EVALUATION OF SOME IMMUNE RESPONSE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

I: INNATE IMMUNITY

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ABSTRACT

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder characterized by the accumulation of mature-appearing lymphocytes in the blood, marrow, and lymphoid tissues. This study aimed to evaluate innate immunity in patients with CLL via estimation of serum levels of Interleukin-1α (IL-1α) and interleukin 8(IL-8), and complement components C3 and C4. Blood samples were collected from 48 patients with CLL (28 treated and 20 untreated). Other 20 blood samples were collected from healthy appearing individuals as control group. Enzyme linked immunosorbent assay (ELISA) was used to estimate serum concentration of IL-1α and IL-8. Single radial immune-diffusion assay was used to estimate the serum levels of C3 and C4. Total white blood cells and absolute lymphocyte count in each blood sample were determined. The study showed a significant decrease in serum concentration of IL-1α and complement components C3 and C4 in treated and untreated patients, whereas, serum concentration of IL-8 and blood absolute lymphocyte count showed significant increase in untreated patients. These data collectively indicated the aberration in innate immunity in CLL patients which can used as a prognosis for the disease.

Key words: CLL, IL-1α, IL-8, C3, C4, Cytokine

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تقييم الإستجابة المناعية في مرضى إبيضاض الدم اللمفي المزمن 1: المناعة المتأصلة

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الخلاصة

إبيضاض الدم اللمفي المزمن هو إختلال في عملية تكاثر الخلايا اللمفاوية ويتميز بتجمع خلايا لمفاوية ناضجة ظاهرياً في الدم، نخاع العظم، والانسجة اللمفاوية. هدفت هذه الدراسة الى تقييم مستوى المناعة المتأصلة لدى مرضى إبيضاض الدم اللمفي المزمن من خلال تقدير المستويات المصلية لانترليوكين 1 الفا، انترليوكين 8 ومكوني المتمم الثالث والرابع. جمعت عينات دم من 48 مريضا بإبيضاض الدم اللمفي المزمن (28 معالجون و20 غير معالجين) فضلاًعن 20 عينة من أشخاص أصحاء ظاهرياً مثلوا مجموعة سيطرة. أستعملت تقنية الاليزا لتقدير التراكيز المصلية لانترليوكين1الفا و8 فيما أستعملت تقنية الانتشار المناعي الشعاعي المنفرد لتقدير مستويي المتممين الثالث والرابع. وحسب العدد الكلي لكريات الدم البيضاء والعدد المطلق للخلايا اللمفاوية في كل عينة دم. أظهرت النتائج إنخفاضاً معنوياً في التراكيز المصلية لانترليوكين 1الفا ومكوني المتمم الثالث والرابع في المرضى المعالجين وغير المعالجين فيما أظهرت التراكيز المصلية لانترليوكين8 والعدد المطلق للخلايا اللمفاوية إرتفاعاً معنوياً في المرضى غير المعالجين. تشير هذه النتائج الى مدى التغيرات الحاصلة في المناعة المتأصلة لدى مرضى إبيضاض الدم اللمفي المزمن والتي يمكن إستعمالها في تحديد مآل المرض.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a neoplastic disease characterized by the accumulation of small mature-appearing $CD19^+$ B-lymphocytes in the blood, marrow, and lymphoid tissues. The causes of this disease are unknown, although genetic factors likely contribute to its development (1). As a hematologic malignancy, involvement with this disease will bring about abnormalities in the normal function of immune system of the patient. Although the main affecter from this abnormalities is antibodies production (humoral immunity), innate immunity do have its allotment from this defect (2).

Interleukin-1α (IL-1α) and interleukin-8 (IL-8) play a pivotal role in innate immunity; IL-1 is a cytokine secreted by monocyte, macrophage, large granular lymphocyte and some other body cells. The principle effects of IL-1α are lymphocyte activation, macrophage stimulation, and increases leukocyte/ endothelial adhesion, pyrexia, and acute phase proteins (3). IL-8 is produced mainly by monocyte and acts on neutrophils stimulating degranulation, chemotaxis, and superoxide releasing factor (4).

The complement system is comprised of large number of proteins and have essential functions in innate immunity among which chemotaxis, opsonization, and activation and lyses of target cells (3). Sever infections can frequently observed in CLL patients who have defects in complement components even with normal levels of immunoglobulin(5). A number of studies indicated an elevation in serum concentration of IL-1α and IL-8 and dropping in some complement components in CLL patients (6,7,8). This study aimed to evaluate innate immunity in treated and untreated CLL patients via estimation of serum levels of IL-1α, IL-8, and the third and fourth complement components (C3 and C4).

