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A Study of Bone Marrow Levels of CD44, IL3 and IL6 in Acute Leukemia and Non-Hodgkin's Lymphoma at Diagnosis and During Remission

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ABSTRACT

The aim of this study was to clarify the diagnostic and prognostic aspect of bone marrow microenvironment elements in Acute leukemia and Non-Hodgkin's lymphoma. In this study, bone marrow plasma soluble CD44 (sCD44) and cytokines IL3 and IL6 were measured by enzyme-linked immunosorbent assay in 20 NHL and 40 Acute leukemia patients at diagnosis and after remission, and 10 bone marrow donors for transplantation as control. All the cases were subjected to clinical assessment, laboratory tests including bone marrow aspiration, immunophenotyping, cytochemistry and trephine biopsy.

Results: The BM sCD44 mean level was significantly elevated in patients with NHL (426.9±146ng/ml) and Acute leukemia (938.5±203.17ng/ml) reaching 2 folds the mean in NHL, while it decreased to near normal levels after remission with no statistical difference when compared to control group. In NHL patients, BM sCD44 >280ng/mL were associated with 2.8 and 4.7 folds higher frequencies of BM invasion and B-symptoms respectively at diagnosis. In NHL, the mean values of BM cytokines (IL-6 and IL3) were significantly increased in: Patients with B-symptoms, in high grade lymphoma, BM involvement and high IPI. Also, in acute leukemia, the mean values of BM cytokines (IL-6 and IL3) were found to be elevated at diagnosis and decreased to near normal during remissions.

Conclusions: It was concluded that sCD44 is useful in evaluating NHL and Acute leukemia disease activity, extent and response to treatment and can be used as a prognostic marker in these patients. Also, it was concluded that BM IL3 and IL6 are good indicators of disease activity and regression, so they can be considered good diagnostic and prognostic factors.

Key Words: *BM microenvironment - Extracellular matrix ECM - CD44 - IL-3, IL-6 - NHL.*

INTRODUCTION

The bone marrow microenvironment supports growth and differentiation of normal hematopoietic cells and can contribute to malignant growth. Since malignant cells localize and accumulate in bone marrow, it is important to understand the influence of the bone marrow microenvironment not only on the growth of the malignant cells, but also on the therapeutic response of malignant cells [1].

Studies have demonstrated that haematopoiesis depends not only on specific cytokines (secreted by stromal cells) but also by adhesion molecules, that allow different cell types to adhere stably to one another or to ECM [2].

In the hematopoietic system, adhesion and migration are essential activities for normal development and function of hematopoietic cells [3].

The lymphocyte homing receptor, CD44, is a polymorphic glycoprotein with a molecular mass ranging from 85 to 250 kda. It represents a family of glycoproteins encoded by a single gene on the short arm of chromosome 11p13 that contains 20 exons [4]. Ten additional exons can be alternatively introduced into a common splice site in different combinations creating various splice variants designated as CD44v [5,6]. The standard form (commonly referred to CD44H or CD44S) of the molecule lacks all variant exons and is expressed on cells of hemopoietic and mesodermal origin [7].

Soluble CD44 has been detected in serum, lymph, arthritic synovial fluid, and bronchoalveolar lavage. Malignant disease and immune activation and inflammation are often associated with increased plasma levels of sCD44, whereas

immunodeficiency correlates with low plasma levels of sCD44 [8].

A high serum sCD44 level and/or tumour cells expression at diagnosis is associated with prognostic criteria and/or unfavourable outcome in childhood lymphoblastic leukemia/lymphoma and not in acute myeloid leukemia [9]. Also a high serum level of sCD44 was reported to be correlated with a poor outcome of aggressive NHL suggesting that it could be a useful prognostic marker [10].

Cytokines are glycoproteins that regulate all the important biological processes including cell growth, cell activation, inflammation, immunity, tissues repair, fibrosis and morphogenesis. Cytokines that influence hematopoiesis can be divided into three categories; direct acting e.g. IL3, IL6, GM-CSF on multipotent progenitors, indirect acting e.g. IL1 and TNF on stromal cells and miscellaneous e.g IL8, IL9, IL10, IL11 and IL12 [11].

This study is conducted to investigate the role of sCD44, IL3 and IL6 in bone marrow microenvironment in acute leukemia and Non Hodgkin's lymphoma at diagnosis and remission after treatment, and to study their use as prognostic markers for disease activity and effect of therapy on BMM.

