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Assessment of CXCL13 plasma level in chronic lymphocytic leukemia and its relation to other prognostic markers

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Abstract:

BACKGROUND: Chronic lymphocytic leukemia is a mature B-cell malignancy where there is a progressive accumulation of leukemic cells with a distinctive immunophenotype as consequence to defective apoptosis and survival signals derived from the microenvironment. CXC chemokine ligand 13 (CXCL13) chemokines have recently emerged as crucial orchestrators for lymphocyte trafficking and activation. These secreted polypeptides exert their function by binding to specific cell surface receptors and can be divided into two categories: homeostatic and inflammatory. The CXCL13 is an efficacious attractant of naive B-cells *in vitro* and has been shown to be produced constitutively by stromal cells in lymphoid follicles of human lymph nodes. The CXCL13-CXCR5 axis has been previously shown to contribute to the progression of several malignancies and possibly CLL relapse.

OBJECTIVE: The aim of this study to compare the plasma level of CXCL13 in a patient with CLL with healthy normal control and to correlate the plasma level of CXCL13 to beta-2 microglobulin (β2 M) and other hematological parameters in CLL.

PATIENTS, MATERIALS AND METHODS: This cross-sectional study was conducted on 50 CLL patients who were newly diagnosed. A total of 30 healthy individuals were included in this study as a control group. Measurement of plasma CXCL13 and beta-2 microglobulin levels was done by the enzyme-linked immunosorbent assay.

RESULTS: Fifty CLL patients were studied and compared with thirty control group of healthy individuals. The mean level of CXCL13 was 67.48 pg/ml in CLL patients while it was 69.2 pg/ml in control, so it is statistically not significant ($P = 0.363$). The mean level of β2 M was 50.89 ug/ml in CLL patients while it was 50.59 ug/ml in control, so it is statistically not significant ($P = 0.702$). The percentage of stage A of CLL patients was 22.44%, stage B was18.36%, and stage C was 10.20%. The percentages of lymphadenopathy, splenomegaly, and hepatomegaly were 66%, 32%, and 16%, respectively. The mean of malignant cell percentage in stage A was 55.18%; in stage B, it was 69.28%; and in stage C, it was 81%, so it is statistically significant ($P < 0.001$). CXCL13 shows no statistically significant between Cd38+ and CD38− and *P* value was 0.950.

CONCLUSIONS: There was no correlation in level of CXCL13 between the CLL group and the control group. There was no correlation in level of CXCL13 and β2 M in the CLL group.

Keywords:

Beta-2 microglobulin, chronic lymphocytic leukemia, plasma CXC chemokine ligand 13 level

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Introduction

Chronic lymphocytic leukemia is a malignancy of mature B-cells characterized by progressive lymphocytosis, lymphadenopathy, splenomegaly, and cytopenia. The progressive accumulation

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of leukemic B-cell is a consequence of defective apoptosis and survival signals derived from the microenvironment. Progressive disease results in dysregulation of the cellular and humeral components of the effector immune system with a resultant increase in the incidence of infectious complication, which constitutes the leading cause of morbidity and mortality in this disease.[1]

Chemokines have recently emerged as crucial orchestrators for lymphocyte trafficking and activation. These secreted polypeptides exert their function by binding to specific cell surface receptors and can be divided into two categories: homeostatic and inflammatory. The CXC chemokine B-cell attracting chemokine 1 (BCA-1)/CXC chemokine ligand 13 (CXCL13) is an efficacious attractant of naive B-cells *in vitro* and has been shown to be produced constitutively by stromal cells in lymphoid follicles of human lymph nodes.[2] Lymphoid chemokines and their counterreceptors are constitutively expressed and control homeostatic trafficking of B- and T-cells to and within secondary lymphoid organs. B-cell-attracting chemokine 1 (BCA-1, CXCL13), which is expressed on putative follicular dendritic cells, is responsible for compartmental homing of CXCR5-bearing B-lymphocytes and also directs T-helper cells into the lymphoid follicle. Expression of this chemokine is considered critical for lymphoid neogenesis.[3]

Beta-2 microglobulin (β2 M) is a light chain of Class I histocompatibility locus antigen (HLA) with 99 amino acids. It is homologous to CH_3 domain of human immunoglobulin. It migrates in beta-2 region on electrophoresis and has a molecular weight of 11.8 kilodalton (K da). It was first isolated from human urine. β2 M is located on the exterior of plasma membranes of all nucleated cells, attached noncovalently to the heavy chain of HLA. Small amounts of β2 M are shed into body fluids regularly, the main source of circulating β2 M being the cells of immune system and liver. Approximately 150–200 mg of β2 M is produced per day.[4] This study aimed to compare plasma level of CXCL13 in patient with CLL with healthy normal control and to correlate plasma CXCL13 to β2 M and other prognostic hematological parameters in CLL.

