



EVALUATION OF IMMUNE RESPONSE IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA. II: HUMORAL IMMUNE RESPONSE

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Abstract

Chronic lymphocytic leukemia (CLL) is a hematologic malignancy characterized by progressive accumulation of lymphocytes. This study aimed to evaluate humoral immunity in patients with CLL via estimation of serum levels of immunoglobulins IgG, IgA, and IgM and two cytokines: interleukin-4 (IL-4) and interleukin-10 (IL-10). Blood samples were collected from 48 patients with CLL (28 treated and 20 untreated). Other 20 blood samples were collected from healthy individuals as control group. Single radial immune-diffusion assay was used to estimate the serum levels of immunoglobulins. Enzyme linked immunosorbent assay (ELISA) was used to estimate serum concentration of IL-4 and IL-10. The study showed insignificant increase in serum concentration IgG and IL-10 while there was insignificant decrease in IgA, IgM, and IL-4 in treated and untreated groups as compared with the healthy control group. These data indicates a little or unimportant effects of IL-4 and IL-10 on immunoglobulin production in studied patients.

تقييم الاستجابة المناعية في مرضى ابيضاض الدم اللمفي المزمن. 2: الاستجابة المناعية الخلوية

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الخلاصة

ابيضاض الدم اللمفي المزمن هو أحد الأمراض السرطانية التي تصيب الدم. هدفت هذه الدراسة إلى تقييم مستوى المناعة الخلوية لدى مرضى ابيضاض الدم اللمفي المزمن من خلال تقدير المستويات المصلية للكلوبيولينات المناعية IgG ، IgA ، IgM، والمحركين الخلوئين انترليوكين 4 (IL-4) وانترليوكين 10 (IL-10) (10) . جمعت عينات دم من 48 مريضاً بابيضاض الدم اللمفي المزمن (28 معالجون و 20 غير معالجين) بالإضافة إلى 20 عينة من أشخاص أصحاء مثلوا مجموعة سيطرة . استخدمت تقنية الانتشار المناعي الشعاعي المنفرد لتقدير المستويات المصلية للكلوبيولينات المناعية فيما استخدمت تقنية الاليزا لتقدير التراكيز المصلية للانترليوكين 4 و 10. أظهرت النتائج ارتفاعاً غير معنوي في التراكيز المصلية لـ IgG و IL-10 وانخفاضاً غير معنوي في المستويات المصلية لكل من IgA ، IgM ، و IL-4 في المرضى المعالجين وغير المعالجين مقارنة مع مجموعة السيطرة. تشير هذه النتائج إلى وجود تأثير قليل أو معدوم لكل من IL-4 و IL-10 على إنتاج الكلوبيولينات المناعية في مرضى ابيضاض الدم اللمفي المزمن قيد الدراسة.

Introduction

Chronic lymphocytic leukemia (CLL) is a hematologic malignancy characterized by progressive accumulation of lymphocytes, starting in lymph nodes and/ or the bone marrow and gradually expanding to most of the hemopoietic system [1]. CLL is primary a disease of moderate-aged and elderly persons and there is a peak occurrence between 50 and 60 years of age [2]. Nearly, three fourth of all B cell CLL patients develop severe hypogammaglobulinemia during the course of their disease[3], while hypergammaglobulinemia, usually polyclonal, is present in 15% of patients, especially females and elderly persons over 60 years of age[4]. Interleukin-4 (IL-4) is a pleiotropic cytokine that is produced by TH₂ cells, natural killer (NK) cells, basophils, and mast cells[5]. It plays a central role in the differentiation of naïve T cells to TH₂cells capable of producing IL-4, IL-5, IL-10 and IL-13. Thus IL-4, through TH₂ differentiation, controls humoral immunity [6, 7, 8].

Interleukin-10 (IL-10) is produced by TH₂ clones [9], macrophages, B cells and keratinocytes. It strongly stimulates proliferation of B cells with production of large amounts of IgM, IgG, and IgA antibodies especially after cross-linking of CD40 by anti-CD40 antibody[10], and this effect is antagonized by IL-4 but enhanced by transforming growth factor beta (TGF-β) especially for IgA secretion [7,8,11]. Chronic lymphocytic leukemia patients in more advanced stages of their disease show higher level of IL-10 compared with the healthy people [12].

Most cases of CLL involve abnormal B-cell proliferation. Hence, patients with this disease have shortage of normal B-cell and decreased anti-body production [2]. The appearance of hypogammaglobulinemia was more common in cases with diffuse infiltration of bone marrow and advance stages of the disease [13].