MATERIAL AND METHODS

Materials:

Study samples were divided into two groups of patients at diagnosis and during remission and controls. They were divided as follows:

Group (Ia): 40 patients with de novo diagnosis of acute leukemia (24 males and 16 females) with a mean age 35 years (range 8-70). Patients of this group were subdivided according to FAB Classification into: (a) ALL (6 cases L_1 and 9 cases L_2) (b) AML (4 cases M_1-7 cases M_2-3 cases M_3-4 cases M_4-3 cases M_5 and - 4 cases M_7).

Group (Ib): Group Ia (35 out of 40 patients) during remission, after induction course of chemotherapy (21 males and 14 females), age range 8-60 years. 5 cases died during follow up (2 cases M_7 & 3 cases M_3).

Group (IIa): 20 patients with de novo diagnosis of Non-Hodgkin's lymphoma (13 males and 7 females with a mean age 50 years (range 20-72). Patients of this group were subdivided after immunophenotyping into B-NHL (16/20) and T-NHL (4/20).

Group (IIb): The same 20 patients of Group IIa during remission.

Group (III) Control group: 10 healthy subjects (8 males and 2 females) with mean age 29 years (range 20-38 years), selected from bone marrow transplantation donors. All the cases were selected from National Cancer Institute and Nasser Institute from 2002 to 2003.

Methods:

Sample collection: Peripheral blood samples were collected for routine lab investigations. (CBC, ESR, LFTs and KFTs). Serum samples were separated and used for the determination of LDH and B2 microglobulin (for NHL cases).

Bone marrow aspiration: This was done for all patients and controls by the standard technique [12]. Diagnosis was done based on morphology and cytochemistry. The remaining aspirate was transferred into a tube containing heparin and centrifuged at 1000xg/ for 10min and plasma was divided into sterile aliquots and stored at -70°C till use.

Immunophenotyping: This was done for all cases of Acute leukemia and NHL by flow cytometry using fluorescent labeled antibodies according to Landy and Muirhead 1989 [13].

Bone marrow trephine biopsy:

BM biopsy was done to fulfill the diagnosis whenever indicated (done only for 10 cases of ALL, 9 cases of AML and all cases of NHL) to study the stromal cells and for staging of NHL, using the standard technique according to Williams and Nicholson, 1963 [14].

Investigations:

1- Serum LDH: Was determined by the kinetic assay on the Beckman-Synchron CX R Systems Chemistry Information (LD-P) kit by Handerson, 1995 [15].

2- Serum B2 microglobulin: Was determined by an immunometric enzyme immunoassay EISA for the quantitative determination in human serum, plasma or urine [16]. The kit produced by ORGEN Tec. Diagnostila GMBH, (Mainz, Germany). The amount of color

is directly proportional to the concentration of Beta-2 microglobulin present in the original sample.

3- Assay of IL-3: Was performed in bone marrow plasma by ELISA technique for quantitative determination of human IL-3 by Fishman, 1990 [17] (manufactured by BioSource International, California, USA). IL3 is expressed in (pg/ml).

4- Assay of IL6: Was measured in bone marrow plasma by a two step sandwich ELISA technique manufactured by Diaclone Research, 2000 [18], (FRANCE) for in-vitro quantitative determination. IL6 is expressed in (pg/ml).

5- Assay of CD44: Was measured in bone marrow plasma by a two step sandwich ELISA technique manufactured by Diaclone Research 2000 [18], (FRANCE) for in-vitro quantitative determination. CD44 is expressed in (ng/ml).

All assays were performed as per the manufacture's instructions. Each sample was assayed in duplicate.

Statistical analysis: [19]

The collected data were tabulated and statistically analyzed (Minitab SPSS statistical software version, 1998) :12-1. For quantitative data, the range, mean and standard deviation were calculated.

The difference between two means was statistically analyzed using the students (t) test. Paired t test was performed to test mean values at diagnosis and at remission.

Chi-square as a non parametric test was used to assess the statistical significance of associations among categorical variables when assumptions for its application were fulfilled.

Pearson correlation coefficient (r) was used to assess the statistical significance of correlation among normally distributed quantitative variables the value of r ranges from –1 to +1, if the value of r positive then the correlation is positive, whereas negative values of r indicate inverse or negative correlation.

Difference, associations and correlations were considered significant when the p-value of the corresponding test is less than or equal to 0.05.

RESULTS

Characteristic and clinical data of patients and controls:

In Group Ia (Acute leukemia patients): Pallor was the most common finding present in 80% of cases, 56% of cases were presented by fever, 48% were presented by purperic rashes & 32% were presented by ecchymosis. Splenomegaly was felt in 60% of cases hepatomegaly in 44% of cases and lymphadenopathy in 60% of cases.

