Assessment of Interleukin-8 in Patients with Chronic Lymphocytic Leukemia in Correlation with the Prognostic Factors: β2-microglobulin, LDH and Binet Stage

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ABSTRACT:

BACKGROUND:

Chronic lymphocytic leukemia is a malignancy of mature B cells characterized by progressive lymphocytosis, lymphadenopathy, splenomegaly, and cytopenia. The progressive accumulation of leukemic cells is a consequence of defective apoptosis and survival signals derived from the microenvironment.

OBJECTIVE:

Is to assess the levels of interleukin-8, β_2-microglobulin and lactate dehydrogenase in chronic lymphocytic leukemia patients and evaluate the prognostic value of interleukin-8 in correlation with β_2-microglobulin, lactate dehydrogenase and Binet stage.

PATIENTS AND METHODS:

A case-control study enrolled 40 newly diagnosed chronic lymphocytic leukemia patients attended at Oncology Teaching Hospital, Medical City with 40 adult healthy volunteers as a control group. Interleukin-8, β_2-microglobulin and lactate dehydrogenase were investigated.

RESULTS:

There were statistically significant relationships between Binet staging and each of following: plasma level of interleukin-8, serum level of β_2-microglobulin and lactate dehydrogenase and (P= 0.005, 0.005 and 0.023, respectively). There were significant correlations between β_2-microglobulin and both interleukin-8 and lactate dehydrogenase level (P= 0.005, both) but correlation between interleukin-8 and lactate dehydrogenase was insignificant.

CONCLUSION:

Interleukin-8 level was positively correlated with β_2-microglobulin, and higher plasma levels were associated with advancing Binet stage which makes it a reliable marker for patients at late clinical stage.

KEYWORDS: chronic lymphocytic leukemia, interleukin-8, β_2-microglobulin, lactate dehydrogenase

INTRODUCTION:

Chronic lymphocytic leukemia (CLL) is a chronic B-cell lymphoproliferative disorder characterized by progressive lymphocytosis caused by the clonal accumulation of small, monomorphous, monoclonal B cells in peripheral blood (PB), bone marrow (BM), and lymphoid organs,[1] in which there will be progressive lymphocytosis, lymphadenopathy, splenomegaly, and cytopenias. Progression of disease results in dysregulation of the cellular and humoral immunity with a resultant increase in the incidence of infectious complications, which constitutes the leading cause of morbidity and mortality in this disease.[2]

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Unusual for tumor cells, CLL cells circulating in the peripheral blood are cell cycle arrested. Ex vivo, CLL cells rapidly die from apoptosis if not co-cultured with immune or stromal cells, suggesting that the malignant cells are in constant need of supportive signals from the lymphoid microenvironment. It is therefore believed that at least a sub-population of the peripheral blood CLL pool is able to recirculate into lymphoid organs in order to receive signals for proliferation and survival. Moreover, retention in these organs appears to favor onset and progression of CLL.[3]
The malignant B cells of patients with CLL constitutively express interleukin-8 (IL-8) and IL-8 receptors. Ex-vivo culture with exogenous IL-8 enhances IL-8 expressions and prolongs leukemia cell survival, partly through increased Bcl-2 expression. IL-8 may function as an

autocrine growth and apoptosis resistance factor in CLL.[4] An essential effect of IL-8 is its initiation of leukocyte infiltration and neovascularization, which precedes invasion and metastasis. This tumor progression may occur as a function of the regulation of angiogenesis, cell motility, immune cell infiltration, cell growth and survival in the microenvironment, and modulation of local antitumor immune responses.[(5)]

β_2-microglobulin (β_2M) is released by CLL cells and its level approximately correlates with tumor mass.[6] Increased serum β_2M level that exceed 2-time the upper limit of normal are associated with increased tumor burden, extensive BM infiltration and shorter treatment–free and overall survival regardless of age or creatinine clearance.[(7,8)] Increased total serum lactate dehydrogenase (LDH) is commonly interpreted as reflecting high tumor burden or tumor aggressiveness and carries a poor prognosis in CLL.[(9)]

PATIENTS, MATERIALS AND METHODS:

The study population consisted of forty adult, newly diagnosed, CLL patients and forty healthy adult controls from April 1, 2016 to November 3, 2016. The patients were attending the Hematology outpatient clinic at Oncology Teaching Hospital of the Medical City. Diagnosis was based on morphology and immunophenotyping of the PB cells and was established in the Teaching Laboratories and Nursing Home Hospital of the Medical City in Baghdad. Immunophenotyping was done using a four-color flow cytometer (BD FACS Canto™II Flow Cytometer, UAS).

