19 and IL-10 in NHL

Interestingly, the results showed that IL-10 in NHL was positively associated with EMAP-II (r=0.827; P<0.001) (Figure 4). No association was found between IL-19 and IL-10 or EMAP-II (r=0.125; P=0.325).

Discussion

It has been postulated that cytokines play important roles in the pathogenesis of lymphomas [36] and disease progression [37]. Serum concentration of the cytokines may be used as a marker of prognosis in cancer [5]. In this study, we chose to measure serum EMAP-II, IL-19, and IL-10 levels in NHL patient. There have been no studies on EMAP-II and IL-19 that analyzed the serum levels of these cytokines in NHL patients.

	IL-10		P value
	Low IL-10 (n=32)	High IL- (n=32)	
Age			
Range	(13-68)	(14-75)	0.877
Mean ± SD	46.93 ± 13.53	46.34 ± 16.75	
Sex			
Male	20(62.5%)	18(56.2%)	0.611
Female	12(37.5%)	14(43.8%)	
Response to treatment			
No treatment	0(0%)	23(71.9%)	**<0.001****
Complete remission	9(28.1%)	0(0%)	**0.002***
Partial remission	6(18.8%)	4(12.5%)	**0.732**
Relapse	17(53.1%)	5(15.6%)	**0.003***
B-NHL histologic subtype			
FCL	4(16.7%)	6(21.4%)	0.828
DLCL	10(41.7%)	13(46.4%)	
CLL	7(29.2%)	7(25%)	
SCL	1(4.2%)	0(0%)	
Others	2(8.3%)	2(7.1%)	
NHL histologic subtype			
B	24(75%)	28(87.5%)	0.200
T	8(25%)	4(12.5%)	
Hb			
Range	(7.4-15.8)	(7.4-15.1)	0.707
Mean ± SD	11.74 ± 1.99	11.56 ± 1.91	
WBCs			
Range	(1.5-20)	(2-15.2)	0.106
Mean ± SD	7.47 ± 4.46	5.75 ± 31	
Platelets			
Range	(60-651)	(50-512)	0.813
Mean ± SD	230.25 ± 122.91	230.93 ± 125.88	
AST			
Range	(13-163)	(14-566)	0.110
Mean ± SD	41.75 ± 32.92	70.41 ± 102.19	
ALT			
Range	(2.5-217	(11-232)	0.058
Mean ± SD	42.67 ± 41.26	619 ± 50.18	
LDH			
Range	(268-1416)	(274-1138)	0.238
Mean ± SD	561.21 ± 235.98	631.56 ± 236.62	
Urea			
Range	(18.7-65)	(14-50)	**0.020***
Mean ± SD	34.22 ± 11.5	27.95 ± 9.46	
Creatinine			
Range	(0.5-2.7)	(0.5-1.3)	0.400
Mean ± SD	0.87 ± 0.4	0.8 ± 0.23	
Hepatomegally			
No	15(46.9%)	13(40.6%)	0.614
Yes	17(53.1%)	19(59.4%)	
Spleenomegally			
No	14(43.8%)	9(28.1%)	0.193
Yes	18(56.2%)	23(71.9%)	
LN enlargement			
No	0 (0%)	0 (0%)	1
Yes	32 (100%)	32 (100%)	

Low: IL-10 was <700 pg/ml; High: ≥ 700 pg/ml; Statistically significant differences (P ≤ 0.05) are indicated with an asterisk (*). Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**). NHL: Non-Hodgkin's Lymphoma; N: Number; IL-10: Interlekin-10; FCL: Follicular Cell Lymphoma; DLCL: Diffuse Large B-cell Lymphoma; CLL: Chronic Lymphocytic Leukaemia; SCL: Small Lymphocytic Lymphoma; Hb: Haemoglobin; WBCs: White Blood Cells; AST: Asparatate Aminotransferase; ALT: Alanine Aminotransferase; LDH: Lactate Dehydrogenase; LN: Lymph Node.

Table 5: Correlation of low versus high IL-10 levels and NHL patient disease characteristics.