In Group IIa (NHL patients): 60% presented by axillary lymphadenopathy, followed by cervical and inguinal lymph nodes enlargement in 45% and 40% respectively. 86% presented by weight loss while 50% presented by fever and night sweat. Bone marrow infiltration was detected in 75%. Splenomegaly and hepatomegaly were present in 90% and 75% of patients respectively. CNS invasion was present in 10% of cases.

Laboratory investigations:

Hematological tests were performed for all cases. In Group Ia patients at diagnosis; 82% represented by anaemia and leucocytosis, while 20% represented by leucopenia and 90% represented by thrombocytopenia. In Group IIa patients at diagnosis; 70% presented by anaemia, 35% by leucocytosis and thrombocytopenia in 40%.

Immunophenotyping: Evaluation of the following markers was done for all cases: CD45, CD34, HLA DR, CD10, CD19, CD20, CD21 CD22, CD23, IgM, SIg Kappa, SIg Lambda, FMC7, CD1, CD2, CD3, CD5, CD7, CD4 and CD8, CD13, CD33, CD14, CD15, MPO, CD41, and CD61.

I- Acute leukemia cases were diagnosed as follows:

A- *Acute lymphoblastic leukemia:* Precursor B-ALL (CD19, CD20, CD22 and cytoplasmic μ positive) in 4/40 (10%) cases L1 and 4/40 (10%) cases L2. Common ALL (CD10, CD19, HLA DR, CD34 and CD22 positive) in 2/40 (5%) cases L1 and 5/40 (12.5%) cases L2.

B- *Acute myeloid leukemia:* CD 13+ve, CD33+ve, MPO+ve in 4/40 cases M1 (10%) and 7/40 (17.5%) cases M 2, 3/40 (7.5%) cases M 3 also HLA DR+ve, 4/40 (10%)

cases M 4 and 3/40 (7.5%) cases M 5 also CD14+ve, CD15, CD34+ve, HLA DR+ve and 4/40 (10%) cases M 7 CD41 +ve and CD61 +ve.

II- *NHL cases were divided into:*

A- B-cell Lymphoma which constituted 80% of patients:

Follicular cell lymphoma (FCL) 8/20 cases (40%) were CD10, FMC7, CD22, SIg Light chain +ve. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) 3/20 cases (15%) were CD19+ve, CD5+ve, CD23+ve and SIg Light chain dim, FMC7 and CD22 negative. Diffuse large cell lymphoma 5/20 cases (25%) were CD 19+ve, CD20+ve and CD21+ve, FMC7 with positive expression of SIg Kappa (3/5 cases) and SIg Lambda (2/5 cases) (mature B cell lymphoma).

B- T-cell lymphoma constituted 20% of patients:

Early T-cell phenotype in 2/20 cases (10%) were CD2+ve, CD5+ve, CD7+ve cytoplasmic CD3+ve with CD4-ve and CD8-ve.

Intermediate T-cell phenotype in 2/20 cases (10%) were CD1, CD2+ve, CD5+ve, CD7+ve, cytoplasmic CD3+ve with CD4 and CD8 coexpression.

NHL cases were further divided according to the grade of disease into: Low grade lymphoma 11 cases (55%), Intermediate grade lymphoma 5 cases (25%) and High-grade lymphoma 4 cases (20%).

I- *Assay of serum level of LDH:*

Fig. (1) shows the serum levels of LDH (U/L) in the studied groups: The mean level of LDH in acute leukemic patients at diagnosis (1250±879) was found to be significantly increased as compared to the mean level in controls.

During remission, the mean level of LDH (541±196.47) was less than its level at diagnosis but it still higher than its level in controls. A comparison of mean serum LDH level between ALL and AML at diagnosis and during remission: Revealed a nonsignificant difference.

In Group IIa (NHL at diagnosis), mean level of LDH was (570.65±161.84U/L), while it decreased (at remission) in Group IIb, where it was (282.80±66.36 U/L), close to that of control group (278±51.22). There was a statistical significant increase at diagnosis when compared to control group ($p>0.001$) and at remission ($p<0.001$).

II- *Assay of serum B2 microglobulin only in NHL patients:*

The mean value of serum B2 microglobulin showed a statistically significant increase in Group IIa (NHL at diagnosis) when compared to control group ($p<0.001$), while Group IIb showed a statistically nonsignificant increase when compared to control group ($p>0.05$).

