Original Article

Access this article online

Quick Response Code:

Website: https://journals.lww.com/ijhm

DOI: 10.4103/ijh.ijh_96_23

Evaluation of plasma CCL3 levels in individuals with newly diagnosed chronic lymphocytic leukemia and it's correlation with clinical and laboratory parameters

Ruaa Hameed Nasif, Maysem Mouayad Abd Al-Mahdi

Abstract:

BACKGROUND: The most prevalent type of leukemia in adults is called chronic lymphocytic leukemia (CLL). is a lymphoproliferative condition is defined by the mature clone's expansion in blood, secondary lymphoid tissues, spleen and the bone marrow. CCL3 is a cytokine that is involved in development, repair, and the acute inflammatory state. CCL3, therefore, should become useful for risk assessment in patients with CLL.

OBJECTIVES: The aims of the study were to estimate the level of plasma CCL3 in newly diagnosed patients with CLL matched to healthy controls and correlate it with hematological parameters and clinical parameters and prognostic markers such as CD38 and LAIR-1.

PATIENTS, MATERIALS AND METHODS: This study was cross-sectional in nature. Patient group included 55 patients with newly established diagnosis of CLL proven by morphology and immunophenotyping and control group included sex and age matched of 30 healthy individuals. The plasma CCL3 levels were measured using an enzyme-linked immunosorbent assay. Microsoft Office Excel 2019 and SPSS version 26 were used for the statistical analysis. The mean and standard deviation of normally distributed numerical values whereas the terms "median" and "range" were used to describe numerical values that are not regularly distributed. P <0.05 was used to determine the degree of significance.

RESULTS: There were statistically significant relationships between CCL3 of patient (median level about 766.42 ng/ml) and control (median level about 453.35 ng/ml) groups (P < 0.001). There was a strong correlation between CCL3 and white blood cell, hemoglobin, and absolute lymphocyte count (P = 0.013, P < 0.001, and P = 0.011, respectively). There was a strong relationship between CCL3 expression with Binet stage and with hepatomegaly (P < 0.001 and P = 0.020, respectively), but no significant relation between CCL3 expression and CD38 and LAIR-1.

CONCLUSIONS: Patients with CLL had high levels of CCL3, which increased with advancing Binet stages. Therefore, our research clarified the useful and simplicity of measurement of CCL3 plasma levels for follow-up in CLL patients and risk assessment.

Keywords:

Chronic lymphocytic leukemia, ELISA, plasma chemokine ligand3 level

Department of Hematopathology, Medical City Teaching Laboratories, Baghdad, Iraq

Address for correspondence:
Dr. Ruaa Hameed Nasif, Medical City Teaching Laboratories, Baghdad, Iraq.
E-mail: ruaahameed49@gmail.com

Submission: 17-12-2023
Revised: 29-01-2024
Accepted: 30-01-2024
Published: 12-03-2024

Introduction

Chronic lymphocytic leukemia (CLL) is a cancer that begins in bone marrow cells and spreads to the blood, usually growing slowly. By the maturation of CD5+ clones and their accumulation in the bone marrow, peripheral blood, and secondary lymphoid organs together with the B-cell antigens CD19, CD20, and CD23.[1] In the most

How to cite this article: Nasif RH, Al-Mahdi MM. Evaluation of plasma CCL3 levels in individuals with newly diagnosed chronic lymphocytic leukemia and it's correlation with clinical and laboratory parameters. Iraqi J Hematol 2024;13:44-9.

recent update of the SEER database, the age-adjusted incidence of CLL in the United States was 4.9 per 100,000 inhabitants per year, which makes CLL one of the most common types of leukemia. The median age at diagnosis is 70 years. Only 9.1% of patients with CLL are younger than 45 years. More male than female patients (1.9:1) are affected, and this gender effect seems to be stable across all ethnicities.[2]