Patients, Materials, and Methods

This is cross sectional study that included 80 individuals divided into two groups consisting of 50 patients with newly diagnosed CLL and of 30 age and sex matched healthy individuals as a control group (based on their history and complete blood count) and were used for comparison with patients study groups for CXCL13 and β2 M. The study was performed over the course of 5-month period from January to May 2023. The patients were attending at medical city in Baghdad. Diagnosis was based on CBC (as absolute lymphocyte count [ALC] is important for the diagnosis), morphology, and immunophenotyping using eight-color flow cytometer (BD FACSCanto 2 flow cytometer, USA) of peripheral blood samples by an expert hematopathologist in the teaching laboratory of medical city in Baghdad. A study was approved by the Review Ethical Committee of Scientific Council of Pathology in Iraqi Council for Medical Specializations. From each patient, consent was obtained for accepting to take peripheral blood samples. After the diagnosis of CLL has been documented, a 2 ml venous blood sample was aspirated under aseptic technique from each patient included in this study, and the samples were collected in K3-EDTA tubes for CBC, blood films, reticulocyte count, and DAT and then plasma was separated after centrifugation for 10 min at 3000 rpm and was divided into two Eppendorf tubes: one to assay for CXCL13 by using 500 μL of plasma that was collected from the supernatant and carefully stored in Eppendorf tube below (−80°C) at the molecular genetic department in teaching laboratory, until plasma CXCL13 level assay was done by sandwich enzyme-linked immunosorbent assay (ELISA). Moreover, the other Eppendorf tube was used to assay for beta-2 microglobulin by ELISA. From controls, 2 ml of blood was collected in K3-EDTA tube (for CBC, CXCL13, and β2 M). Blood samples were examined for CBC using automated hematology analyzer (ADVIA analyzer/Siemens, Germany).

CD38 and malignant cell percentage were taken from the flow cytometry department in Baghdad Medical City. Statistical analyses were performed using SPSS Statistical Set for Social Science (version 26.0 for windows, SPSS, IBM Corp., Armonk, New York, USA). Differences between the groups were assessed with ANOVA, unpaired *t*-test, Mann–Whitney, Kruskal–Wallis, and Fisher's exact tests. $P < 0.05$ was regarded as statistically significant.

Results

The study included 50 newly diagnosed CLL patients and 30 controls, the mean age of patients was 62 years, and males were 35 patients while females were 15 patients. Most of patients are in stage A, and most frequent presenting sign was lymphadenopathy. In Table 1, Mann–Whitney utilizes the comparison of hematological parameters between patients and controls. No statistically significant difference in CXCL13 and B2-microglobulin was observed between patients and controls, *P* value for CXCL13 = 0.363 and *P* value for B2-microglobulin = 0.702.

In Table 2, Kruskal–Wallis test was utilized the comparison of ALC, smudge cells, malignant cell %, CXCL13, and B2-microglobulin of patients according to stages of disease. ALC shows statistically significant according to stages of diseases A, B, and C, $P = 0.024$. Malignant cell % shows statistically significant according to stages of diseases A, B, and C, $P \leq 0.001$, while smudge cells, CXCL13, and B2-microglobulin show statistically not significant according to the stages of disease.

In Table 3, a study compared ALC, smudge cells, malignant cell%, CXCL13, and β2 M of patients according to CD38. The results show no statistically significant between them.

Table 1: Comparison of hematological parameters (absolute lymphocyte count, smudge cells, CXC chemokine ligand 13, and beta-2 microglobulin) between patients and controls

Parameter	Patients (*n*=50)	Controls (*n*=30)	*P**
ALC (×10³/μL)			
Mean±SD	57.09±43.08	-	-
Median (range)	41.75 (6.5–192)	-	
Smudge cells%			
Mean±SD	21.08±17.49	-	-
Median (range)	15 (1–69)	-	
CXCL13 (pg/mL)			
Mean±SD	67.48±20.27	69.2±16.52	0.363
Median (range)	63.64 (30.71–127.99)	68.26 (32.51–107.38)	
β2 M (ng/mL)			
Mean±SD	50.89±13.66	50.59±15.2	0.702
Median (range)	48.75 (30.93–85.83)	47.78 (30.89–101.75)	

**P* value by Mann–Whitney test. SD=Standard deviation, ALC=Absolute lymphocyte count, CXCL13=CXC chemokine ligand 13, β2 M=Beta-2 microglobulin