III- *Assay of bone marrow level of IL3 (pg/ml):*

In Fig. (2) the mean level of BM IL3 (pg/ml) in Acute leukemic patients at diagnosis Group Ia (360.6±45.7) was found to be statistically significantly increased as compared to the mean level in controls (28.4±10.5) i.e Group Ia was nearly 13 folds higher frequencies of Group III ($p<0.001$).

During remission of acute leukemia, Group Ib, the mean level of BM IL3 (185±26.35) was 50% less than its level at diagnosis but still higher than in controls i.e. group Ib was 6.5 folds of group III ($p<0.001$). A comparison in mean level of BM IL3 level between ALL and AML at diagnosis and during remission revealed a nonsignificant statistical difference ($p>0.05$).

In NHL Group IIa, the mean level of BM IL3 (95.85±49.34) pg/ml at diagnosis, showed a statistically significant increase when compared to the mean level during remission (Group IIb) and controls (Group III) ($p<0.001$) and ($p<0.001$), respectively. In the meantime, the mean level of BM IL3 in Group IIb showed a nonsignificant statistical difference when compared to control Group III ($p>0.05$).

IV- *Assay of bone marrow level of IL6 (pg/ml):*

In Fig. (3), the mean level of BM IL6 in acute leukemic patients at diagnosis Group Ia (59.4±12.5) was found to be statistically significantly increased as compared to the mean level in controls (18.6±6.3) i.e. Group Ia was 3 folds higher than control. During remission, in Group Ib, the mean level of BM IL6 (28.2±5.19) was less than that at diagnosis but still higher than controls.

A comparison in mean levels of BM IL6 level between ALL and AML at diagnosis and during remission showed a nonsignificant statistical difference.

In NHL Group, the mean level of BM IL6 showed a statistically significant increase in Group IIa at diagnosis (49±19.3) when compared to Group IIb during remission (20.1±7.83) and control ($p<0.001$) and ($p<0.001$) respectively. While the mean level of Group IIb showed a nonsignificant statistical difference when compared to control group ($p>0.05$).

V- *Assay of bone marrow level of CD44s (ng/ml):*

Fig. (4) shows the BM levels of CD44s (ng/l) in Acute leukemia and NHL. The mean level of CD44s in acute leukemic patients at diagnosis (938.5±203.17) was found to be statistically significantly increased as compared to the mean level in controls (194.6±61.55) i.e. Group Ia was 5 folds higher frequencies of group III.

During remission in Group Ib, the mean level of CD44s (238.7±45) was less than its level at diagnosis but it was still higher than its level in controls i.e. Group Ib was higher than control group. A comparison of CD44s level between ALL and AML at diagnosis and during remission showed a nonsignificant statistical difference.

In NHL group, the mean level of BM CD44s was statistically significantly increased in Group IIa at diagnosis (426.9±146) when compared to control group and during remission in Group IIb (205.2±70.2) ($p<0.001$) and ($p<0.001$) respectively. While during remission, there was a nonsignificant statistical difference when compared to control group ($p>0.05$).

A coefficient correlation study was done in Acute Leukemia Group Ia at diagnosis between the studied levels of BM IL3, BM IL6, BM CD44s, and HB level, LDH, total leukocyte count, % of blasts in peripheral blood and in bone marrow.

There were significant positive correlations between BM CD44s levels and total leucocytic count, % blasts in both peripheral blood and BM ($p<0.05$). There were significant positive correlations between BM IL3 and BM IL6 with TLC and LDH respectively. There were non-significant correlations between levels of BM IL3 as well as BM IL6 and rest of the studied parameters ($p>0.05$) (Table 2).

A correlation matrix was done between the studied parameters in NHL at diagnosis Group IIa revealing the following:

There was a positive correlation between BM IL-6 and IL3, Serum LDH and B2 microglobulin with percentage of blasts in peripheral blood and bone marrow and IPI (International NHL Prognostic index).

BM CD44s level showed a significant positive correlation with each of BM IL-6, LDH, B2 microglobulin, Hb level, total leucocytic count and % of blast cells in peripheral and bone marrow ($p<0.05$).

The relation between the different cytokines and symptoms & signs of BM and CNS invasion in NHL cases was studied showing the following:

In Group IIa the symptoms and signs of BM invasion were present in 13/17 (76.5%) of NHL cases with a BM plasma level of IL-3 above >50pg/ml (highest level in control group), and in 2/3 cases (66.7%) with BM IL3 less than <50pg/ml, a non significant statistical difference ($p>0.05$).