The inflammatory cytokine CCL3 is a member of the CC chemokine family. The majority of mature hematopoietic cells are able to finish the synthesis of MIP-1α/CCL3. CCL3 is known to be secreted by many mature hemopoietic cells. It participates in osteoclast activation, wound healing, and the acute inflammatory state, both nonhematological and hematological carcinogenesis and metastasis.[3] B lymphocytes generate elevated quantities of CCL3, which in turn lead to the leukemia niche being populated by cells of the macrophage lineage. The inflammatory substances produced by the recruited cells, such as tumor necrosis factor alpha, eventually trigger the expression of VCAM-1 by stromal cells. VCAM-1 is a ligand for CD49d, and it interacts with CD49d to transmit prosurvival signals to CLL cells. Therefore, CCL3 can promote the leukemia niche's development, which is crucial for CLL cells to survive.[4] The aims of the study were to estimate the level of plasma CCL3 in newly diagnosed patients with CLL in contrast healthy controls and to correlate it with clinical and hematological parameters and with prognostic markers CD38 and LAIR-1.

Patients, Materials, and Methods

This research was cross-sectional composed of 55 adults, newly diagnosed, CLL patients with different clinical stages. It was done from October 1, 2022, to March 8, 2023. The patients were in the Baghdad Teaching Hospital of the Medical City's Haematology Outpatient Clinic. The diagnosis was made in the Flow Cytometry Unit of the Medical City in Baghdad's National Centre for Teaching Laboratories based on the morphology and immunophenotyping of the peripheral blood cells. Flow cytometry was done for the diagnostic markers using an eight-color flow cytometry (BD FACS Can't Flow Cytometer, USA). This study, approved by Ethics Committee of Iraqi council were obtained from all of the patients who participated in the study. The study was approved by the Review Ethical Committee of the Scientific Council of Pathology at the Iraqi Council for medical specialization and written informed consent was taken from all of the individuals who were included in the study.

Data were assembled for each patient using a questionnaire form detail from each patient were obtained, such as name, age, sex, primary symptoms, and physical indicators, particularly the existence of lymphadenopathy, splenomegaly, and hepatomegaly in addition to hematological markers. The ages of the included patients were newly diagnosed from <50 to >70 years old. The 30 sex- and age-matched control group of healthy individuals.

Blood sampling a 3 ml venous blood sample from each of the study's patients and controls, obtained under aseptic technique by venipuncture from antecubital fossa. These 3 ml of blood were added to K3-EDTA tubes for examination of complete blood count, blood film and reticulocyte count in National Centre for Educational Laboratories then centrifugation for 10 min at 3000 rpm Plasma was separated and then was stored in Eppendorf tube at − 80°C up to 6 months for measurement of CCL3 by ELISA in National Centre for Educational Laboratories using CCL3 ELISA kit from Bioassay.

Results

Fifty-five patients of CLL were studied in contrast to 30 sex- and age-matched control group of healthy individuals. No statistically strengthen relation of age was observed between patient and control, $P = 0.083$.

No statistically strengthen relation of sex distribution was observed between patient and control, $P = 1.000$ [Table 1].

Clinical features

splenomegaly and lymphadenopathy are the most prevalent clinical characteristics at the time of presentation for CLL patients in this research were (58.2% and 32.7%, respectively), followed by, hepatomegaly (16.4%) [Figure 1].

Patient distribution for CLL based on Binet staging of the 55 cases studied; 26 were Stage A, 18 for Stage B and 11 for Stage C.

