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Evaluation of Serum Level of CXCL12 in Correlation with Several Clinical and Hematological Parameters in Patients with Chronic Lymphocytic leukemia

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Abstract:

Background: Chronic lymphocytic leukemia (CLL) is the commonest leukemia in western countries. The disease typically occurs in elderly patients and has a highly variable clinical course, transformation is initiated by specific genomic changes that impair apoptosis of clonal B-cells. C-X-C Motif chemokine Ligand 12(CXCL12) is a chemotactic factor for T cells and monocytes, and in B cell lymphopoiesis and bone marrow myelopoiesis. CXCL12 is a 68-amino acid small (8 kDa) cytokine that belongs to the CXC chemokine family. It had been reported as key mediator for angiogenesis and inflammation, also it expressed by bone marrow stromal cells in CLL patients and it is responsible for CLL cells chemotaxis in to the tissue microenvironments. **Aims of the study:** To assess CXCL12 expression in patients with chronic lymphocytic leukemia and to correlate their expression with different clinicopathological and hematological parameters . Materials and Methods: the study included 80 subjects divided in to two groups: Patient group included 40 adult patients with CLL and control group included 40 healthy age and gender-matched controls. All hematological parameters were done by automated hematological analyzer. Serum CXCL12 was estimated in both patients and control subjects by ELISA technique. Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences-version 25). Results: Serum CXCL12 expression was significantly higher in CLL patients compared to controls .There was significant inverse correlation between CXCL12 expression and hemoglobin level and significant direct correlation between its expression and total W.B.C. Count and absolute lymphocyte count , significant direct correlation had been also found between CXCL12 expression and advanced Ria stage .**Conclusions:** CXCL 12 expression was significantly higher in CLL patients than control group, there is significant inverse correlation between CXCL 12 and hemoglobin levels, significant direct correlation between its expression and total W.B.C. count and absolute lymphocyte count , there was no correlation between CXCL12 expression and platelets count and Serum level of CXCL12 is directly correlated with advanced Ria stage.

Key words: *chronic lymphocytic leukemia, chemokines ,CXCL12*

Introduction:

Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world, characterized by the accumulation of monoclonal B cells with the appearance of small mature looking lymphocytes. One of the most interesting features of the disease is its clinical heterogeneity with some patients

progressing rapidly with early death, whereas others exhibit a more stable non progressive disease lasting many years.(1)

Chemokines play an important role in the evolution of cancers as they are involved in tumor growth, angiogenesis, metastasis and immune evasion. (2)

The expression of chemokines and their receptors is altered in many malignancies and subsequently leads to aberrant chemokine receptor signaling. This alteration occurs due to inactivation of the tumor suppressor genes or constitutive activation of the oncogenes that play a role in the regulation of the chemokines.(3,4)

The stromal cell-derived factor 1 (SDF1), also known as C-X-C motif chemokine 12 (CXCL12), is a chemokine protein that in humans is encoded by the CXCL12 gene on chromo-

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some 10. It is ubiquitously expressed in many tissues and cell types .(5)

In CLL, stromal cells in the bone marrow secrete CXCL12, which bind to a variety of corresponding receptors on the CLL cell surface. (6,7)

These interactions lead to CLL cell chemotaxis into the tissue microenvironments, where the malignant cells are then subject to survival and proliferation signals through the cell receptor and other pathways. Once in the tissues, the CLL cells are surrounded by a supportive microenvironment that provide additional survival and anti-apoptotic signals to those cells. moreover, higher serum level of CXCL12 are detectable in patients with B-CLL compared to the normal controls ,in addition elevated level of CXCL12 has been associated with aggressive clinical behavior , suggesting a role in the pathogensis of BCLL. (8,9)

Patients, Materials and Methods:

It is a cross-sectional study, samples from eighty subjects were included in this study, and they were classified in to two groups: - 1- forty adult patients group, newly diagnosed, CLL patients, 29 of them were males and 11 were females.2- forty healthy subjects as a control group. a total venous blood sample of 3 ml by venipuncture from antecubital fossa was obtained from the patients and control groups under aseptic technique, The drawn sample was put in serum separator tube (SST) and samples were allowed to clot for two hours at room temperature, centrifugation for 15 minutes at 1000 ×g. Serum were collected in Eppendorf tubes in 2 aliquot for each sample. The serum stored at - 80˚C for up to 6 months, and used for measuring serum CXCL12 level by enzyme linked immunosorbent assay (ELISA).

