Soluble CD14: a new Prognostic Factor in Patients with Chronic Lymphocytic Leukaemia

Sabreen Hameed Zghair[1], Najlaa B. Alawadi[2], Athraa Falah Alshimerry[3]
[1, 2, 3]Department of Pathology, College of Medicin, Babylon University, Babylon, Iraq
Email: sabreenhalgubory@gmail.com

Abstract
Background: CLL is presently an incurable disease characterized by progressive accumulation of mature lymphocyte in bone marrow and peripheral blood cause heterogeneous clinical course ranging from indolent to aggressive disease. So, many efforts focus on finding reliable indicators that can help to predict the outcome or explain clinical variability. Cluster of differentiation 14 (CD 14) has been emerged as one of these indicators, But it needs thorough assessment for its prognostic capacity in CLL. This gave us the ardor to carry out this study. Objective: the current study was conducted to assess the level of CD14 in serum of CLL patients and evaluate its relation to clinical stage and other prognostic parameters and study its probable prognostic role in CLL patients. Patients and methods: forty five Iraqi newly diagnosed B-CLL patient and forty five age and sex matched healthy control were included in this study for evaluation of serum CD14 by Enzyme Linked Immunosorbent Assay'' ELISA''. Results: The mean serum CD14 level was found to be significantly higher in patients' serum than control and it was higher among patients with advanced stage (Binet stage C and high risk (III-IV) Modified Rai Stage). There had been a significant relation between sCD14 level and patients with organomegaly and those with specific complications. There were no mean differences between CD14 level and patient's age or gender and there was no significant relation to hematological parameters. Conclusions: sCD14 level was significantly higher in CLL patients than control group.
Keywords: CLL, CD14, Prognostic factor.

1. Introduction

Chronic lymphocytic leukemia (CLL) is a lymphproliferative disorder recognized with accumulated small mature lymphocytes at lymph nodes, bone marrow, blood, liver, spleen, or often at other organs. These lymphocytes are marked with mature morphology and immature biology [1]. CLL is a prevalent leukaemia that affects adults in the Western world, accounting for about 30% of all leukaemias and approximately 10% of all haematological neoplasms [2]. There are approximately 21,250 new cases of CLL and responsible for around 4,320 fatalities, accounting for a quarter of all new leukemia cases in USA according to the American Cancer Society's estimates for CLL for 2021. Men have approximately twice the rate of occurrence as women. CLL is less common in African or Asian people [3]. According to the most up-to-date issued Iraqi Cancer Boared Registry in 2019, leukemia was the fifth of top 10 most common cancers in Iraq accounting for 5.51% with 1,977 new case/ year. Unfortunately there is no registration for incidence of each type of leukemia. In a local study in Karbala province in Iraq, CLL was the least common type of leukemia which represented only 15.7% of all leukaemia types [4]. The etiology is poorly understood, yet it is considered as an acquired disorder. Conversely there is a suggestion of genetic factors contribute to disease susceptibility; first-degree relatives of patients with CLL have an 8.5-fold increased risk of developing this disease, and the concordance of CLL is higher among monozygotic twins than among dizygotic twins [5]. CLL is distinguished by a typical apoptosis failure; one of the hallmarks of cancer is the ability to evade the apoptotic program, which is a key factor in clinical resistance to treatment. That is especially true for CLL [6]. The dormant leukemic cells in the blood are unable to activate their apoptotic program. This is dependent on a variety of factors, including impaired apoptotic machinery in CLL cells and an excess of survival signals from the micro-environment (complex mix of T- cells, nurse-like cells, macrophages and stromal cells), which generate chemokines and interleukins that activate survival pathways such as NF-kB. In CLL cells, these pathways are constantly active, resulting in the production and overexpression of critical anti-apoptotic proteins like myeloid cell leukemia-1(MCL-1), B-cell lymphoma-2(BCL-2), X-linked inhibitor of apoptosis protein(XIAP)and Bcl-2 homologous antagonist/killer(BAK) [7] CLL is marked by progressive immunological dysregulation in the cellular, humoral, and innate immune components, as well as an early increase in the absolute number of circulating T-cells, particularly immunosuppressive T-regulatory cells and myeloid-derived suppressor cells [8, 9]. Aberrant functioning immune cell contribute to the pathogenesis of CLL through release of cytokines, chemokines and small extracellular vesicles that promote CLL growth and survival [10]. Many patients are diagnosed as a result of incidental finding of a persistent lymphocytosis, whereas the others have symptoms. Peripheral blood morphology and immunophenotyping are