This study aimed to evaluate humoral immune response in patients with CLL via estimation of serum levels of IgG, IgM, and IgA associated with serum levels of IL-4 and IL-10.

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 used for this study . These patients were divided into two groups: twenty-eight (43-74 years old, 20 males and 8 females) treated patients (Endoxan or Leukeran and Prednisolone), and twenty (43-73years old, 14 males and 6 females) untreated patients (newly diagnosed). In addition, twenty (42 -59 years old, 12 males and 8 females) healthy individuals from outside the hospital were used as a healthy 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 put in plain tube where serum is separated, put in Eppendorf tubes and stored at -20C°.

Immunological assays:

Single radial immunodiffusion assay (Biomeghreb , Tunisia) was used to estimate the serum levels of IgG, IgM, and IgA. Enzyme-Linked immunosorbent assay (maptech, USA) was used to estimate the serum levels of IL-4 and IL-10 in each sample.

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

Immunoglobulin G (IgG):

There was un-significant elevation of serum levels of IgG in untreated group (1255.18±142.74 mg/dL) as compared with treated group (1364.36±196.44 mg/dL) and healthy control group (1226.84±138.6 mg/dL) .

Immunoglobulin A (IgA):

Serum levels of IgA shows un-significant dropping in treated and untreated group (156.46±31.32 and 217.72±105.41 mg/dL respectively) as compared with healthy control group (230.54±29.11 mg/dL).

Immunoglobulin M (IgM): Healthy control group has the highest serum levels of IgM (

165.78±42.63 mg/dL) and differed un-significantly from both untreated and treated groups (99.80±53.85 and 80.07±20.36 mg/dL respectively with no significant difference between the later two groups (Figure 1).

Interleukin-4 (IL-4):

Untreated group had lowest serum levels of IL-4 (952.66±158.11pg/mL) and differed un-significantly from treated group (1323.63±848.31 pg/mL) and healthy control group(1491.46±827.39 pg/mL).

Interleukin 10 (IL-10) :

Serum levels IL-10 severely dropped in untreated group (27±11.47 and 0.65±0.23 pg/mL respectively) and differed significantly from

treated (1323.63±848.31 pg/mL respectively) and healthy control group (1715.66±1014 pg/mL respectively) with no significant difference between the later two groups (Figure 2) .Estimation of serum levels of immunoglobulins has been suggested as a prognostic factor in patients with CLL [14]. The pathogenic mechanism of hypoglobulinemia probably results from impaired B cell function. Decreased in vitro immunoglobulin synthesis to polyclonal mitogens or antigens has been reported [15]. Furthermore, Natural killer (NK) cells from patients with CLL with hypogammaglobulinemia were found to cause decrease in immunoglobulin secretion by normal B-cell [16].