Diagnosis of CLL was settled according to International Workshop criteria:[10]

i. Presence in peripheral blood of $>5\times10^9$/L monoclonal B-lymphocytes persisting for at least 3 months.

ii. Demonstration of the clonality of the population (i.e., κ/λ analysis).

iii. Characteristic immunophenotype (IPT): shows CD19+, CD5+, CD23+, FMC7 absent/weak, SmIg (κ/λ) weak and CD79b absent/weak, giving a score of 4 - 5.

Serum and plasma samples were separated from peripheral blood samples; serum was put into two Eppendorf tubes one for LDH level assessment within six hours (stable for three days at room temperature) and the other tube was stored at -80°C with another plasma Eppendorf tube for assessment of β_2M and IL-8, respectively, by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Human Interleukin-8 ELISA Kit Biological Technology Co., Ltd. and Human β_2M ELISA Kit Demeditec Beta-2-Microglobulin ELISA). Serum LDH was measured by Dimension® clinical chemistry system LDI Flex® reagent cartridge (for LDH). Preparation of samples and reagents were conducted according to manufacturing company leaflet.

Statistical Analyses were performed using SPSS statistical package for Social Sciences (version 17.0). Data are presented as median for quantitative variables and as number and percentage for qualitative variables. Kruskal-Wallis and Mann-Whitney *U* tests were used for the difference between multiple groups. Pearson correlation and Spearman's rho were used to test the correlations between different variables Qualitative data relations were analyzed by Chi square test.

Statistical significance was considered whenever the *P* value was less than 0.05.

RESULT:

The mean age of CLL patients included in this study was 62.4 ± 10.8 (mean ±SD) years and median age of CLL patients was 64.50 years were the control group mean age was 55.9 ± 11.0 years while the median age was 56 years. CLL patients 23/40 (57.5%) of them were males and 17/40 (42.5%) were females with an M: F ratio of 1.4: 1. According to Binet stages at diagnosis; 20/40 (50%) of patients were stage A while 9/40 (22.5%) were stage B and 11/40 (27.5%) were stage C.

The median plasma level of IL-8 was 115.3 pg/mL in CLL patients whereas in the control group it was 123.7 pg/mL. There was statistically insignificant difference (*P*= 0.597) while median level of β_2M was 4.754 mg/L in CLL patients whereas control group it was 2.299 mg/L; there was statistically significant difference (*p*= 0.005) Table 1.

Table 1: Comparison of median plasma level of IL-8 β_2M between control and CLL group.

	Group				P value*
	CLL		Control		
	Median	IQR**	Median	IQR	
IL-8 pg/mL	115.310	52.9-220.3	123.735	60.6-161.8	0.597
β_2M mg/L	4.754	3.4-9.2	2.299	1.9-2.5	0.005

*Mann-Whitney U test
** IQR: Inter quartile range

The LDH level was not performed for the control group and comparison was with the normal range (125- 220 u/L)[11] where 33 of CLL patients had elevated LDH level as illustrated in Table 2.

Table 2: Level of LDH in CLL patients

Level of LDH	Number of CLL patients	percent
< 220 u/L (normal)	7	17.5
≥ 220 u/L (increased)	33	82.5
Mean ± SD: 402.72 ± 164.87 Median: 403.3 u/L IQR: 151-889		

There were statistically significant relationships between Binet staging and each of following: plasma level of interleukin-8, serum level of β_2-microglobulin and lactate dehydrogenase with *P*= 0.005, 0.005 and 0.023, respectively (Table 3).

Table 3: Relationship between IL-8, β_2M and LDH with Binet stage.

Stage		A	B	C	P value
IL-8 ng/L	N(number of patients)	20	9	11	0.005*
	Median	64.65	78.75	362.76	
	Minimum	2.500	11.900	179.302	
	Maximum	207.237	195.000	1534.500	
□□□ mg/L	N(number of patients)	20	9	11	0.005*
	Median	3.43	4.76	12.75	
	Minimum	1.541	3.836	3.583	
	Maximum	5.496	10.000	16.7	
LDH u/L	N(number of patients)	20	9	11	0.023*
	Median	352.5	408.0	500.0	
	Minimum	151	200	235	
	Maximum	889	563	633	

Kruskal-wallis test
*Significant difference between stages.

The median of absolute lymphocyte count (ALC) for CLL patients was 69.8 $\times 10^9$/L and IQR (29.7-131.7). There were significant positive correlations between median of ALC and the median levels of IL-8 and LDH (*P*= 0.004 and 0.001, respectively), while no significant correlation was found with β_2M (Table 4).

Table 4: Pearson Correlations of IL-8, β_2M and LDH with ALC.