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important diagnostic procedures. Prognosis can be determined using clinical staging, lymphocyte doubling time, and biomarkers. Because CLL has generally been thought to be incurable, the goals of therapy are to increase survival time and control symptoms [11]. Despite considerable gains in CLL diagnosis, prognosis, and therapy over the last decades, little is known about how these advancements have influenced patient survival at the population level, particularly among newly diagnosed patients [12]. CD14 is one of several markers being studied for prognostic assessment in CLL patients. CD14 is a cell surface glycoprotein expressed mostly in innate immune response cells like monocytes, macrophages, neutrophils, and B cells [13, 14]. This protein exists in two forms: membrane molecule (mCD14) and a soluble form (sCD14) [15]. The survival of CLL cells is linked to NFκB constitutive activity. Monocytes aid CLL cell survival by secreting soluble CD14, which activates NFκB in these cells, while CLL cells actively alter their microenvironment by promoting accessory monocytes to secret CD14 [16]. The CD14 molecule is expressed on the surface of monocytes and macrophages which functions as an LPS receptor, coupled to LPS binding protein, to mediate LPS-induced tumor necrosis factor (TNF) production. Additionally, this glycoprotein is seen in various amounts on the surface of CLL cells that can release IL1. However, the significance of the soluble version of CD14 is still being contested [17].

2. Patients and Methods

This case control-study was approved ethically and carried out in pathology department, College of Medicin- Babylon University. It was conducted on a total of 90 Iraqi adult individuals. Forty five adult patients were newly diagnosed with chronic lymphocytic leukaemia based on physical examination by a specialist, morphological assessment of peripheral blood films, as well as flowcytometric immunophenotypic profile were attending the outpatient clinic of haemtology in Baghdad Teaching Hospital and Marjan Teaching Hospital, together with forty five age- and sex-matched participants without CLL were included and served as a normal control for comparison with the patients study group. For reliable assessment of CD14, alcoholic patients and those who have severe trauma, subsequent malignancy, tuberculosis, chronic liver disease, AIDS and infection at the time of blood collection were excluded from this study. Also, Patients with small lymphocytic leukemia (SLL), monoclonal B cell lymphocytosis, Richter's syndrome and B-cell pro-lymphocytic leukemia were not included in this study. The patients divided into patients with typical CLL and those with atypical subtype and all staged according to modified Rai and Binet staging systems. All participants were informed about the study objectives and their consents were obtained, then blood samples were collected and the following investigations were done: CBC, blood film and serum CD14 assay. Human CD14 ELISA kit (BT LAB; E0283Hu) was used for serum CD14 measurement, according to manufacturer's instructions. CBC was done by automated hematology analyzer and the blood film examined by specialist haematopathologist.

3. Results

Clinicopathological distribution of patients

The mean age of patients was (59.64 ± 8.01) years with older patient was 75 year and younger patient was 40 year. Majority of patients were males (N=32, 71.1%). distribution of patients with CLL using Modified Rai staging system revealed the majority of patients (N=23, 51.1%) presented with moderate risk and according to Binet system, the majority of patients (N=19, 42.2%) presented with stage B. Lymphadenopathy was the commonest clinical finding presented in 86.7% of patients and typical CLLwas the commonest subtype.