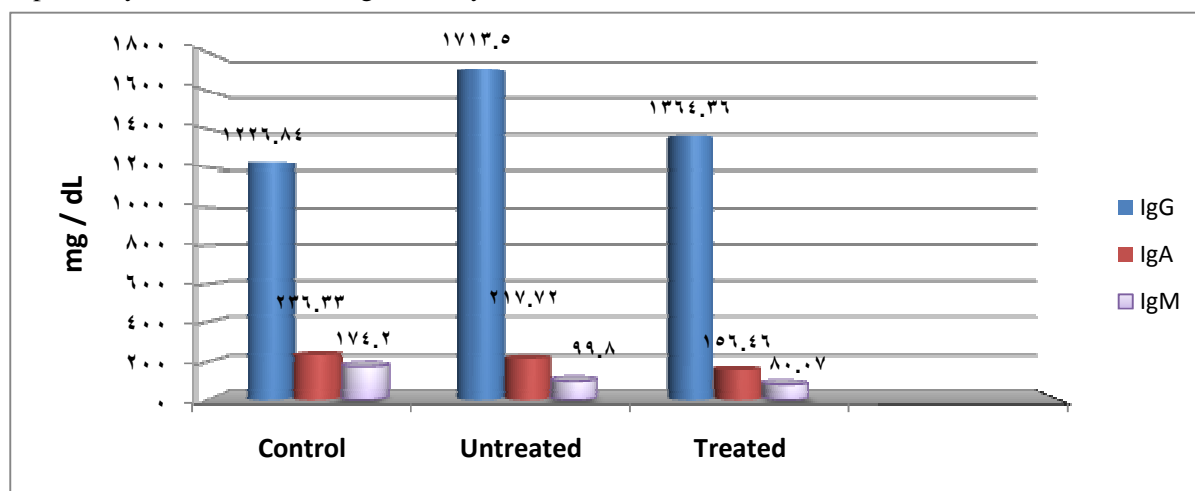


Figure 1: Mean values of IgG , IgA and IgM in three studied groups

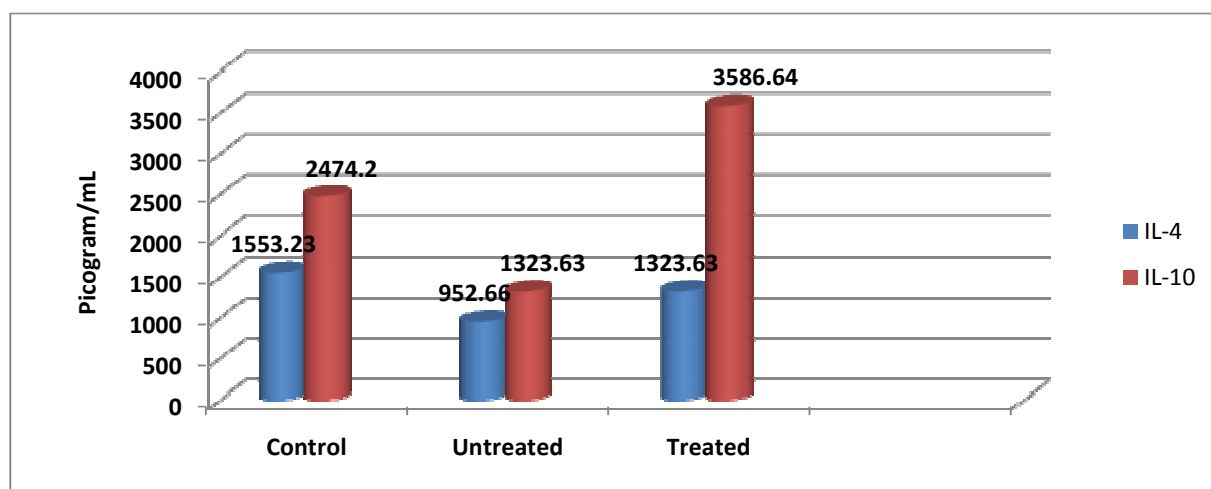


Figure 2: Serum concentration of IL-4 and IL-10 in the three studied groups

The relatively higher serum levels of IgG in treated and untreated groups compared with those of healthy control may be related to the stage of the disease and/or age of the patients as most patients involved in this study were elderly people. Although the real cause of hypergammaglobulinemia in CLL patients is unknown and represents only 15% , this phenomena was reported among elderly and female patients [4]. On the other hand, serum level of IgG was found to be inversely correlated with the stage of the disease. This explain the relatively higher serum level of IgG in untreated group.

Dropping in serum levels of IgA and IgM in treated and untreated groups (although insignificant) were expected because the impaired function of B-cells in leukemic patients. Colovic et al. [13] found that low levels of IgA and IgM occur in 30% and 10% of CLL patients respectively at the time

of diagnosis, but the clinical and prognostic significance of these two immunoglobulins are less important than those of IgG. However, some authors reported significant correlation between serum level of IgA and survival rate [15 ,16].

The slight decrease in serum levels of IL-4 in treated and untreated groups was inconsistent with the results of some investigators [17] who found that serum level of IL-4 elevated significantly in 47% of cases with B CLL. This elevation was not attributed to the activity of malignant cells, because freshly isolated B CLL cells did not express mRNA for IL-4 but express mRNA for IL-4 receptors. The effect of IL-4 on B CLL cells was a controversial issue. Defrance et al. [11] demonstrated that this

cytokine has a direct killing effect on B CLL cells may be through inhibiting DNA synthesis with simultaneous decrease in IL-6

(TNF- α) [18]. Nevertheless, Douglas et al. [19] had shown that IL-4 may protect B CLL cells from apoptosis; the effect which may be modulated by bcl2 protein. When used clinically to treat CLL patients, this cytokine was found to increase number of blood lymphocytes during the course of the therapy which may confirms the anti-apoptotic effects of IL-4 for B CLL cells [20]. Interleukin-10 (IL-10) is a pleiotropic cytokine produced by various normal body cells as well as

neoplastic B-cells [21]. The later cells may be the main cause of its

and tumor necrosis factor alpha elevation in untreated and treated groups compared with healthy control group. Serum level of IL-10 has been found to be important prognostic factor in some hematologic malignancies [22]. High serum levels of this cytokine were associated with unfavorable phenotype features in CLL such as elevated lactate dehydroginase, previous treatment, elevated β_2 -microglobulin and advanced stages of the disease. Increased serum levels of IL-10 in untreated and treated groups were accompanied by increased serum levels of IgG but not IgA and IgM which supposed to be so.

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