Discussion

In this study, CLL was observed more in males (70%) than in females (30%), with an male/female (M: F) ratio 2.3:1 which is near to studies reported previously in Iraq[5,6] and comparable to that of Western countries and other world studies.[7] It is known that CLL is higher incidence in men than women. The reason for this imbalance is unclear.[8] Regarding Binet stages of CLL patients in this study, 44% of patients were in stage A, 36% of patients were within stage B, and the rest were within stage C (20%). Other Iraqi studies also show a high percentage of stage A over stage B and stage C,[9] while other studies show a higher percentage of stage C by Alqasim and Kareem.[10] Other studies in India also show a higher percentage of stage C by Kunnumbrath *et al*.[11] our study was showed higher percentage of stage A, that is probably due to improvement of diagnostic tools like present of flow cytometry in public and private laboratories and better awareness of patients seeking medical services.[12] There was no significance in level of CXCL13 between CLL patients and controls ($P = 0.363$); these results compare with other Egyptian studies and studies of Texas University,[13] but these studies show significance in level of CXCL13 between CLL patients and controls. Our study found no difference in CXCL13 level between different disease stages. This contradicts other studies do in Egypt and Texas university, which reported higher CXCL13 level in advance stages. Also, we observed no significant correlation between CXCL13 and beta2- macroglobulin levels. Again this differs from the Egyptian and Texas university studies which show higher level of CXCL13 in higher level beta2- macroglobulin. There was no significant correlation emerged between CXCL13 level and WBC count. This also contradicts studies done in Egypt and Texas university which showed higher CXCL13 level associated with higher WBC count.

Table 2: Comparison of parameters (absolute lymphocyte count, smudge cells, malignant cells %, CXC chemokine ligand 13, and beta-2 microglobulin) of patients according to the stage of disease

Parameter	Stage A (*n*=22)	Stage B (*n*=18)	Stage C (*n*=10)	*P**
ALC (×10³/μL)				
Mean±SD	38.81±21.77	64.58±44.54	83.8±59.41	0.024*
Median (range)	35.9 (6.5–79.8)	54 (20–186)	68.5 (22.5–192)	
Smudge cells (%)				
Mean±SD	26.27±18.25	15.89±12.88	19±21.24	0.096*
Median (range)	24.5 (3–69)	10 (2–48)	10 (1–55)	
Malignant cell (%)				
Mean±SD	55.18±20.39	69.28±10.1	81±6.15	<0.001**
Median (range)	57.5 (13–83)	71 (48–85)	81.5 (72–90)	
CXCL13 (pg/mL)				
Mean±SD	73.39±24.27	64.05±13.79	60.65±18.51	0.238*
Median (range)	70.37 (39.14–127.99)	63.07 (30.71–91.5)	53.2 (39.81–96.86)	
β2 M (ng/mL)				
Mean±SD	46.18±9.52	51.94±12.75	59.36±19.02	0.100*
Median (range)	47.22 (30.93–61.85)	51.39 (34.41–78.48)	58 (35.6–85.83)	

**P* value by Kruskal–Wallis test, ***P* value by ANOVA. SD=Standard deviation, ALC=Absolute lymphocyte count, CXCL13=CXC chemokine ligand 13, β2 M=Beta-2 microglobulin

Table 3: Comparison of the parameters of patients according to CD38

Parameter	Positive (*n*=5)	Negative (*n*=45)	*P*
ALC (×10³/μL)			
Mean±SD	66±50.67	56.1±42.7	0.706**
Median (range)	48 (20–147.5)	39.9 (6.5–192)	
Smudge cells (%)			
Mean±SD	24±20.38	20.76±17.38	0.788**
Median (range)	15 (6–55)	15 (1–69)	
Malignant cell (%)			
Mean±SD	71±10.91	64.8±18.56	0.615**
Median (range)	72 (59–86)	70 (13–90)	
CXCL13 (pg/mL)			
Mean±SD	67.99±21.11	67.42±20.42	0.950**
Median (range)	78.32 (42.22–91.5)	63.15 (30.71–127.99)	
β2 M (ng/mL)			
Mean±SD	51.05±21.93	50.87±12.81	0.987*
Median (range)	38.25 (34.41–83.33)	48.75 (30.93–85.83)	

**P* value by unpaired test, ***P* value by Mann–Whitney test. SD=Standard deviation, ALC=Absolute lymphocyte count, CXCL13=CXC chemokine ligand 13, β2 M=Beta-2 microglobulin

There was no significant in CXCL13 level in compare with smudge cells percentages among patients. This contradict Egyptian study that shows higher level of CXCL13 in those patients who have high percentages of smudge cells. The interpretation of these unexpected results may related to infections, and many infections lead to an increase in plasma level of CXCL13.[14] Also may related to limited number of patient we studied, if there were more patients may get appropriate results. Beta-2 microglobulin show no correlation between the CLL group and the control group in contrast to other studies,[15] and this may related to small sample size. There was a significant correlation between β2 M and platelets ($P = 0.001$), but we could not find any studies to explore this correlation. CD38 was expressed in only 10% of cases of CLL which is lower than other Iraqi studies.[16] These differences may be due to the choice of different cutoff values for the number of CD38+ve cells or due to the size of the group. Malignant cells percentage were high in advance stages of CLL, so it is statistically significant (P>0.001).

Conclusions

This study revealed no correlation in CXCL13 level between patients and controls and also showed no correlation between CXCL13 level and β2 M, CD38, Binet staging, organomegaly, and lymphadenopathy. β2 M expression was statistically significant in correlation with platelets.

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Conflicts of interest

There are no conflicts of interest.

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