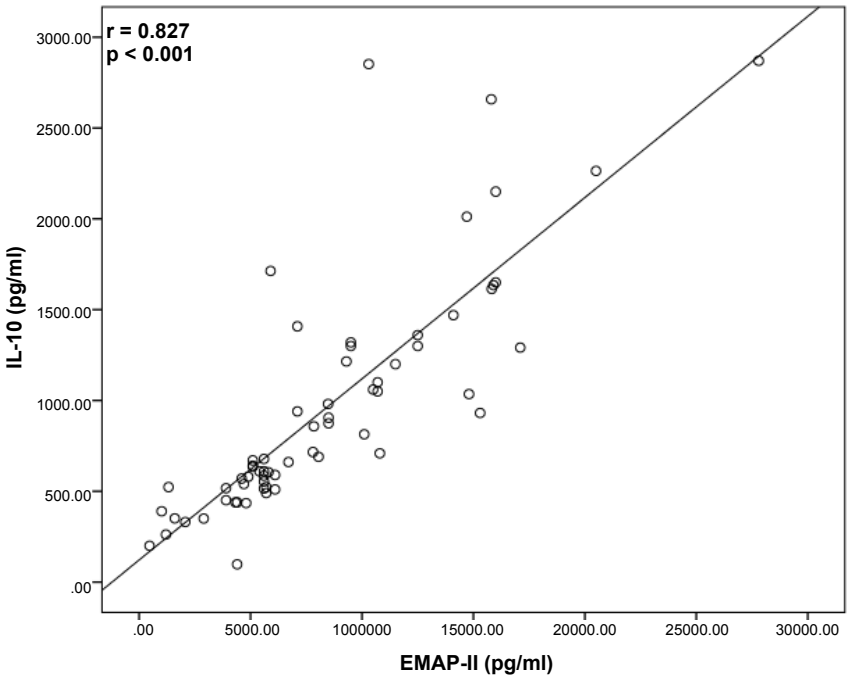

Figure 4: Association between serum EMAP-II and IL-10 in NHL patients. Significant strong positive correlation between serum levels of EMAP-II and IL-10 in NHL patients. r=0.75-1 (strong correlation); P-value<0.001; NHL: Non-Hodgkin's Lymphoma; EMAP-II: Endothelial Monocyte Activating Polypeptide-II.

The present study showed elevated serum levels of EMAP-II in patients with NHL. In a previous study, serum EMAP-II levels were significantly higher in patients than in controls [13]. There were no significant associations between serum EMAP-II levels and various clinicopathologic and phenotypic parameters which are consistent with previously reported findings [13]. However, Serum EMAP-II levels showed a significant negative correlation with WBCs. Higher EMAP-II level ≥ 7000 pg/ml showed significant correlation with ALT. Patients who achieved CR had a lower serum EMAP-II level than those who achieved PR or did not respond to therapy. This notice is consistent with that of Sen et al. [13], who reasoned that high serum EMAP levels is of possible predictive value. In the present study, an association between higher pretreatment serum levels of EMAP-II in newly diagnosed patients with NHL was observed. These data suggest the idea that EMAP-II may have a role as a biomarker of treatment response and outcome in NHL. Elevation of EMAP-II levels in patients with NHL might be linked to factors controlling its release, such as hypoxia, as a result of vascular insufficiency [22].

There was a high IL-19 concentration in sera of patients with NHL compared to controls. Our results seemed to be uniform with other works which showed upregulation of IL-19 in tumors compared to normal tissues and an association of high IL-19 expression in tumor tissues with advanced tumor stage [14].

In the present study, patients had a high level IL-19 level at the time of diagnosis, which was associated with higher AST levels, CD23, and Bcl2. In former studies, Bcl-2 and CD23 positive patients were related with poor prognosis [38,39]. In our present subject, IL-19 levels were statistically significantly higher in patients with relapse. This determination was confirmed by other studies which demonstrated the association between high IL-19 expression and high tumor metastasis and poor clinical outcome [14,40]. This involves the meaning of using IL 19 for the early detection of recurrence, suggesting earlier treatment and longer endurance.

Former subjects have shown that increased serum IL-10 levels influence the forecast of various subtypes of lymphoma [17,41,42]. The present study showed elevated serum levels of IL-10 in patients with NHL at diagnosis when likened with the levels in healthy controls, and after treatment, those levels decreased substantially which is in conformity with previous results [41]. The drop in IL-10 levels after treatment can be explained by a therapy-induced disruption in IL-10 signalling. IL-10 may affect antitumor immunity and, proliferation and resistance to apoptosis [27]. So, it is easy to explain the correlation between IL-10 treatment response.

In this study, none of the parameters analyzed was significantly correlated with serum IL-10 levels. Even so, we found a statistically significant correlation between serum IL-10 levels and WBCs levels and blood urea level. Furthermore, serum IL-10 levels showed a significant correlation with the response to therapy. Patients who achieved CR had a lower median serum IL-10 level than those who achieved PR or with relapse. This observation confirmed that IL-10 is a strong predictor of treatment response in NHL, suggesting a key role for IL-10 in the pathogenesis and progression of this disease.

In this article, a strong association was demonstrated between EMAP-II and IL-10 in NHL. This finding is supported by higher serum levels of EMAP-II and IL-10 before therapy and its decrease significantly thereafter in all patients.

In summary, it was observed that pretreatment serum levels of EMAP-II and IL-10 were associated with relevant laboratory findings in patients with NHL and that these cytokines decreased significantly after treatment for the disease. Higher IL-19 serum levels seem to be associated with treatment failure and relapse. We, therefore, suggest, introducing them into routine panel, for the useful follow up of NHL patients, assessment of their response to therapy and early detection of recurrence for an improved survival.

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