MATERIALS AND METHODS

Patients: A total of 48 in and outpatients with CLL from both sexes in Baghdad teaching Hospital during the period from December 2009 to August 2010 were enrolled in this study. These patients were divided into two groups: twenty-eight (43-74 years old, 20 males and 8 females) treated patients with CLL (Endoxan or Leukeran and Prednisolone), and twenty (43-73 years old, 14 males and 6 females) untreated patients with CLL (newly diagnosed).
In addition, twenty (42 -59 years old, 12 males and 8 females) healthy individuals from outside the hospital were chosen as a healthy appearing control group. All study population didn't receive blood transfusion for more than one month before the time of sampling.

Samples: Five ml of venous blood were collected from each individual. Each blood sample was divided into two parts: one in ethylene diamine tetra acetic acid (EDTA) tube for total leukocyte count and absolute lymphocyte count and the other in plain tube for immunological tests.

Immunological assays: Enzyme-Linked immunosorbent assay (ELISA) (Immunotech, France) was used to estimate the serum levels of IL-1α and IL-8 in each sample. Single radial immunodiffusion assay (Biomeghreb, Tunisia) was used to estimate serum levels of C3 and C4 on Diffu-Gel plated (Oxford) with maximal diffusion method.

Total White Blood Cell count (TWBC): Total white blood cells in liter of blood was counted using Neubauer's chamber.

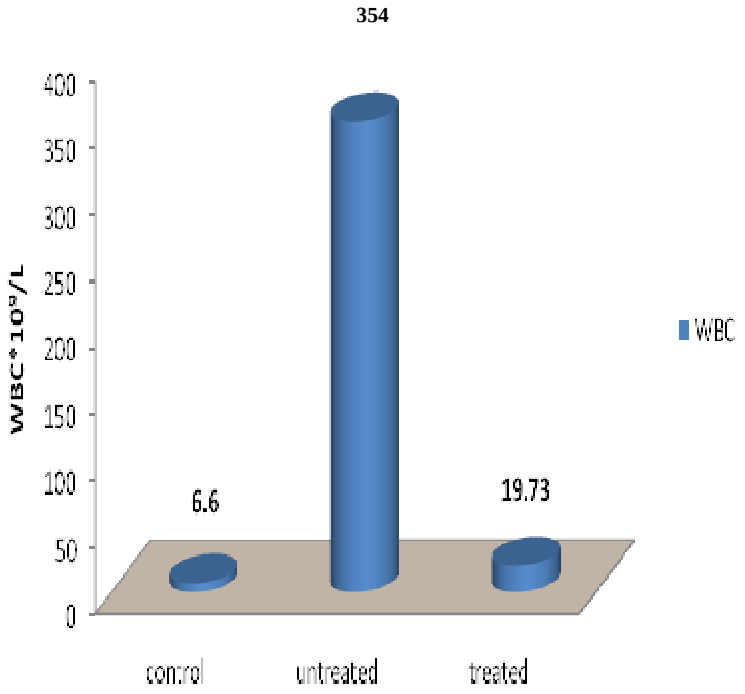
Absolute Lymphocyte Counts (ALC): Blood films were used for total differential leukocyte count (TLC), and ALC was obtained by multiplying lymphocyte percentage by TWBC.

Statistical analysis: Mean values and standard deviation (SD) of the parameters recorded were calculated. Statistical package for the social sciences (SPSS) software was used to find least significant differences among means of group. Statistical probability of $p<0.05$ was considered significant.