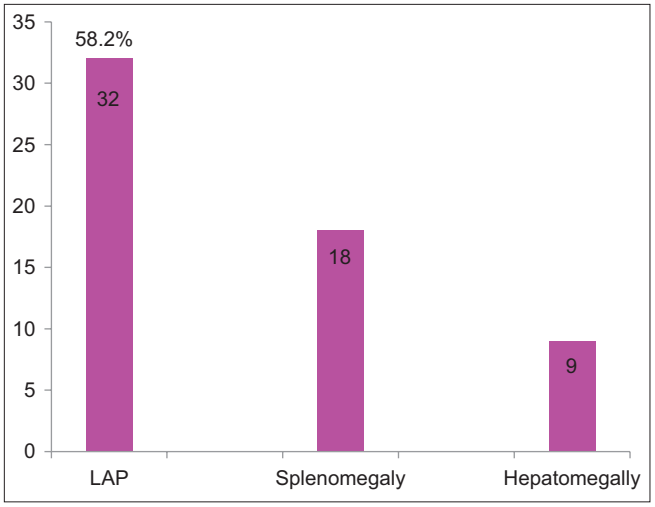

Figure 1: Clinical feature of patients ($n = 55$). LAP = Lymphadenopathy

Hematological parameters of patients and controls

Comparison of hematological data between 55 patients and 30 controls as Table 2 show there are statistically significant relation of hematological parameters (white blood cell [WBC], hemoglobin [Hb], platelet [PLT]) were determined between patients and control $P < 0.001$.

- CLL patients with median plasma CCL3 level was (766.42 ng/mL) which is significantly raised than that in control group (453.35/mL) with a $P < 0.001$
- In CLL patients absolute lymphocyte count (ALC) and smudge cells of median (39.9×10^9/l, 17%) respectively, while in control there were no lymphocytosis and smudge cells.

Comparison of CCL3 (chemokine ligand 3) according to sex of patients

CCL3 levels did not significantly relate with sex with $P = 0.893$ [Table 3].

Comparison of CCL3 with the presenting signs in chronic lymphocytic leukemia patients

Significant statistical correlation between CCL3 with hepatomegaly of $P = 0.020$.

There was no significant statistical correlation between chemokine ligand 3 (CCL3) with lymphadenopathy (LAP) and splenomegaly in patients.

Comparison of chemokine ligand 3 according to stages of disease in patients

The median plasma CCL3 level was found to be raised among Binet C patients than Binet A and Binet B. A statistically significant relation was found between CCL3 and higher clinical stages of CLL with $P < 0.001$ [Figure 2].

Relation of CCL3 to CD38 and to LAIR-1

No significant relation was found between CCL3 and CD38 [Table 4] and also with CCL3 and LAIR-1 [Table 5].

Relations of CCL3 with age and hematological parameters in patients and control

CCL3 expression showed a highly positive correlation with hemoglobin level, WBC count, and ALC ($P < 0.001$, $P = 0.013$, and $P = 0.011$, respectively).

However, it showed no significant relation with smudge cells ($P = 0.155$).

While correlation with PLT of patient was no significant ($P = 0.067$).

Finally, age did not significantly correlate with CCL3 in patients shown in Table 6.

Table 1: Comparison of patient and control group demographic data

Parameter	Patients (*n*=55)	Controls (*n*=30)	*P*
Age (years)			
Mean±SD	62.67±12.07	58.67±4.3	0.083*
Median (range)	62 (35–87)	59 (48–65)	
Sex, *n* (%)			
Male	37 (67.3)	20 (66.7)	1.000**
Female	18 (32.7)	10 (33.3)	

*Unpaired test, **Fisher's exact test. SD=Standard deviation

Table 2: Comparison of hematological data between patients and control

Parameter	Patients (*n*=55)	Controls (*n*=30)	*P**
Hb (g/dL)			
Mean±SD	12.41±2.46	14.86±1.34	<0.001
Median (range)	12.7 (5.4–16.63)	15.1 (12–16.52)	
WBC ($\times 10^9$/L)			
Mean±SD	69.44±52.8	7.85±1.82	<0.001
Median (range)	51.7 (10.3–228)	7.4 (5.12–11)	
Platelets ($\times 10^9$/L)			
Mean±SD	196.43±108.74	283.7±70.17	<0.001
Median (range)	164.7 (49.6–661)	289.5 (150–409)	
CCL3			
Mean±SD	866.64±369.28	480.48±140.44	<0.001
Median (range)	766.42 (373.23–1635.57)	453.35 (216.55–945.57)	
ALC ($\times 10^9$/L)			
Mean±SD	58.12±44.04	-	-
Median (range)	39.9 (6.5–192)	-	
Smudge cell (%)			
Mean±SD	22.32±17.7	-	-
Median (range)	17 (1–69)	-	