The symptoms of CNS invasion were present in only 2/17 (11.76) of cases with BM IL-3 >50pg/ml. While no symptoms of CNS invasion were present in NHL cases who had BM IL-3 ≤50pg/ml 3/3 (100%), and 15/17 (88.24%) with BM IL3 >50pg/ml.

As for IL6, the symptoms and signs of BM invasion were present in 14/17 (82.3%) NHL cases at diagnosis with a BM plasma level of IL-6 above >28pg/ml, (highest level in control group), and in only 1/3 cases (33.3%) with BM IL6 less than ≤28pg/ml, a nonsignificant statistical difference ($p>0.05$).

The symptoms of CNS invasion were present in only 2/17 (11.76) of cases with BM IL-6 >28pg/ml. In the meantime, no symptoms of CNS invasion were present in NHL cases, who had BM IL-6 ≤28pg/ml 3/3 (100%) and in 15/17 (88.24%) with BM IL6 >28pg/ml, a statistical nonsignificant difference.

The relation between BM adhesion molecule sCD44 and symptoms & signs of bone marrow and CNS invasion in NHL was studied revealing the following:

In Group IIa, 82.35% of patients who had a BM sCD44 level >280ng/ml (highest level in control group), suffered bone marrow invasion compared with 33.3% of those who had BM sCD44 level ≤280ng/ml, the difference was not statistically significant ($p>0.05$).

The symptoms of CNS invasion in relation to BM CD44 showed the same results as those for BM IL6.

The mean value of BM IL6 and CD44 showed a highly significant statistical increase in patients with extranodal site >1 than those patients with extra nodal sites ≤1 at diagnosis and remission with a p value ($p<0.001$).

The mean values of BM cytokines (IL6 and IL3), serum LDH and B2 microglobulin were increased in patients with high grade lymphoma than those with low/intermediate grade lymphoma at diagnosis and after remission with no statistical significance ($p>0.05$).

In the meantime, the mean value of BM CD44 was statistically significantly increased with high grade lymphoma than those with low/intermediate grade lymphoma at diagnosis and after remission ($p<0.05$). The mean values of BM cytokines (IL3 and IL6) were more increased in patients with BM invasion than those without BM invasion at diagnosis and remission with no statistical significant difference ($p>0.05$) for each.

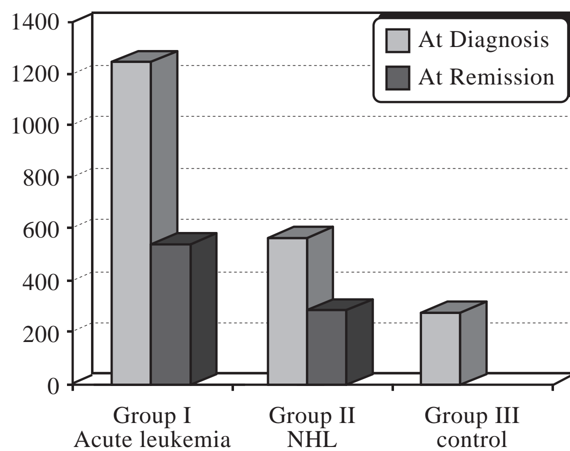

Fig. (1): Comparison between Serum LDH levels in Acute Leukemia and NHL at diagnosis and remission.

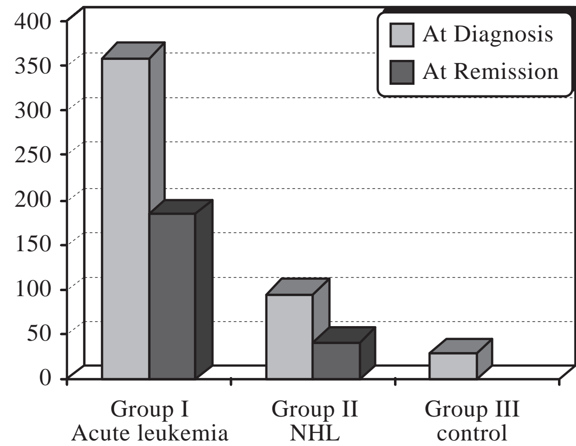

Fig. (2): Comparison between BM IL-3 in Acute Leukemia and NHL at diagnosis and remission.