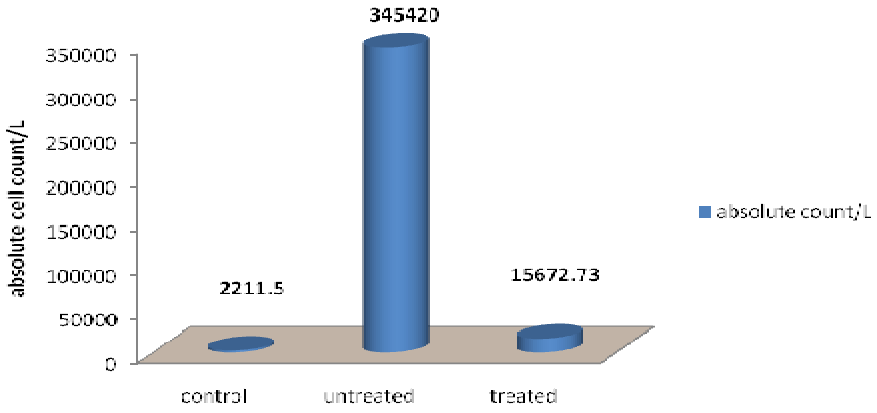
RESULTS AND DISCUSSION

Total White Blood Cell count and Absolute Lymphocyte Counts: Untreated group has the highest mean of TWBC and ALC (354×10^9 ±143.13/L and 345420±140142.4/L respectively) and differed significantly from both treated group (19.73×10^9±4.49/L and 15672.73±4383.3/L respectively) and healthy control group (6.6×10^9±0.769/L and 2211.5±228.43/L respectively) Figure(1،2).

The natural course of hematologic neoplasia often includes a phase of impaired host defense among which host innate immunity. Of course there are other very important components of innate immunity apart from those involved in this study such as neutrophils, but their function generally remains normal (9). One of the most obvious feature of CLL is the moderate to high increase in WBC count. In most patients the TWBC count is 10000 to 30000/µL (10].The relatively low TWBC count in treated group as compared with untreated group is referred to the action of chemo- and radiotherapy. The median lymphocyte count at diagnosis is 30×10^9/L and in most patients there is a continuous increase in the lymphocyte count over the time (11), although cyclic fluctuation of up to 50×10^9 can occur in the lymphocyte count of untreated patients (12).



Figure(1) : Total WBC counts in the three studied groups.



Figure(2) : Absolute lymphocyte counts in the three studied groups.

IL-1α: Serum level of IL-1α in untreated, treated , and healthy control groups were 6.59± 0.63 pg/ml, 8.91 ± 0.85 pg/ml, and 16.77± 4.2 pg/ml respectively with significant differences among the three groups.

IL-8: The highest concentration of IL-8 (23.67± 8.0 pg/ml) was in untreated group which differed insignificantly from both treated group (18.50 ± 1.21 pg/ml) and healthy control group (19.22 ± 3,4 pg/ml) Figure(3).

IL-1 was initially described as lymphocyte activating factor (LAF), and endogenous pyrogen (EP) for its pro-inflammatory, immune-stimulant, and chemotatic properties. The genes of the IL-1 complex code for three proteins: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra) (13). Of a particular interest, IL-1 is known to be up-regulated in many tumor types and has been implicated as a factor in tumor progression via the expression of metastatic and angiogenic genes and growth factors. A number of studies have reported that high IL-1 concentrations are associated with more virulent phenotype (14). It seems from this study that B CLL cell do not produce IL-1α and its serum levels in CLL patients (specially in untreated) has decreased significantly, and this may referred to malfunction of normal secretor cells of this cytokine. This is in accordance with some previous works(6).In fact , what is well documented is that CLL cell produce IL-1β and IL-1Ra which prevent B CLL cells from undergoing apoptosis, but not IL-1α(14).

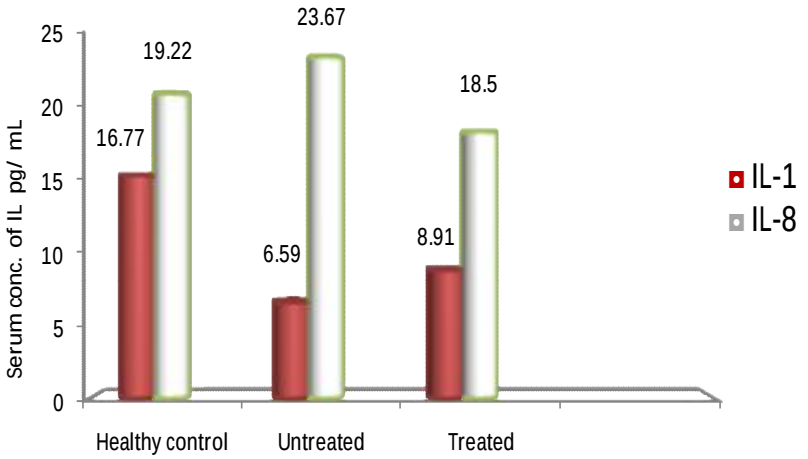
IL-8, a 72-77 amino acid 6-8 KDa inflammatory cytokine(15), has been reported to be increased in serum CLL patients(7). However, Molica *et a*l (16) found this elevation only in 25.8% of B-CLL patients, whereas Wierda *et al*. (15) reported that only advanced stage of CLL were associated with high plasma IL-8 levels.

