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Prognostic values of β2 microglobulin, interleukin-4, and interleukin-6 in patients with different stages of chronic lymphocytic leukemia

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Abstract:

BACKGROUND: Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by relentless accumulation of monoclonal B-cell. Beta-2 microglobulin (β2m) is an extracellular protein that is bound to the chain of Class 1 major histocompatibility complex molecule, which is present on all nucleated cells. Interleukin-4 (IL-4) is a T-cell-derived pleiotropic cytokine which is involved in the mechanism of survival of leukemic B-cell. IL-6 is a pleiotropic cytokine produced in the site of inflammation; it plays a major role in the acute-phase response. β2m, IL-4, and IL-6 may have prognostic value in patients with CLL.

OBJECTIVES: The aims of this study were to estimate the levels of β2m, IL-4, and IL-6 in different stages in patients with CLL and to know if there is prognostic significance of β2m, IL-4, and IL-6 in those patients.

PATIENTS AND METHODS: This study was conducted on fifty patients from March 2013 to March 2015 including 36 males and 14 females; all were newly diagnosed CLL. Those patients were divided into two groups depending on the stages of disease: Group I (21) patients and Group II (29) patients according to the Rai staging system. Diagnosis of CLL was determined for all those patients by complete blood count and blood films and flow cytometry. The estimation of β2m, IL-4, and IL-6 was done by enzyme-linked immunosorbent assay technique.

RESULTS: The male-to-female ratio was 2.3:1; there were 29 (58%) patients in Group I (Rai Stages 0, I, and II) and 21 patients (42%) in Group II (Rai Stages III and IV). β2m, IL-4, and IL-6 were elevated in patients with advance stage of the disease as the levels of these prognostic markers was higher in Group II compared to Group I.

CONCLUSION: The elevation of β2-m, IL-4, and IL-6 indicates bad prognosis of the disease and poor outcome because the serum level of β2m, IL-4, and IL-6 is increased in the advanced stages of the disease.

Keywords:

Beta-2 microglobulin, chronic lymphocytic leukemia, interleukin-4, interleukin-6

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Introduction

Chronic lymphocytic leukemia (CLL) is a monoclonal disorder characterized by relentless accumulation of monoclonal B-cell. The onset of CLL is usual; it may be discovered incidentally.[1] Patients with CLL may present with enlarged lymph node, and other patients may present with a wide range of signs and symptoms.[2] CLL accounts for about one-quarter of new cases of leukemia patients in the USA, where the risk of the disease is 1:200 in the general population.[3] CLL is considered to be a disease of the elderly, with a median age at the diagnosis of 70 years.[4]

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Beta-2 microglobulin (β2m) is an extracellular protein that is noncovalently bound to the chain of the Class 1 major histocompatibility complex molecule, which is present in all nucleated cells.[5] Elevated serum levels of this protein correlate with worse prognostic factor, such as advanced CLL stage and high tumor burden.[6] Interleukin-4 (IL-4) is a T-cell-derived pleiotropic cytokine and is involved in the mechanism of survival of leukemic B-cell in CLL.[7] A previous study suggested that IL-4 plays a role in the proliferation and differentiation of normal B-cell after activation.[8] IL-6 is a pleiotropic cytokine produced in the site of inflammation and plays a major role in the acute-phase response as shown by different biological and clinical features such as secretion of acute-phase proteins.[9] IL-6 is produced by a variety of cell types including fibroblasts, endothelial cells, monocytes, normal hematopoietic cells, and lymphocytes. The plasma level of IL-6 varies significantly among different stages of patients with CLL.[10] A previous study indicated that IL-6 plays a role in the development of malignant lymphoma and elevated level of IL-6 correlates with bad prognosis in patients with lymphoma and CLL.[11] The aims of this study were to estimate the levels of β2m, IL-4, and IL-6 in different stages in patients with CLL and to know if there is prognostic significance of β2m, IL-4, and IL-6 in those patients.

Patients and Methods

This cross-sectional study was done on fifty patients with newly diagnosed CLL from March 2013 to March 2015. The study included 36 males and 14 females, and the age of the patients ranged from 45 to 75 years. The study was approved by the ethical committee of Iraqi Council for Medical Specialization. Written informed consent was obtained from every patient before enrollment into the study. Diagnosis of CLL was done through complete blood picture and blood film looking for mature lymphocyte and smudge cells, and then confirmed by bone marrow aspiration and biopsy and flow cytometry on peripheral blood. These patients were attending four centers in Baghdad; these centers are Medical City Complex, National Center of Hematology/Mustansiriyah University, Al-Yarmouk Teaching Hospital, and Al-Imamain Al-Kadhimain Medical City.

From each patient, 10 ml of blood was aspirated from a suitable vein after efficient disinfection over the injection site, and then 5 ml of the blood sample was transferred to an ethylenediaminetetraacetic acid (EDTA) tube and used for:

1. Two milliliters of blood was immediately transferred into the EDTA tube, and complete blood picture (CBC) and blood film were done for each sample by using a CELL-DYN RUBY HEMATOLOGY autoanalyzer-Abbott Park, Illinois, U.S.A.
2. One milliliter of EDTA blood for immunophenotyping done by using four-color flowcytometer (Partec Cyflow® Cube 6, Norderstedt Germany).