*Mann–Whitney test. CCL3=Chemokine ligand 3, Hb=Hemoglobin, WBC=White blood cell, SD=Standard deviation, ALC=Absolute lymphocyte count

Table 3: Comparison of chemokine ligand 3 according sex of patients

Parameter	Male (*n*=37)	Female (*n*=18)	*P**
CCL3			
Mean±SD	866.66±388.9	866.6±336.01	0.893
Median (range)	775.88 (373.23–1635.57)	764.05 (452.46–1406.75)	

*Mann–Whitney test. CCL3=Chronic lymphocytic leukemia, plasma chemokine ligand 3, SD=Standard deviation

Table 4: Comparison of chemokine ligand 3 according to presence of CD38 in patients

Parameter	Positive (*n*=4)	Negative (*n*=51)	*P**
CCL3			
Mean±SD	1122.8±329.55	846.55±367.56	0.136
Median (range)	1054.36 (799.53–1582.95)	761.69 (373.23–1635.57)	

*Mann–Whitney test. CCL3=Chronic lymphocytic leukemia, plasma chemokine ligand 3, SD=Standard deviation

Table 5: Comparison of chemokine ligand 3 according to presence of LAIR-1 in patients

Parameter	Positive (*n*=26)	Negative (*n*=29)	*P**
CCL3			
Mean±SD	853.61±363.25	878.33±380.64	0.833
Median (range)	735.97 (373.23–1635.57)	775.88 (453.05–1635.57)	

*Mann–Whitney test. CCL3=Chronic lymphocytic leukemia, plasma chemokine ligand 3, SD=Standard deviation

Table 6: Correlation of chemokine ligand 3 with age and heamatological parameters in patients

Parameter	CCL3 patients
Age	
r	0.238
P	0.080
Hb	
r	−0.556
P	<0.001
WBC	
r	0.332
P	0.013
Platelets	
r	−0.249
P	0.067
ALC	
r	0.339
P	0.011
Smudge cells (%)	
r	−0.194
P	0.155

CCL3=Chronic lymphocytic leukemia, plasma chemokine ligand 3, Hb=Hemoglobin, WBC=White blood cell, ALC=Absolute lymphocyte count

Discussion

With time, in applying prognostic parameters are interest which may predict survival and direct the plan of management in patients with CLL has been increased. This increasing interest is the result of the difficulty in detected the time of onset and choice of therapy because CLL shows a highly heterogeneous clinical course.[5] The purpose of the current study was to elucidate how CCL3 expression in risk assessment and follow-up of CLL patients.

The median age of CLL patients at diagnosis in this study included was 62 (35–87) years; this result comparable to other Iraqi study in 2018[6] and was similar to other study in 2011[4] and likewise comparable to reports from nearby nations such as Iran.[7] In comparison within Western countries, the median age at diagnosis was significantly higher.[8] This dissimilarity may be due to biological, genetics, and possible risk factors in Iraqi patients.[8]

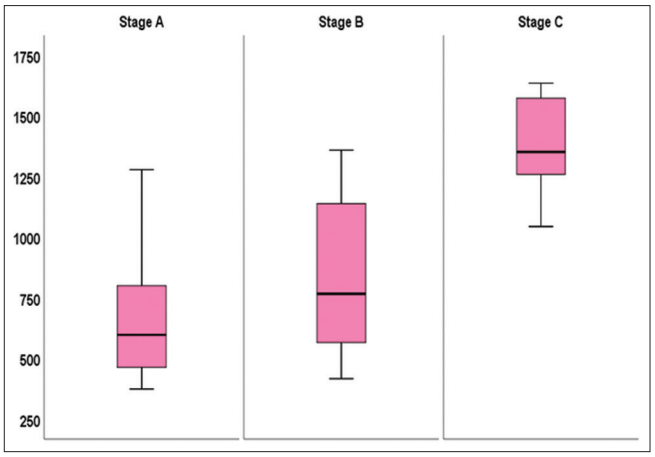

Figure 2: Box plot chart of chronic lymphocytic leukemia 3 in patients according to stage