Inclusion criteria

Adults' patients, patients diagnosed with CLL not receiving treatment.

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means or ANOVA test for difference among more than two independent means. Statistical significance was considered whenever the P value was equal or less than 0.05.

Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation.

Results:

Forty CLL patients were recruited in this study. There were 29 men (72.5%) and 11 women (27.5%). At the time of diagnosis, patient ages ranged from 42 to 88 years (mean 63.3±11.4 years). The mean ± SD for hemoglobin, total leucocyctic count, lymphocytes count and platelets for CLL patients were (11.50±2.49 gm/dl), (82.50±84.25 x109/L), (49.19±49.75 x109/L) and (163.85±76.01 x109/L) respectively.

CXCL12 mean level was 5.55 ± 2.42 pg/ml (mean ± SD) in CLL patients, the range was 1.82-11.08 pg/ml, this level was significantly higher in CLL patients compared with the age and sex-matched healthy control group (P value was 0.0001*)as shown in (table1)

Table (1) CXCL12 expression in CLL patients and control group (no=80).

(CXCL 12 (pg/mL	CLL patients		Healthy controls		P value
	No	%	No	%	
pg/mL 1>	-	-	2	5.0	*0.0001
1.0---	1	2.5	14	35.0	
2.0---	7	17.5	22	55.0	
3.0---	6	15.0	2	5.0	
4.0---	3	7.5	-	-	
5.0---	6	15.0	-	-	
6.0---	6	15.0	-	-	
7.0---	5	12.5	-	-	
8.0---	2	5.0	-	-	
9.0---	2	5.0	-	-	
pg/mL 10<=	2	5.0	-	-	
(Mean±SD (Range	(1.82-11.08) 5.55±2.42		(0.60-3.97) 2.24±0.72		
*Significant difference between proportions using Pearson Chi-square test at 0.05 levels.					

Regarding the relation of CXCL12 expression with age and gender, there was no significant correlation between CXCL12 and age or gender in CLL patients. As shown in (table 2):

Table (2) Relation of CXCL12 expression with age and gender in CLL patients and control (no=80)

	CXCL 12 (pg/mL)					
	CLL patients		Healthy controls		P value	
	No	Mean±SD (Range)	No	Mean±SD (Range)		
Age (years)	40---49	5	(2.16-5.50) 3.74±1.36	10	(1.32-2.77) 2.27±0.60	0.011*
	50---59	9	(1.82-11.08) 5.32±3.14	13	(1.40-2.84) 2.32±0.53	0.003*
	60---69	9	(2.77-8.64) 6.24±1.93	10	(0.60-3.97) 2.22±1.10	0.0001*
	70---79	14	(2.17-10.18) 5.87±2.61	6	(1.29-2.77) 1.97±0.66	0.002*
	years 80<=	3	(5.56-6.14) 5.79±0.31	1	2.77±	0.013*
	P value		0.436		0.828	
Gender	Male	29	(2.16-11.08) 5.50±2.40	22	(1.40-3.97) 2.37±0.65	0.0001*
	Female	11	(1.82-9.18) 5.71±2.57	18	(0.60-2.77) 2.08±0.80	0.0001*
	P value		0.810		0.212	
*Significant difference between two independent means using Students-t-test at 0.05 level						
#Significant difference among more than two independent means using ANOVA test at 0.05 level						

Regarding the relation of CXCL12 expression with different clinical characteristics, there was no significant relation between CXCL12 and different clinical sign and symptoms. As shown in (table 3)