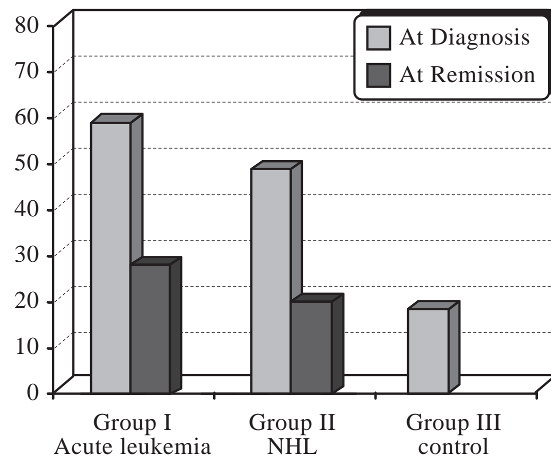

Fig. (3): Comparison between BM IL-6 in Acute Leukemia and NHL at diagnosis and remission.

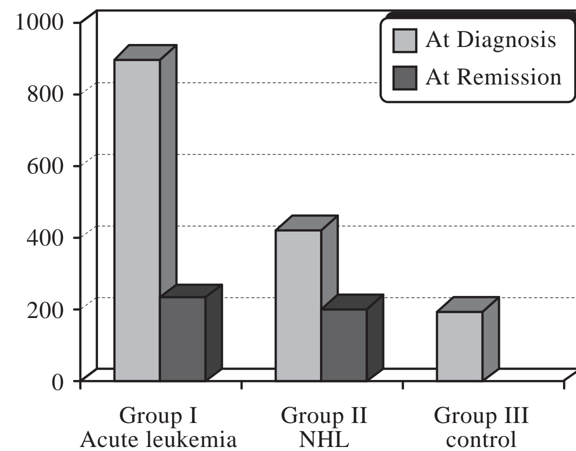

Fig. (4): Comparison between BM SCD44 in Acute Leukemia and NHL at diagnosis and remission.

Table (1): Classification of studied cases.

Group	No. of Patients	Diagnosis
Group Ia	40	De novo Acute leukemia patients before receiving chemotherapy
Group Ib	35	Same Acute leukemia patients during remission
Group IIa	20	De novo NHL patients before receiving chemotherapy
Group IIb	20	Same NHL patients during remission
Group III	10	Donors of bone marrow transplantation as controls

Table (2): Correlation coefficient (*r*) of Acute Leukemia at diagnosis Group Ia.

	HB	TLC	% PB BLASTS	% BM BLASTS	LDH
LDH:					
r	–0.032	0.174	–0.032	0.090	0.371*
p	0.846	0.284	0.847	0.607	0.018
CD44:					
r	0.003	0.770*	0.750*	0.670*	–0.032
p	0.987	0.000	0.001	0.001	0.845
IL3:					
r	–0.184	0.405*	0.158	–0.193	-0.044
p	0.255	0.009	0.332	0.265	0.788
IL6:					
r	–0.744*	0.003	0.046	0.050	0.279*
p	0.001	0.987	0.777	0.773	0.018

*: Significant. *r*: Pearson correlation.

Table (3): Correlation coefficient (*r*) of NHL at diagnosis Group IIa.

Variables	LDH		IL6		sCD44		B2 microglobulin		IL-3	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
IL6	0.666	0.001*								
S-CD44	0.744	0.001*	0.505	0.023*						
B2-microglobulin	0.654	0.002*	0.168	0.479	0.59	0.006*				
IL3	0.781	0.001*	0.465	0.039*	0.804	0.001*	0.66	0.002*		
Hb	–0.714	0.001*	–0.746	0.001*	0.860	0.001*	–0.772	0.001*	–0.745	0.001*
TLC	0.516	0.020*	0.581	0.007*	0.459	0.042*			0.631	0.003*
% Blasts in PB	0.715	0.001*	0.759	0.001*	0.757	0.001*	0.485	0.007*	0.701	0.001*
% of Blasts in B.M	0.671	0.001	0.520	0.019	0.621	0.003	0.239	0.310	0.675	0.001
ESR 1st hour	0.864	0.001*	0.727	0.001*	0.828	0.001*	0.828	0.001*	0.833	0.001*
ESR 2nd hour	0.837	0.001*	0.699	0.001*	0.819	0.001*	0.823	0.001*	0.822	0.001*
IPI	0.523	0.018*	0.590	0.006*	0.692	0.001*	0.593	0.006*	0.621	0.003*

*: Significant. *r*: Pearson correlation.

DISCUSSION

Hematological malignancies including acute leukemia and NHL are clonal disorders resulting from the neoplastic transformation of progenitor cells. Similar to their normal counterparts transformed hematological progenitor cells remain dependent on signals from the microenvironment for survival and proliferation during their malignant progression. These cells can also induce reversible changes in the marrow stroma that will further faster the development of malignant cells. This dynamic reciprocal interaction between the microenvironment and the malignant hematological cells continues throughout disease progression [20].

