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Evaluation of interleukin-6 level in patients with chronic lymphocytic leukemia and its correlation with the stage of the disease

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Abstract:

BACKGROUND: Chronic lymphocytic leukemia (CLL) is not an uncommon hematological malignancy which affects elderly individuals primarily. It is more common in developed world. Interleukin-6 (IL-6) is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis. Due to its major role in initiation as well as resolving inflammation, deregulation of IL-6 is a mainstay of chronic inflammatory and autoimmune diseases. In addition, IL-6 has been shown to be implicated in the pathogenesis of many human malignancies.

OBJECTIVES: The aim of the study was to measure the plasma level of IL-6 in newly diagnosed pretreated CLL patients and its association with disease stage and peripheral blood indices.

PATIENTS, MATERIALS AND METHODS: A case–control study included 60 patients who are newly diagnosed untreated CLL patients classified into three stages according to the Binet classification system as a patient group and 20 healthy individuals as a control group. The samples were collected from the patients during their attendance at the laboratories of the Hematology Center of the Medical City in Baghdad. For the determination of plasma level of IL-6 in both patient and control groups, an ELISA-based method was used.

RESULTS: IL-6 plasma level was higher significantly in the patient's group than in the control group ($P < 0.001$), with the highest IL-6 level found in Stage C and the least IL-6 level found in Stage A ($P = 0.043$). The white blood cell count shows a high correlation to IL-6 level when compared to other peripheral blood elements (hemoglobin and platelet count) but is still not statistically significant. Regarding age and gender, they did not have a statistical significance ($r = 0.081$, $P > 0.05$) on the mean plasma level of IL-6 within both the patient and control group.

CONCLUSIONS: In this study, IL-6 plasma levels are significantly correlated with the stage of the disease as its level is the highest in CLL patients with advanced disease. IL-6 is an independent prognostic factor in the prediction of the activity and the stage of the disease in these patients.

Keywords:

Binet classification system, chronic lymphocytic leukemia, interleukin-6

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Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common hematological malignancies which primarily affects elderly individuals. It is more common in developed world than in developing countries. Some patients experience rapid disease progressions, whereas others exist for a decade without any treatment; however, a number of patients are asymptomatic at the time of diagnosis and are only identified by the incidental finding of lymphocytosis on routine investigation. The staging systems are useful to delineate the disease extent and to predict the likelihood of survival.[1]

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Interleukin-6 (IL-6) may promote disease progression either directly through effects on cell survival and proliferation or indirectly through effects on the microenvironment. Cytokines are intercellular short-acting soluble mediators that are involved in the pathogenesis of cancer. Cytokines can either be produced by or exert effects on neoplastic or reactive cells, cytokines may be utilized as a marker of immunity status and/or prognosis in cancer. IL-6 is a multifunctional cytokine involved in the regulation of various cellular functions, among them proliferation, apoptosis, angiogenesis, differentiation, and regulation of immune response, being also implicated in the pathogenesis of several lymphoproliferative disorders suggested an important role of IL-6 in the survival of CLL cells.[2]

Cytokines are soluble factors, whether originated from the neoplastic cells (autocrine) or the surrounding cells in the microenvironment (paracrine), were assumed to play a substantial role in the growth mechanism of the neoplastic clone.[3]

The aim of the study was to assess the plasma level of IL-6 in pretreated newly diagnosed CLL and evaluate its association with disease stage and peripheral parameters.

Patients, Materials and Methods

A case–control study was performed on 60 patients newly diagnosed and untreated CLL patients during the period from November 2022 to May 2023.

The diagnosis was based on both peripheral blood and bone marrow morphology and immunophenotype reports.

Exclusion criteria were patients on chemotherapy or any other treatment for CLL, patients with other hematological or nonhematological neoplasms, hemolyzed sample, and any intercurrent illness such as any infection or inflammation.

A total of 20 apparently healthy individuals were included in this study (samples were collected from control subjects only if the subjects had no fever within 1 week, not receiving any medications, not known to be pregnant, and did not have a history of any chronic or acute illnesses).

Clinical and laboratory information regarding age, gender, if the patient received treatment or not, reports of blood film, bone marrow aspirate and biopsy, and flowcytometry and reports of U/S and computed tomography scan were obtained from patients recording files at diagnosis. The staging was done according to Binet classification, stages A, B, and C.[4]

From each participant blood was withdrawn into two (K_2 ethylenediaminetetraacetic acid [EDTA]), tubes each one containing 2 mL. The first EDTA tube for measuring the basic hematological parameters, including (hemoglobin [Hb], white blood cell [WBC] count, and platelets count), blood film evaluation, and manual reticulocyte count if needed. The second EDTA tube for the determination of the plasma level of IL-6 using ELISA-based method. The drawn samples were centrifuged for 20 min at 3000 rounds per minute (RPM). The plasma is stored at –80°C, then thawed before using. Generally, IL-6 is not significantly detected within the systemic circulation. That's why normal plasma levels of IL-6 can be considered up to 7 pg/mL (ng/L).[5] 1 pg/mL = 1 ng/L.[6]

An assay of the IL-6 uses ELISA based on the Biotin double antibody sandwich technology. The plasma of both patients and control were added to the wells, which were precoated with IL-6 monoclonal antibody and then incubated. After that, anti-IL-6 antibodies labeled with biotin were added to unite with streptavidin-horseradish peroxidase, which forms an immune complex. Unbound enzymes were removed after incubation and washing. Substrates A and B were added. Then, the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of IL-6 are positively correlated. Assay range: 2–600 ng/L.