Table (1): characteristics of CLL patients

Characteristic	Patients
Age:	
Range	40-70year
Mean	59.64 ± 8.01year
median	60.00year
Gender	
Male	32(71.1%)
Female	13(28.9%)
M:F ratio	2.46:1
Clinical finding	
Lymphadenopathy	
Yes	39(86.7%)
no	6(13.3%)
Organomegally	
Yes	30(66.7%)
No	15(33.3%)
Modified Rai Stage	
Low risk	7(15.6%)
Moderate risk	23(51.1%)
High risk	15(33.3%)
Binet stage	
A	12(26.7%)
B	19(42.2%)
C	14(31.1%)
CLL subtype	
Typical	43(95.6%)
Atypical	2(4.4%)
Complication	
Yes	4(8.9%)
no	41(91.1%)

Comparison of hematological parameters of the studied group

There were a significant differences in the means of (Hb level, ALC, TWBC Count and platelet) between CLL and control groups (p-value <0.001)

Table (2): The mean differences of hematological parameters among study groups (N=90)					
Hematological parameter	Study group	N	Mean ± SD	t-test	P-value
Platelet count (×109/L)	CLL	45	169.79 ± 73.03	-5.496	<0.001*
	Control group	45	245.44 ± 56.48		
Hb level (g/dl)	CLL	45	11.76 ± 2.46	-5.212	<0.001*
	Control group	45	14.03 ± 1.57		
Absolute lymphocyte count(×109/L)	CLL	45	54.72 ± 51.34	6.859	<0.001*
	Control group	45	2.21 ± 0.82		
Total WBC count (×109/L)	CLL	45	68.99 ± 64.52	6.401	<0.001*
	Control group	45	7.40 ± 1.74		
*P ≤ 0.05 was significant.					

CD14 marker

There were significant differences in CD14 mean values between research groups.

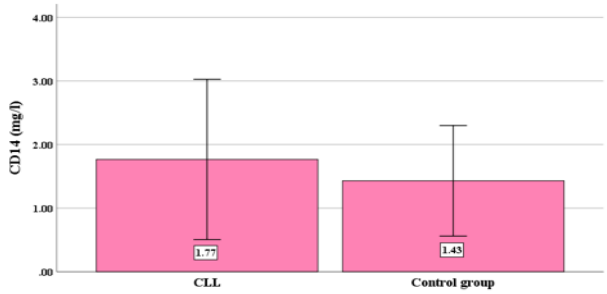

Figure (1): mean differences in CD14 levels (mg/l) according to research group (N=90, P=0.004)*

Relation of sCD14 level with modified Rai and Binet stages

There were significant differences in means of CD14 (mg/l) according Modified Rai and Binet staging systems with the higher level demonstrated with advanced stages (high risk Rai stage III-IV and binet stage C).

Table (3-A): The mean differences of CD 14 (mg/l) according to Modified Rai stage					
Study variables	Modified Rai stage	N	Mean ± SD	F	P-value
CD 14 (mg/l)	Low risk	7	1.41 ± 0.32	4.166	0.022*
	Moderate risk	23	1.65 ± 0.73		
	High risk	15	2.11 ± 0.38		

Table (3-B) The mean differences of CD 14 (mg/l) according to Binet stage					
Study variables	Binet stage	N	Mean ± SD	F	P-value
CD 14 (mg/l)	A	12	1.46 ± 0.28	3.629	0.035*
	B	19	1.73 ± 0.81		
	C	14	2.08 ± 0.39		

Relation of CD14 to hematological and clinical parameters in CLL patients

The comparison between CD 14 (mg/l) and haematological variables revealed there was a negative correlation between Hb level and platelet count with CD14 concentrations as the concentration increased with lower Hb level and platelet count, though this was statistically not significant. Regarding clinical parameters, this study showed significant differences between means of CD14 (mg/l) and organomegaly.