Table (3) Relation of CXCL12 expression with different clinical characteristics

CLL patients		CXCL 12 (pg/mL)		P value
		No	Mean±SD (Range)	
Signs and Symptoms				
Fever	Yes	13	(3.57-9.18) 5.92±1.69	0.509
	No	27	(1.82-11.08) 5.38±2.71	
Fatigue	Yes	26	(1.82-10.18) 5.54±2.30	0.968
	No	14	(2.16-11.08) 5.58±2.71	
LN enlargement	Yes	15	(1.82-11.08) 6.05±2.56	0.321
	No	25	(2.16-9.18) 5.26±2.33	
Bleeding tendency	Yes	4	(5.50-8.64) 6.81±1.32	0.280
	No	36	(1.82-11.08) 5.42±2.48	
Diagnosed during routine check up	Yes	7	(2.16-9.09) 4.09±2.54	0.077
	No	33	(1.82-11.08) 5.87±2.31	
Hepatomegaly	Yes	5	(1.82-6.69) 5.23±1.96	0.752
	No	35	(2.16-11.08) 5.60±2.50	
Splenomegaly	Yes	8	(1.82-9.18) 6.45±2.32	0.248
	No	32	(2.16-11.08) 5.33±2.42	
LN adenopathy	Cervical	8	(2.55-11.08) 6.12±2.57	0.611
	Multiple	7	(1.82-10.18) 5.97±2.74	
	No	25	(2.16-9.18) 5.26±±2.33	
*Significant difference between two independent means using Students-t-test at 0.05 level				
#Significant difference among more than two independent means using ANOVA test at 0.05 level				

Regarding the relation of CXCL12 expression with disease stage, For correlation of marker expression to Ria stage , the level of CXCL12 was significant with Ria stage and increased with direct correlation with increasing stage of CLL patients (P value 0.023).As shown in(table 4)

Table (4) Relation of CXCL12 expression with disease stage

CLL patients		CXCL 12 (pg/mL)		P value
		No	(Mean±SD (Range	
Ria Stage	0	15	(2.17-8.69) 4.37±2.06	0.023*
	1	6	(2.55-7.74) 5.10±1.86	
	2	3	(1.82-6.69) 4.75±2.58	
	3	6	(5.56-11.08) 7.70±2.11	
	4	10	(2.16-10.18) 6.56±2.44	
Significant difference between two independent means using Students-t-test at 0.05 level*				
Significant difference among more than two independent means using ANOVA test at 0.05 level#				

Regarding the relation of CXCL12 expression with hematological parameters, there was inverse correlation between CXCL12 level and hemoglobin level in which CXCL12 was significantly higher in patients with low hemoglobin level (p value 0.0001)as shown in (table 5)

Table (5): Relation of CXCL12 expression with hematological parameters

CLL patients		Mean CXCL 12 (pg/mL)	
		Healthy controls	
(Age (years	r	0.228	-0.100
	P	0.156	0.540
Hemoglobin	r	-0.626**	-0.001
	P	0.0001	0.993
WBC count	r	0.376*	0.028
	P	0.017	0.863
Lymphocytes count	r	0.366*	-0.136
	P	0.020	0.403
Platelets count	r	-0.121	0.023
	P	0.455	0.887
**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level (2-tailed).			

According to(table 5),There was significant direct correlation between CXCL12 (pg/ml) and total

W.B.C. count and absolute lymphocyte count, CXCL12 level was significantly higher in patients with elevated W.B.C. count and lymphocyte count (p value 0.017 and 0.020 respectively), There was no significant correlation between CXCL12 and platelet count.

Discussion:

In this study, the mean age of all patients included was 63.3±11.4 SD, with a range of (42-88) years old. These results were comparable to other Iraqi studies(10, 11, 12) and Egyptian study in 2015. (13) While there were higher median ages of presentation in the studies of western countries, it was reported to be 71 years old. (14, 15) this discrepancy may be attributed to the effects of environmental factors and genetic predisposition between Iraq and western countries.

In this study, CLL cases were observed more in male (72.5%)Than in females with an male/female (M: F) ratio of 2.6:1 which is near to studies reported previously in Iraq (10,11) but this finding is higher than that reported in western studies like that reported by (Cantu ES etal). Which is nearly 1.5? (16) This difference can be due to variation in population structure between two countries and due to differences in sample size. But in all studies the finding of CLL male predominance was obvious which might be related to genetic base. (16)

The two most frequent signs include lymphadenopathy and fever (37.5% and 32.5%, respectively) followed by splenomegaly and hepatomegaly (20% and 12.5% respectively).