Ethical consideration

This study was approved by Review Ethical committee from the Arab Council for Health Specialization. Written informed consent was obtained from each participant before the collection of the sample.

Statistical analysis

We used SPSS version 26 (IBM Corp., Armonk, NY) with standard approaches of percentages and frequencies for the qualitative variables and mean with standard deviation (SD) for quantitative variables. P <0.05 was considered a significance level for any test throughout the study.

Results

Sixty patients with CLL (newly diagnosed and untreated) were included in this study using the morphological features (blood film and bone marrow) and the immunophenotyping, they were 40 males and 20 females. The patients' age ranged between 33 and 87 years with 60.87 ± 12.448 years as a mean age. Most of them, 85% (51/60), were between 45 and 80 years old.

Twenty healthy normal controls were included, the age of these subjects ranged between 39 and 77 years and they were 11 males and 9 females. Both groups (patients and control) were age and sex-matched.

The stages of CLL, including the total number of cases, the distribution between male and female patients, and the mean age with standard deviation (±2 SD) for each stage were mentioned in Table 1.

The patient groups were summarized in Table 2 according to their symptoms and signs at presentation, lymphadenopathy was the most common presentation, whereas asymptomatic patients were discovered by accident during routine investigations or for other complaints unrelated to the disease.

The correlation was not statistical significance between the patient's age and mean plasma level of IL-6 within the patient group ($P > 0.05$, $r = 0.081$), also within the control group, the age was not significantly correlated ($r = 0.21$, $P > 0.05$).

Sex did not have a significant effect on the plasma level of IL-6 in patients ($P > 0.05$) or within the control group ($P > 0.05$).

Plasma mean level of IL-6 in the cases group was 68.2121 ± 24.24249 ng/L whereas in the control group was 5.05 ± 0.99657 ng/L. This difference had a statistical significance ($P < 0.001$).

Plasma level of IL-6 was higher significantly in Stages B and C compared to those with Stages A ($P = 0.043$) [Table 3].

The total WBC count shows a high correlation to IL-6 level when compared to other peripheral blood parameters (Hb and Platelet count) but is still not statistically significant as shown in Table 4. The correlation was statistically significant at 0.05 level (two tailed).

No statistically significant correlation was found between peripheral blood parameters and plasma IL-6 among patients in CLL stages A, B, and C.

Table 1: Age and sex distribution of the patients according to the stage of 327 the disease

Stage	Stage, *n* (%)	Male, *n* (%)	Female, *n* (%)	Mean age±2SD (years)
A	23 (38.3)	15 (65.2)	8 (34.8)	62±11.314
B	25 (41.6)	16 (64)	9 (36)	56.92±12.688
C	12 (20)	9 (75)	3 (25)	66.92±12.079

SD=Standard deviation

Table 2: The presenting features of the patients included in the study

The complaint	The number of patients (%)
Asymptomatic	16 (26.7)
Splenomegaly	23 (38.3)
Lymphadenopathy	38 (63.3)
Hepatomegaly	14 (23.3)

Some patients may be presented with multiple features such as lymphadenopathy and hepatosplenomegaly

Table 3: Mean plasma interleukin-6 level in patients within the stages of chronic lymphoid leukemia

The stage of CLL	Mean IL-6 (ng/L)	*P*
A	62.2679	0.043
B	66.4404	
C	83.2965	

CLL=Chronic lymphocytic leukemia, IL-6=Interleukin-6

Discussion

Chronic lymphocytic leukemia (CLL) progresses slowly in most cases while generally considered incurable. For many years, many patients with CLL may have normal and active lives. Because of its indolent course, generally, the early stage of CLL does not require treatment and requires only monitoring over time to detect any progression. Rai and Binet staging systems are helpful regarding how and when to treat the patient. While Efforts to discover new markers that are helpful regarding this decision are ongoing; plasma IL-6 might be a reasonable choice.[7]

CLL represents 22%–30% of all leukemias with an incidence between <1 and 5.5 per 100,000 people.[8] In the Iraqi population, CLL constitutes about 15.7% of all leukemia.[9] This could be explained that some cases are undiagnosed (especially the asymptomatic patients) due to the absence of early detection and screening programs in the primary healthcare system in Iraq.

The patients' mean age was 60.87 ± 12.448 years. Most of them, 85%, were between 45 and 80, and none of the patients at the age of 30 years or below, this mean age was also reported in an Iraqi study in Karbala[9] and Iran.[10] This is younger than the mean age in the USA where the Black patients were diagnosed at a younger age compared to white patients (mean age for CLL/small lymphocytic lymphoma is 70–67 years in white versus Black patients),[11] also in Iceland, the mean age was 70.9 years at the time of diagnosis.[12] This can be explained by the epidemiologic distinctions between different regions and races. Furthermore, the quality and ability to access the health systems can explain the geographical differences.