The patients age group within (60–69) years are the highest percentage which was similar to a previous Iraqi study in 2015, and 2011.[4,7]

However, male CLL patients in the current study was higher than female percentage and male/female ratio about (2:1) identical to that reported in western study.[9] and similar to previous Iraqi study in 2018.[6]

About clinical presentation, lymphadenopathy most common feature in our study then splenomegaly followed by hepatomegaly These results were comparable to other westren study in 2017 and 2007.[10,11]

In Binet Stage A the majority of our patients presented. The remaining for each Binet B and C this result was similar to western studies It revealed a higher percentage of patients in stage A and a lesser percentage of patients falling into Stage C, But dissimilar to Iraqi study reported higher percentage of patients within Stage C in 2018[6] probably due to improvement of diagnostic tools and better awareness of patients seeking medical services.[12,13]

For the hematological parameters of the patients.

The median Hb of patients was comparable to a previous study of Western countries.[14] may be due to distribution of 47% of patients in current study in Stage A.

The median level of ALC and platelatpheresis count was comparable with another Iraqi study.[15]

The marker that was aimed to be assessed in this study is CCL3.

In CLL patients the plasma CCL3 levels of our study were considerably greater than that of the typical control group ($P < 0.001$) These were comparable to

the results obtained by western studies done in 2009, 2011, 2016.[4,16,17]

Their levels were higher in CLL patients than in healthy individuals. Because CCL3 is secreted by CLL cells in response to B-cell receptor activation therefore, CCL3 plasma levels presumably reflect the activation of the CLL clone.[18]

No significant statistical relationship was detected between CCL3 and sex ($P = 0.893$) and between CCL3 and age ($P = 0.080$) were in agreement with that reported in 2019 study.[19]

Significant agreements were found between CCL3 and each of WBC, ALC with (0 $P = .013$ and $P = 0.011$), this result was consistent with that reported by study in 2011.[4]

Significant versus correlation was found with Hb level with ($P < 0.001$), while no significant correlation found in PLTs with ($P = 0.06$) but we couldn't find any studies to explore these correlations of their levels with CCL3 in CLL patients.

Patients with Binet stage C had significantly higher levels of CCL3 with ($P < 0.001$) in our study Similar to that study in 2011.[4] CLL cells secrete CCL3 in response to B-cell receptor activation so more CLL cells in stage C secrete more CCL3.[18] We found no significant relation between plasma levels of CCL3 and poor prognostic markers, such as CD38 and LAIR-1 of ($P = 0.136$ and $P = 0.833$) in contrast to that study in 2011.[4] May be due to small number of patient samples or may be due to CD38 and LAIR-1 have different physiological role in CLL than CCL3, so this considers independent marker in CLL.

Conclusions

Patients with CLL had high levels of CCL3, which increased with advancing Binet stages. CCL3 secretion show a significant positive correlation with WBC and ALC and Hb but no significant correlation with platelet count and show significant relation between CCL3 secretion and hepatomegaly, but no significant relation between CCL3 and CD38 and LAIR-1. This study demonstrates the useful and simplicity of measurement of CCL3 plasma levels for follow-up in CLL patients and risk assessment.