These results were comparable to that published by Mohammed S. et al.(11) who reported that the most frequent sign is lymphadenopathy.(Jasim HN et al.) (12) also reported that most common presenting sign was lymphadenopathy, also western studies reported that lymphadenopathy was more common sign than splenomegaly and hepatomegaly. (17,18)

By applying Rai staging system in this study, 37.5% of the patients were Rai stage 0, 25% were within Rai stage IV, 15% were within Rai stage I,III, and the remaining cases were within Rai stage II (7.5%), this result is comparable with Egyptian study published by(Nashwa N., Omnia A. et al.) (13) who reported that 35% of patients were within stage II,42.5% of patients were within stageIII and 22.5% were within stage IV . In contrast, to the western countries studies that showed lower percentage of patients that fell within stage III and IV (19) , this may be attributed to the regular checkup of their patients and advanced presentation of our patients.

For the hematological parameters of the patients, the mean Hb level was 11.50±2.49 g/dl (Mean ±SD) and the mean platelet count was 163.85±76.10x 109 /L (Mean ± SD) and absolute lymphocyte count in the peripheral blood was 49.19 ± 49.75 × 109 / L (Mean ± SD) , these results were comparable to the Iraqi studies results (11,20) also by Western study by (Cavalcanti Júnior GB et al. 2005). (21) Bone marrow involvement by leukemic cells or by other pathology like splenic pooling are the most probable causes of anemia and thrombocytopenia in these patients . To exclude autoimmune hemolytic anaemia DAT was performed on all patients included in this study and was negative in all cases.

Regarding CXCL12 expression, among 40 newly diagnosed CLL patients with 40 healthy controls) age and sex matched patients), CXCL12 level was much higher in CLL patients compared with control group, and these were comparable to the results obtained by Egyptian study done by(Nashwa N.Omnia ,et al 2015). (13) This elevation in serum CXCL12 noted in B –CLL patients attributed to the alternation in the expression of chemokine and their receptor (CXCR4) and that subsequently leads to aberrant chemokine receptor signaling mainly due to inactivation of tumor suppressor gene or activation of oncogene (which regulate the chemokines action) .

The circulating CLL cells in the peripheral blood typically expressing high levels of surface (CXCR4) so if stimulation of(CXCR4) by CXCL12, signaling through the CXCR4 receptor has pleotropic effects on CLL cells, and activation occur through different pathways as serine phosphorylation of signal transducer and activator of transcription 3 (STAT3). For correlations of CXCL12 with hemoglobin, there is inverse correlation between CXCL12 level and hemoglobin level in which CXCL12 was significantly higher in

patients with low hemoglobin level and this could explain the higher risk of disease progression with decrease overall survival if elevated CXCL12 expression in CLL patients which mentioned in other study(Burger etal 2005) who demonstrated that CLL cells migration in response to CXCL12/CXCR4 depend on CD38 expression on cell surface and this mean that CD38 synergies with CXCL12 /CXCR4 pathway. (22)

The primary role of CXCL12 appears to be the mobilization of hematopoietic stem cells (including erythroid lineages) and the establishment of the cancer stem like cell niche. For CXCL12 correlation with total WBC count and absolute lymphocytes count there is direct correlation between the CXCL12 and WBC count ,this result is comparable to the study done by(Dao-ung LP etal 2004). (23)

For marker correlations with Rai staging, there was progressive increase in CXCL12 level in correlation to the stage of the disease. Specifically; patients having a higher CXCL12 level had a shorter survival time . this effect was independent of another prognostic factor. concordant results were reported by (Ghobrial IM etal 2004)(19) .who show significant correlation between the CXCL12 level and Ria staging system, other study done by (Barretina J,etal 2003). (24) show no correlation of (SDF1alpha)/CXCL12 with Binet staging of the disease.

There is no significant correlation found between CXCL12 expression and other clincopatholgical and hematological parameters in this study (age, organomegally, platelet count … etc.), this could be attribute to small sample size, no study about such association has been reported till now

Conclusions:

CXCL 12 expression was significantly higher in CLL patients than control group. There is no significant correlation was found between CXCL12 expression and clinical characteristics of the disease. this study revealed significant inverse correlation between CXCL12 and hemoglobin levels, significant direct correlation between its expression and total W.B.C. count and absolute lymphocyte count and there was no correlation between the marker expression and platelets count. Regarding relation with the Ria stage, Serum level of CXCL12 is directly correlated with advanced Ria stage.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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