The patients were divided into two groups according to Rai staging: Group I included Rai Stages 0, I, and II, in which the hemoglobin concentration was ≥11 g/dL and the platelet counts were ≥100,000 cells/L. Whereas Group II included Rai Stages III and IV, in which the hemoglobin concentration was 11 g/dL and the platelet counts were <100,000 cells/L.

The remaining 5 ml of the blood sample was used to measure the serum levels of β2m, IL-4, and IL-6, which were determined for all patients by enzyme-linked immune sorbent assay (ELISA; ELISA Reader version Stat FAX, USA).

Statistical analysis

Analysis of the data was done by using the Statistical Package for the Social Sciences version 22 (SPSS, IBM Corp. Armonk, NY, USA). The significance of difference between means was tested by using Student's *t*-test for difference between two means. $P < 0.05$ was considered statistically significant.

Results

Among the fifty patients enrolled in this study, there were 19 patients (38%) below the age of 55 years, whereas there were 31 patients (62%) above the age of 55 years. The mean ± standard deviation was 59.4 ± 10.0 with a range from 42 to 78 years. The male-to-female ratio was 2.3:1. There were 29 (58%) patients in Group I and 21 patients (42%) in Group II.

Table 1 and Figure 1 represent the comparison of β2m in the sera of Group I and Group II patients with CLL. There was a significant difference in the sera of β2m in Group II patients with CLL when compared to those in Group I.

Table 2 and Figure 2 represent the comparison of IL-4 mean value in the sera of Group I and Group II patients with CLL. There was a significant difference in the value of IL-4 in patients with CLL in Group II when compared to Group I.

Table 1: Level of sera β2 microglobulin in Group I and II patients with chronic lymphocytic leukemia

β2 microglobulin (ug/ml)	Group I (*n*=21)	Group II (*n*=29)
Mean±SD	1.606±0.907	3.239±0.972
Standard error of mean	0.198	0.180
Range	0.314-3.752	0.303-4.972
50th (median)	1.582	3.214
P	0.0001*	

*Significant difference means using Student's *t*-test for difference between two independent means at 0.05 level. SD=Standard deviation

Table 3 and Figure 3 represent the comparison of IL-6 mean value in the sera of Group I and II patients with CLL. There was a significant elevation in the IL-6 in the sera of patients with CLL in Group II when compared to those in Group I.

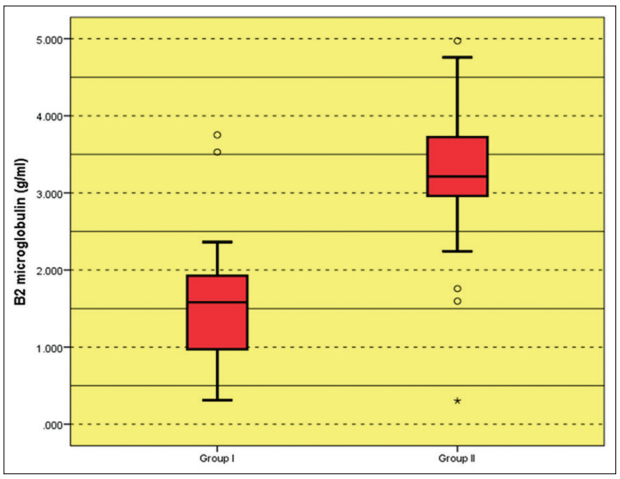

Figure 1: Distribution of β2 microglobulin (mean ± standard deviation) in Group I and II patients with chronic lymphocytic leukemia

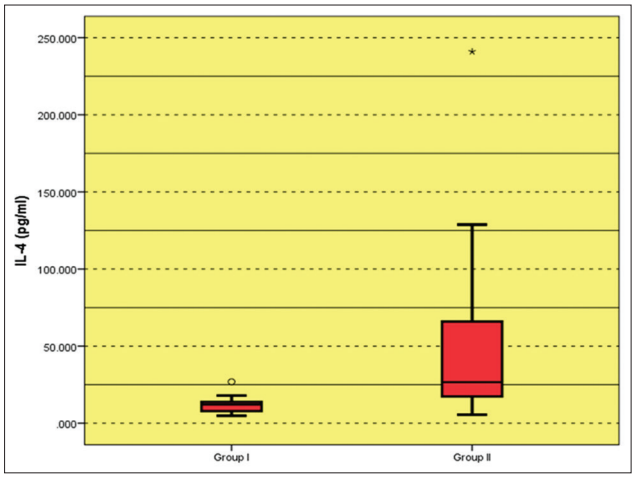

Figure 2: Distribution of IL4 level (mean ± standard deviation) in Group I and II patients with chronic lymphocytic leukemia