Table (4): CD14 mean differences according to haematological parameters in CLL patients (N=45).					
Study marker	Hematological parameter	N	Mean ± SD	R	P-value
CD 14 (mg/l) (1.77±0.63)	Platelet count (×109/L)	45	169.79 ± 73.03	-0.134	0.38
	Hb level (g/dl)	45	11.76 ± 2.46	-0.259	0.086
	Absolute lymphocyte count(×109/L)	45	54.72 ± 51.34	0.185	0.223
	Total WBC count (×109/L)	45	68.99 ± 64.52	0.218	0.151
*P ≤ 0.05 was significant					

Table (5): CD14 mean differences in CLL patients according clinical findings (N=45)					
clinical finding	Complication	N	Mean ± SD	t-test	P-value
CD 14 (mg/l)	Yes	4	2.75 ± 1.04	3.743	0.001*
	No	41	1.66 ± 0.49		
clinical finding	LAP	N	Mean ± SD	t-test	P-value
CD 14 (mg/l)	Yes	39	1.81 ± 0.65	1.369	0.178
	No	6	1.44 ± 0.34		
Clinical finding	Organomegaly	N	Mean ± SD	t-test	P-value
CD 14 (mg/l)	Yes	30	1.92 ± 0.69	2.528	0.015*
	No	15	1.44 ± 0.27		
*P ≤ 0.05 was significant.					

4. Discussion

This study found the mean age of patients was 59.64 year which is comparable to that reported by other Iraqi workers where the mean age in their studies were 57.18 and 59.24 respectively [18, 19], While it was lower than that reported in western countries [20, 21]. These differences may be related to the effect of geographical, environmental factors and population structure between Iraq and Western countries that made life expectancy lower among Iraqi population. The incidence of registered cases of CLL in our study which included 32 (71.1%) male and 13(28.9%) female was higher in male; where the male: female ratio was 2.4:1. Some local research in Baghdad and Erbil found the same result with M: F ratio of 2.5:1 and 2.7:1 respectively [22, 23]. While it was higher than that found in western study [24] where the ratio was 1.4:1. The real cause of male predominance in CLL disorder is unknown [25], But it may be related to some oncogenic influences like dietary habitudes and cigarette smoking or even

hormonal abnormalities. We discovered that sCD14 levels were significantly greater in patient group compared to control group (p-value =0.004). This result was in line with that of other researchers in many previous studies [16, 17, 26, 27] in the present study, the level of sCD14 concentration was greater in females than males, however, the difference is not significant. In addition there was no significant correlation between sCD14 level and the age of patients. Similar result was reported by other studies [27]. sCD14 level significantly associated with advance stage of disease as the level was markedly higher in advanced stages including high risk group (Modified Rai Stage III-IV) and Binet stage C that coincident with the result of other studies [16, 17, 26, 27]. There is a study found a direct link between the expression of CD14 and advanced clinical stage which are associated with shorter overall survival. This strong relation reflects the importa nce of CD14 as prognostic factor in CLL. Regarding haematological parameters, We found a negative correlation of CD14 with haemoglobin level and platelet count as the concentration increased with decreased haemoglobin level and platelet count although it was not significant (The non-significant association may be due to few number of patient with low hemoglobin and platelet count in our study and the negative correlation can be explained by higher concentration of CD14 in advanced CLL stage that associated with lower haemoglobin level and platelet count). There were no significant correlation with total WBC and absolute lymphocyte counts. In the assessment of CD14 relation to clinical parameters in this study, there was a significant correlation of CD14 to organomegaly (P=0.015), but there was no considerable association with lymphadenopathy. One study showed that, there was no significant relation between CD14 levels any and clinical, laboratory or haematological parameters [26], other study discovered a significant negative correlation of CD14 to haemoglobin level [27] These differences may be related to sample number, different technique used, work environment and the way of patient selection. There was no significant correlation with CD14 and CLL subtype, although the level was higher in atypical than typical cases. This finding is original and didn't take in consideration in previous studies. Some patient (N=4, 8.9%) in this study presented with specific complication (three with auto immunehaemolytic anaemia and one with red cell aplasia) and statistical analysis showed significant high CD14 concentration among these complicated cases. This finding is original and didn't take in consideration in previous studies

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