In the current study, there was male predominance with male: female ratio of 2:1. For most patients, 66.7% were males, whereas 33.3% were females, which was the same ratio reported in previous studies in Iraq[13] and India,[14] compared to 1.23 ratio in previous study in Karbala, Iraq[9] and 1.15–1.25 ratio in Japan.[15] This male predominance might be due to occupational exposure to toxic chemicals.

Table 4: The correlation between peripheral blood parameters and mean plasma level of interleukin-6

Peripheral blood parameters vs IL6	Correlation values	Value	*n*	Significant (two-tailed)
IL-6 concentration × Hb (g/dL)	Pearson correlation	−0.181	60	0.167
IL-6 concentration × platelets count (10^9/L)	Pearson correlation	−0.093	60	0.481
IL-6 concentration × total WBC (10^9/L)	Spearman's rho correlation coefficient	0.243	60	0.062

Hb=Hemoglobin, WBC=White blood cell, IL-6=Interleukin-6

Peripheral lymphadenopathy was the most clinical presentation about 63.3% which is similar to the study in Iraq[16] and Turkey,[17] while splenomegaly which was the second-most common in this study and was the most clinical presentation in a study in Erbil, Iraq[18] and Thailand.[19] These results might suggest that routine complete blood count analyses are less common in countries that are still developing or that hospitals in this area fail to implement the right procedures for assessment and management and that patients with incidental lymphocytosis are not referred to them fast enough.

According to the Binet staging system, the distribution of the patients in this study showed that the highest number of the patients (25 patients, 41.6%) fell into Stage B of the disease, which has moderate risk in CLL patients and Stage A (23, 38.3%) which is considered as a low risk, whereas patients with the lowest number (12, 20%) fell into Stage C of the disease, which has the highest risk among all CLL stages. The same results were reported in a previous study in Babil, Iraq;[7] but in Iran[10] and India,[14] the majority are in Stage C. In Molica *et al*.'s study, the majority of the patients were Stage A.[20] This discrepancy might be due to differences in inclusion criteria, study population, and sample size, also improvement in diagnostic tools and better awareness of patients seeking medical services.

Regarding the patient group, the patient's age did not have a statistical significance ($r = 0.081$, $P > 0.05$) on the mean plasma level of IL-6, also within the control group, the age did not have an effect ($P > 0.05$, $r = 0.21$). Gender did not have a significant effect on plasma level of IL-6 ($P > 0.05$) within both groups (patient group and control group). These findings were also reported in a study in Babil, Iraq.[7]

IL-6 plasma level was significantly higher in the patients group than the control group ($P < 0.001$), which is also reported in Iraqi[2] and Egyptian studies.[21] Some studies in the 1980s[22] and the 1990s[23] had found that IL-6 levels were reduced in CLL patients which could be explained by the poor sensitivity of tests at that time utilized for the detection of IL-6. Our study results confirm that IL-6 level has a strong prognostic value in the different stages of CLL. This discrepancy could be explained by the possibility that plasma IL-6 production in patients with advanced stages of the disease is done by other cell types such as macrophages and T-cells in addition to the neoplastic B-cells. The other possibility is that *in vivo* IL-6 production could not be reflected by the *in vitro* experiments.

In this study, the highest IL-6 level was found in Stage C and the lowest IL-6 level was found in Stage A. The mean of plasma IL-6 was found significantly higher in CLL cases presented with B and C in comparison with those with Stage A ($P = 0.043$). This was also found in two previous studies in Iraq where the highest IL-6 was in stage (Rai Stages III and IV) and lowest in (Rai Stages 0, I, and II).[24] This may be explained by a greater bulk of tumor cells in the later stages of the illness.

Recent evidence indicates poor prognosis and outcome of the disease, accompanied by increased levels of IL-6 in the advanced stages of CLL.[25] In a study in Finland, the IL-6 production decreases in advanced stages of the disease.[26] Our results confirm the strong prognostic value of IL-6 in the different stages of CLL. In some studies like fayad *et al*., IL-6 levels correlate significantly with the survival rate.[27] Although WBC count shows a high correlation to IL-6 concentration when compared to other peripheral blood parameters (Hb, platelet count); it is still not statistically significant. In Lai *et al*.'s study, increasing levels of IL-6 significantly correlated with Hb level and WBC count but not the platelets count.[25] Regarding this matter, it is probably that thrombocytopenia, anemia, and leukocytosis in cases with advanced CLL cannot be explained by the aberrant levels of IL-6 alone; abnormal levels of other cytokines may also be responsible.

Conclusions

This study concluded that IL-6 plasma level plays a prognostic factor and the newly diagnosed untreated CLL patients have greater plasma levels of IL-6 than normal control individuals and plasma IL-6 levels correlate significantly with the stage of the disease as its level is the highest in CLL patients with advanced stage of the disease.

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Conflicts of interest
There are no conflicts of interest.

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