The aim of the present work is to study the bone marrow microenvironment (BMM) elements in Acute leukemia and NHL patients at diagnosis (before treatment) and after complete remission to evaluate the impact of chemotherapy on these patients. For this purpose we estimated BM cytokines (IL3 and IL-6), BM adhesion molecule (sCD44) in 40 Acute Leukemia and 20 NHL patients at diagnosis and in remission with 10 apparently healthy bone marrow donors as a control group.

BM aspiration and Trephine biopsy were performed for all patients at diagnosis and after remission for evaluation of BM, also to examine infiltration of BM by lymphoma cells, its types, and degree to be compared with during remission.

Descriptive analysis of our patients showed male predominance (62%) in comparison to females who represented (38%) with male to female ratio 1.63. Mean age of patients in this study was 35 years in acute leukemia group and 50 years in NHL group.

The pathological examination revealed that 85% of cases had B-cell lymphoma and 15% had T-cell lymphoma, Among B-cell lymphoma cases, 35% were follicular lymphoma, 30% were diffuse large cell lymphoma and 15% were B-CLL, while all cases of T-cell lymphoma were of Lymphoblast lymphoma type.

Cytokines are a master element of BMM, they are glycoproteins that regulate proliferation, differentiation, maturation and survival of hematopoietic stem cells & progenitor cells [21]. Tumor necrosis factor (TNFα), interleukin 3 (IL3) and interleukin 6 (IL6) are cytokines produced by cells of the immune system after immunologic stimuli and are vital to normal immunity, they act in a network of factors directing both immune and malignant response.

IL3 appears to have effects predominantly on stem and progenitor cells and minimal effect on more mature cells [22].

IL6 plays a role in mediation of inflammation and immune response. It enhances the formation of multilineage blast cells colonies in vitro and to support the formation of lineage restricted cell types. IL6 also, has an effect on thrombopoiesis [23].

In the present study, it was found that the BM levels of IL3 and IL6 were significantly higher in acute leukemic patients at diagnosis than healthy controls. Their levels (IL3 and IL6) during remission were noticeably lowered but still slightly more than normal controls.

Also in NHL patients, the BM IL-6 level was increased in (80%) at diagnosis above the upper limit of control group (28pg/ml). The mean value of BM IL-6 was statistically significantly increased in NHL in patients at diagnosis when compared to control group. While after remission, it was more decreased than its level at diagnosis with a statistically significant difference, but still to be more than that level in control group with no stastically significant difference ($p>0.05$).

A high level of IL3, in sera from acute leukemia patients, was reported after comparing its level to healthy subjects and it was lowered towards normal after achieving complete remission [24]. All the findings were in agreement with other authors as [25,26].

In NHL group, it was found that 16 patients (80%) had a bone marrow IL-3 level more than reference level at diagnosis. The mean value of BM IL-3 was statistically significantly increased at diagnosis when compared to control group, while after remission, it was more decreased than its level at diagnosis with a statistically significant difference, but still more than its level in control group with no statistically significant difference ($p>0.05$).

In the present study the mean value of BM IL-3 was higher in NHL patients with B symp-

toms (at diagnosis) than without with a statistically significant difference ($p<0.05$). Also the mean values of BM IL-3 were higher in patients with extra nodal sites than those with extra nodal site ≤1 at diagnosis and after remission with no significant difference at diagnosis ($p>0.05$). It was, also, increased in patients with BM involvement than those without BM involvement at diagnosis and after remission with a statistical significance difference ($p<0.05$).

The mean value of BM IL-3 was increased in high grade lymphoma than low/intermediate grade lymphoma with no statistical significant difference ($p>0.05$). Also it was increased in patients with high IPI than those with intermediate/high, intermediate/low and low IPI patients at diagnosis and after remission with statistical significance difference ($p<0.05$).

The results of this study showed that there were positive correlations between BM IL-3 and each of IL-6, sCD44, serum LDH and B2 microglobulin, IPI, total leukocytic count, and % of blast cells in peripheral blood, while it showed negative correlation with Hb level.

The mean value of BM IL-6 was increased in patients with B-symptoms at diagnosis with a statistical significant difference ($p<0.05$). BM IL-6 >28pg/ml was associated with 5.45 folds higher frequencies of B-symptoms than those patients with BM IL-6 <28pg/ml at diagnosis with no statistical significant difference ($p>0.05$). This is in agreement with others who reported that IL-6 was increased in patients with B-symptoms than those without B - symptoms **[27]**.