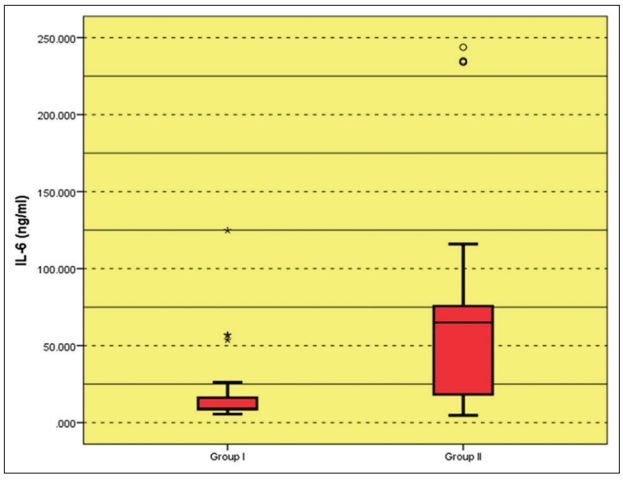

Figure 3: Distribution of interleukin -6 (mean ± standard deviation) in Group I and II patients with chronic lymphocytic leukemia

Discussion

CLL is the most common form of leukemia in the Western countries. This disease occurs mainly in elderly patients. In the present study, the disease was found mainly in individuals aged above 55 years, which was compatible with Parikh *et al*. study which showed that individuals aged 55 years and above were more susceptible to be affected by CLL.[12]

In the present study, the distribution of the disease according to the gender was with a 2.3:1 male-to-female ratio, which agreed with the data by Okaly *et al*. but disagreed with that of Nabhan *et al*. who found that the CLL disease of the elderly was at a male-to-female ratio of 1.9:1.[13,14]

Patients with CLL have different prognostic factors which affect their survival; one of these factors is β2m.[15] The current study showed that the value of β2m might be useful for detecting the prognosis of the disease as the value of β2m was higher in the Group II when compared with Group I, and high value of β2m is associated with bad prognosis. The study observation agreed with a previous result which indicated that elevation of the value of serum β2m may correlate with bad prognosis, such as advanced stage of the disease or high tumor burden, present in the bone marrow.[16] Another study on untreated patients with CLL indicates that lower serum β2m level was associated with a higher rate of complete remission after chemotherapy treatment.[17]

Table 2: Level of interleukin-4 in Group I and II patients with chronic lymphocytic leukemia

IL-4 (pg/ml)	Group I (*n*=21)	Group II (*n*=29)
Mean±SD	11.904±4.805	49.053±48.611
Standard error of mean	1.049	9.027
Range	4.792-26.876	5.490-240.987
50th (median)	12.170	26.613
P	0.001*	

*Significant difference means using Student's *t*-test for difference between two independent means at 0.05 level. IL=Interleukin, SD=Standard deviation

Table 3: Level of sera interleukin-6 in Group I and II patients with chronic lymphocytic leukemia

IL-6 (ng/ml)	Group I (*n*=21)	Group II (*n*=29)
Mean±SD	22.845±28.708	75.480±73.379
Standard error of mean	6.265	13.626
Range	5.637-124.877	4.663-243.765
50th (median)	8.987	64.987
P	0.003*	

*Significant difference means using Student's *t*-test for difference between two independent means at 0.05 level. IL=Interleukin, SD=Standard deviation

IL-6 is an important cytokine which regulates the host defense response; it regulates the major physiological reactants for the production of acute-phase protein including C-reactive protein by the liver and also its function as direct promoter of maturation of megakaryocytes.[18] In the current study, we identified that increasing level of IL-6 correlates with the stage of disease and increased level is associated with bad prognosis, and the result agrees with previous result which identified that high level of IL-6 had a good relationship with the overall survival.[19] Patients with CLL may not have a significantly higher median level of IL-6 compared with normal control, but when the disease is advanced, increasing level of IL-6 correlates with shorter survival. This is confirmed by a multivariate analysis that showed IL-6 to be an independent prognostic factor in these patients.[20]

High level of IL-6 predicts poor outcome in patients with CLL as the disease advances. The explanation is that high IL-6 in patients with advanced CLL is the consequence of a severely defective immune system, such that the biologic refractoriness to IL-6 or the negative feedback on IL-6 is impaired.[18,20]

IL-4 acts on different types of cells by displaying either agonistic or antagonistic effect, and it may effect on cell signal lineage at different stages of differentiation. It is a T cell-derived pleiotropic cytokine and involved in the mechanism of leukemic B-cell survival.[21] In the present study, the level of IL-4 was higher in group II in comparison to group I and this correlates with progression of the disease since IL-4 value in patients with Group II CLL when compared to Group I. This result agrees with Key *et al* study which indicates that IL-4 level increases with disease progression..[22]

Conclusion

Elevation of β2m, IL-4, and IL-6 levels in patient with CLL indicate that patient may progress to advance stage of disease which reflect bad prognosis and poor outcome.

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Conflicts of interest
There are no conflicts of interest.

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