			P-value of these marker with ALC	
ALC $\times 10^9$	N(number)	40	r*	1
	Median	69.8	p**	
	IQR	29.7- 131.7		
IL-8 ng/L	N (number)	40	r	.444
	Median	115.3	p	.004
	IQR	52.9- 220.3		
β_2M mg/L	N (number)	40	r	.164
	Median	4.7	p	.312
	IQR	3.4- 9.2		
LDH u/L	N (number)	40	r	.492
	Median	403.3	p	.001
	IQR	246- 499.5		

*r= Pearson Correlation
**p= Correlation is significant at the 0.001 level (2-tailed).

Correlations between IL-8, β_2M and LDH with each other: the correlation between median of IL-8 and median of β_2M levels was significant positive correlation (*P*= .000**)**, also positive correlation between median of β_2M and median of LDH levels with *P*-value = 0.005 but correlation between median of IL-8 and median of LDH insignificant *p*-value =0.214 (Table 5).
The median and IQR of IL-8, β_2M and LDH same of table 4.

Table 5: Correlation and relationship of IL-8, β_2M and LDH with each other's.

		IL-8	β_2M	LDH
IL-8	r*	1		
	P**	-		
β_2M	r	.542	1	
	P	.000**	-	
LDH	r	.201	.433	1
	P	.214	.005**	-

*r= Correlation Coefficient
**p= Spearman's rho Correlation is significant at the 0.01 level (2-tailed).

DISSCUSION:

B-CLL is a heterogeneous disorder characterized by a variable clinical course. Some patients have an aggressive disease requiring early therapy, whereas other patients show a more stable, indolent disease with no benefit from palliative chemotherapy. Several prognostic markers have been introduced to identify patients with a poor prognosis and to facilitate the clinical management of B-CLL.[12]

In this study, level of IL-8 in CLL patients insignificantly different from IL-8 in health controls, this was similar to that reported by an Italian study[13] in 1999 but it was in contrast to Turkish study[14] in 2007, Canadian study[15] in 2012 and Texas study[4] in 2003. The latter studies reported significant difference between patients and controls. This may be explained by the larger sample size of the other studies.

Serum level of β_2M showed statistically significant difference between patient and control which was comparable to an Egyptian study in 2016.[16]

The statistically significant relationship between IL-8 plasma level and Binet staging (*P*= 0.005) is comparable to that reported by El-Morshdy MS et al. in Egypt 2016,[17] and Wierda et al. in Texas (2003).[4]

The plasma level of β_2M is thought to reflect both tumor burden and renal function, and has been shown to be a very important predictor of response to chemotherapy, time to treatment, duration of response and overall survival However, in current study patients with advance Binet stage had elevated level of β_2M with p-value = 0.005 and this is agreement with reported in Egypt 2012,[12] Canada study in

2012.[15] patients who have high level of β_2M have a shorter treatment-free survival than those with normal levels, suggesting that other factors apart from tumor load can increase β_2M levels.[15]

LDH has statistically significant relationship with Binet staging (p-value = 0.023) which is comparable to that reported by El-Hussiny MN et al. in Egypt 2012[12], Shen QD et al. in China 2007 [18]

Staging system provide a basic framework for estimation of prognosis and are factored into the current international workshop on CLL guidelines for initiation of treatment, based on these guidelines, individual with stage C would meet the criteria for therapy.[19]

The association of IL-8 with other poor prognostic parameters as ALC and higher β_2M level. The correlation of IL-8 with ALC was statistically positive correlation with p-value=0.004 this result is comparable with that to reported by El-Morshdy MS et al. in Egypt 2016.[17] But in contrast to that reported by Molica et al. 1999 in Italy [13] which comparison patients stage A only.

Correlation of serum level of β_2M with ALC was statistically insignificant with p-value=0.312 this result was consistent with the study of Shen QD et al. in china 2007.[18]

Correlation of LDH with ALC was statistically positive correlation with p-value = 0.001 this result is consistent with that of Tsimberidou AM et al. in Texas 2007. [20]

The significant positive correlation of IL-8 with β_2M level was comparable to a Canadian study in 2012,[15] and to Texas study in 2003.[4] This combination of IL-8 and β_2M levels can be used to delineate groups of patients with either very bad or very good prognosis, thus providing the potential for stratification of CLL patients for better therapeutic approaches.[4]

Correlation between IL-8 and LDH was statistically insignificant, similar results were reported by Molica et al.[13] in Italy (1999).

Correlation of LDH with β_2M was statistically positive correlation with p-value =0.005 which comparable to that reported by El-Hussiny MN et al. in Egypt 2012 [12] high level of serum LDH which measure of tumor burden and turnover is associated with rapid disease progression and worse clinical prognosis in CLL patients in which Shen et al.[18] found that the overall survival time in group of elevation of both LDH and β_2M levels was shorter than that in group of normal levels of both LDH and β_2M

CONCLUSION:

Interleukin-8 level was positively correlated with advancing Binet stage which makes it a reliable marker for patients at late clinical stage.

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