Also the mean values of BM IL-6 were increased in patients with extranodal sites than those with extranodal site ≤1 at diagnosis and in patients with BM involvement than those without BM involvement at diagnosis and after remission with no statistical significance difference ($p>0.05$) for each.

Also, patients with BM IL-6 >28pg/ml at diagnosis were associated with 1.5 fold higher frequencies of BM invasion than those with IL-6 ≤28pg/ml. Patients with BM IL-6 >28pg/ml at diagnosis were associated with 2.83 fold higher frequencies of BM invasion than those patients had BM IL-6 ≤28pg/ml.

In the present study the mean value of BM IL-6 in patients with T-cell lymphoma is more than that in patients with B-cell lymphoma at diagnosis and after remission showing a statistical significant difference ($p<0.05$).

The result of this study showed that there were significant positive correlation between IL-6 and each of serum BM IL-3, sCD44, serum LDH, IPI, total leukocytic count and % of peripheral blast sales while it shows strong negative correlation with Hb level. This in agreement with Fayed et al., 1998 **[28]**.

As regard CD44s in the present study: The estimated BM levels of CD44 in acute leukemic patients (both ALL & AML) at diagnosis were found to be significantly higher than their levels in controls ($p<0.05$). Also, in complete remission (C.R.) CD44s levels were significantly lowered than their levels at diagnosis ($p<0.05$), and not significantly different from controls ($p>0.05$).

There was no statistical significant difference in CD44 levels between ALL and AML cases neither at diagnosis ($p>0.05$) nor during remission ($p>0.05$).

In agreement with our study, Zitterman et al., 2001 **[29]** found that acute leukemic patients, before starting their treatment, had four folds higher level of serum CD44 than normal controls involved in the study. Also, Gadhoum et al., 2003 **[30]** found that BM levels of CD44 were significantly elevated in acute leukemic patients at diagnosis than controls.

It was found that 16 patients (80%) in NHL patients at presentation showed BM sCD44 more than the upper limit level of control (280ng/ml). The mean value of BM sCD44 was statistically significantly increased in NHL patients at diagnosis when compared to control group, while after remission, it was more decreased than its level of diagnosis with a statistically significant difference, but still more than that level in control group with no statistically significant difference ($p>0.05$).

This is agreement with Ristamaki et al., 1997 **[31]** who explained that sCD44 level changes in parallel with treatment response in malignant lymphoma and that the origin of soluble sCD44 probably originates from the tumor cell, the strong association between SCD44 levels and stage change of SCD44 parallels with treatment response.

The result of this study showed that the mean value of BM sCD44 in patients with B symptoms is much more than in patients without B-symptoms at diagnosis and after remission and there was high statistical significant difference between them (p<0.05).

Also CD44s was increased in NHL patients with BM involvement than those without BM involvement at diagnosis with statistical significant difference, NHL patients with BM CD44s level >280ng/mL were associated with 4.7 and 2.8 folds higher frequencies of B-symptoms and bone marrow invasion respectively at diagnosis extranodal site than those ≤280ng/mL.

The result of this present study showed that there was significant increase in BM sCD44 level in patients with high grade lymphoma than low/intermediate lymphomas if compared with control or compared to each other at diagnosis and after remission (p<0.05).

The mean value BM CD44s was significantly increased in patients with extranodal sites >1 than those who had extra nodal sites ≤1 at diagnosis and after remission (p<0.05). Also the mean value of BM CD44s was significantly increased in patients with high IPI in comparison to patients with intermediate/high, intermediate/low and low IPI score at diagnosis and after remission (p<0.05).

This is in agreement with Ponta et al., 2003 [32] who explained that sCD44 molecule is shed from the cell surface and released in some solid tumors and is correlated with poor outcome and could be a useful prognostic marker for some hematological malignancies as aggressive lymphoma. Also Niitsu and Ljima, 2002 [10] reported that a high level of sCD44 was correlated with a poor outcome of aggressive NHL suggesting that sCD44 levels could be a useful prognostic marker for aggressive lymphoma.

These results confirmed previous studies that concluded that a high serum sCD44 level and/or tumor tissue expression at diagnosis is associated with poor prognostic criteria and/or unfavorable outcome in childhood leukemias and lymphomas [9].

It was concluded from this study that evaluation of BMM elements including (IL3, IL6 and sCD44) in Acute leukemia and NHL patients, can be useful in evaluating disease activity, extent and response to treatment and they can be used as prognostic markers in these patients.

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