Acknowledgment
The authors would like to thank Mustansiriyah University/Faculty of Medicine, particularly Department of Pathology and Forensic Medicine for the support during the work.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Hallek M. Chronic lymphocytic leukemia: 2017 update on diagnosis, risk stratification, and treatment. Am J Hematol 2017;92:946-65.
2. Hallek M, Al Sawaf O. Chronic lymphocytic leukemia: 2022 update on diagnostic and therapeutic procedures. Am J Hematol 2021;96:1679-705.
3. Maurer M, von Stebut E. Macrophage inflammatory protein-1. Int J Biochem Cell Biol 2004;36:1882-6.
4. Sivina M, Hartmann E, Kipps TJ, Rassenti L, Krupnik D, Lerner S, *et al*. CCL3 (MIP-1α) plasma levels and the risk for disease progression in chronic lymphocytic leukemia. Blood 2011;117:1662-9.
5. Cramer P, Hallek M. Prognostic factors in chronic lymphocytic leukemia-what do we need to know? Nat Rev Clin Oncol 2011;8:38-47.
6. Hasan KM. Clinical aspects, immunophenotypic analysis and survival rate of chronic lymphocytic leukaemia patients in Erbil City, Iraq. Sultan Qaboos Univ Med J 2018;18:e461-7.
7. Payandeh M, Sadeghi E, Sadeghi M. Survival and clinical aspects for patients with chronic lymphocytic leukemia in Kermanshah, Iran. Asian Pac J Cancer Prev 2015;16:7987-90.
8. Eichhorst B, Robak T, Montserrat E, Ghia P, Niemann CU, Kater AP, *et al*. Chronic lymphocytic leukaemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2021;32:23-33.
9. Molica S. Sex differences in incidence and outcome of chronic lymphocytic leukemia patients. Leuk Lymphoma 2006;47:1477-80.
10. Strati P, Parikh SA, Chaffee KG, Achenbach SJ, Slager SL, Call TG, *et al*. CD49d associates with nodal presentation and subsequent development of lymphadenopathy in patients with chronic lymphocytic leukaemia. Br J Haematol 2017;178:99-105.
11. Inamdar KV, Bueso-Ramos CE. Pathology of chronic lymphocytic leukemia: An update. Ann Diagn Pathol 2007;11:363-89.
12. Yoon JY, Lafarge S, Dawe D, Lakhi S, Kumar R, Morales C, *et al*. Association of interleukin-6 and interleukin-8 with poor prognosis in elderly patients with chronic lymphocytic leukemia. Leuk Lymphoma 2012;53:1735-42.
13. Li H, Yi SH, Xiong WJ, Liu HM, Lyu R, Wang TY, *et al*. Chronic lymphocytic leukemia prognostic index: A new integrated scoring system to predict the time to first treatment in Chinese patients with chronic lymphocytic leukemia. Chin Med J (Engl) 2017;130:135-42.
14. Eichhorst B, Dreyling M, Robak T, Montserrat E, Hallek M, ESMO Guidelines Working Group. Chronic lymphocytic leukemia: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2011;22 Suppl 6:i50-4.
15. Nabhan C, Aschebrook Kilfoy B, Chiu BC, Smith SM, Shanafelt TD, Evens AM, *et al*. The impact of race, ethnicity, age and sex on clinical outcome in chronic lymphocytic leukemia: A comprehensive surveillance, epidemiology, and end results analysis in the modern era. Leuk Lymphoma 2014;55:2778-84.
16. Zucchetto A, Benedetti D, Tripodo C, Bomben R, Dal Bo M, Marconi D, *et al*. CD38/CD31, the CCL3 and CCL4 chemokines, and CD49d/vascular cell adhesion molecule-1 are interchained by sequential events sustaining chronic lymphocytic leukemia cell survival. Cancer Res 2009;69:4001-9.

17. Hartmann EM, Rudelius M, Burger JA, Rosenwald A. CCL3 chemokine expression by chronic lymphocytic leukemia cells orchestrates the composition of the microenvironment in lymph node infiltrates. Leuk Lymphoma 2016;57:563-71.
18. Herishanu Y, Pérez Galán P, Liu D, Biancotto A, Pittaluga S, Vire B, *et al*. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. Blood 2011;117:563-74.
19. Tate C, Burgess M, McMillan NA, Saunders NA, Cheung C, Gill D, *et al*. High serum levels of CD178 (soluble FasL) predict for inferior progression free survival in chronic lymphocytic leukemia treated with fludarabine-based chemotherapy. Leuk Lymphoma 2019;60:2563-7.