This study showed that untreated patients had the highest level of IL-8, while treated group had the lowest level. Elevated serum level of IL-8 in untreated CLL patients is, unduobtly, referred to the malignant cells themselves which are constitutively express IL-8 and IL-8 receptors. As an autocrine cytokine, IL-8 production from malignant cells induces more production of this cytokine from malignant cells and perhaps from normal body cell especially monocytes (7). However, decreased serum level of IL-8 in treated group may referred to the action of chemotherapy which reduced malignant cells.

Referred to its normal functions, increased IL-8 production is supposed to be of benefit for patient defense mechanism. This is not the actual situation. The fact that IL-8 prolongs CLL cell survival and protects them from apoptosis is well established (15,16). Interestingly, circulating levels of IL-8 paralleled those of intracellular bcl-2(17) which confirming the antiapoptotic effect of IL-8.

Finally, estimation of IL-8 in CLL patients may have a prognostic value. Wierda *et al*. (15) found that higher plasma levels of IL-8 were associated with shorter survival period. They reported that when plasma level of IL-8 exceeds 26.2 pg/ml it will associate with more than sevenfold increase in risk of death patients had a median survival of 9.3 months compared

with widely longer period (undetermined) for patients who had IL-8 below this value. This indicates that our patients (treated and untreated) have good prognosis.



Figure(3): Serum concentration of IL-1α and IL-8 in the three studied groups.

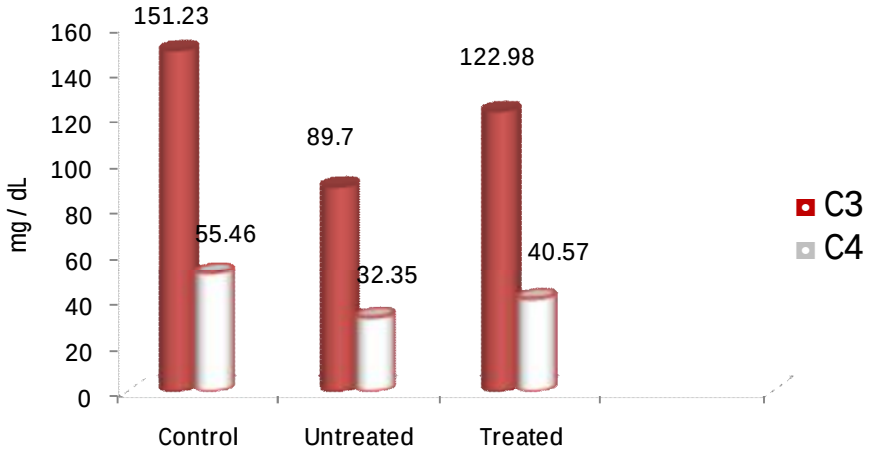
The third complement component (C3): There is significant decrease in serum levels of C3 in CLL patients either in untreated (89.7±12.98mg/dL) or treated (122.98±7.38mg/dL) as compared with healthy control group (151.23 ± 13.18 mg/dL with significant difference between untreated and treated groups Figure(3).

The fourth complement component (C4): Untreated group had the lowest mean serum level of C4 (32.35 ± 4.2 mg /dL) and differed significantly from healthy control group (55.46 ± 7.81 mg / dL) and insignificantly from treated group (40.56 ± 7.99 mg/ dL) Figure(4).

Estimation of complement components and/or complement activity in hematologic malignancy is not only useful for evaluation of immune status of the patient, but also for prognosis of the disease. It has been shown that low complement levels occur mainly in early stages of the disease, and there is a strong positive correlation between initial classical pathway activity and some complement components in one hand and CLL patient survival in the other(2). This correlation may be explained by the increased susceptibility to infection and deficient handling of immune complexes.

This study revealed that untreated CLL patients (newly diagnosed, and usually at early stages of the disease) have the lowest serum levels of both C3 and C4, whereas treated patients have (usually at advanced stages of the disease) higher values for these components which may be referred to the action of chemotherapy and/or the progress of the disease to advanced stages.

The liver is the primary site for the synthesis of most circulating complement proteins. However, extrahepatic synthesis of a wide range proteins of the complement system occurs in a variety of organs/tissues and cells (18). Some authors (19) reported that transforming growth factor-β (TGF-β) which secreted by B-CLL cells can down-regulate complement production by human proximal tubular epithelium. Thus, it appears, as there is no direct involvement of the liver in CLL(9), that dropping in the production of C3 and C4 in treated and untreated CLL patients may be referred to activity of leukemic cells on extrahepatic production site of complement.



Figure(4): Serum concentration of C3 and C4 in the three studied groups.

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