



Interplay of IL4 and CCL2 / CCL3 axis in Patients with chronic lymphocytic leukemia

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Abstract

Background:

The most prevalent leukemia in adulthood is chronic lymphocytic leukemia (CLL), which is a gradual and progressive build up of monoclonal B cells. A favorable microenvironment with the presence of cytokines and chemokines has a strong impact on this process. Among them, IL-4, CCL2, and CCL3 are critical factors that contribute to increasing the leukemic cell survival and reducing the inflammatory contact, as well as influencing the communication between the malignant cells and stroma.

Aim:

The purpose of the study was to determine serum concentrations of IL-4, CCL2 and CCL3 in untreated patients with newly diagnosed CLL, compare them with normal subjects and determine their diagnostic value and the relationship between them and hematological and clinical variables.

Methods:

It was a case-control study that comprised of 60 newly diagnosed CLL patients and 30 healthy controls. Hematological parameters were analyzed with the help of an automated analyzer (WBC count, lymphocytes, neutrophils, and monocytes). ELISA was used to measure serum IL-4, CCL2 and CCL3 using standard manufacturer procedures. Group comparisons, ROC curve analysis, and correlation tests were performed to compare the groups statistically with the significance value of $P \leq 0.05$.

Results:

The patients of CLL showed a great increase in the count of WBC, lymphocyte and monocyte counts than the healthy individuals. The levels of serum IL-4, CCL2 and CCL3 were significantly elevated in the group of patients ($P < 0.001$).

ROC showed that CCL2 showed an excellent diagnostic ability (AUC = 0.905), CCL3 was good (AUC = 0.870), and IL4 was average (AUC = 0.707).

The level of CCL2 was found to be more so in patients with a disease period of more than 5 years but the same was not reflected in the level of IL-4 and CCL3. The three biomarkers were also found to have strong correlations with one another, which indicates management of pathogenesis of CLL.

Conclusion:

The results suggest that the high-levels of serum IL-4, CCL2, and CCL3 can be considered the evidence of immune dysregulation in CLL and can be a beneficial tool to diagnose CLL and assess the disease progression.

Keyword: Chronic lymphocytic leukemia, IL-4, CCL2, CCL3, cytokines

Introduction

Chronic lymphocytic leukemia (CLL) starts in the bone marrow and is later spread to the blood stream where it is typified by uncontrolled proliferation of malignant B lymphocytes (Kharas et.al.2021). Most patients do not show any symptom till several years later when the disease advances, and the leukemic cells increase and spread to other secondary locations including liver, lymph nodes, and spleen (Shadman.2023). CLL occurs in one of every 100,000 people annually in the United States, and hence it is considered to be one of the most common forms of leukemia (Hallek and Al-Sawaf. 2021). In Iraq, as per the 2006 Iraqi Cancer Registry, the leukemias as a whole are the third most frequently occurring malignancies and the CLL represents approximately 4.85% of the total number of leukemia (Abdulridha et.al.2021). The median age of diagnosis is approximately 70 years with approximately 9.1 percent of the patients being under 45 years old. The disease presents a significant male preponderation with a male to female ratio of 1.9: 1, which cuts across ethnic lines (Hallek and Al-Sawaf. 2021).

There are a number of genetic and epigenetic changes that lead to the development and progression of CLL. The cytokines are among the most powerful regulators of the disease biology, having a significant effect on the host immune response and the outcome of treatment (Al-Zaydi et.al.2022). Interleukin-4 (IL-4) is one of the major microenvironment-induced survival signals that dramatically changes the gene and protein expression in CLL cells and develops resistance against both natural and drug-mediated apoptosis (Andreescu et.al.2023). Produced by Th2 cells, mast, basophil, eosinophil, as well as NK-T cells, IL-4 may have agonistic or antagonistic impact on various types of immune cells. Being a pleiotropic cytokine, IL-4 is essential to the existence of the leukemic B-cells and it has an impact on disease pathophysiology (Keegan et.al.2021). It is important to note that the IL-4 prevents apoptosis of CLL cells caused by various agents, including fludarabine and chlorambucil and CLL cells seem to be more susceptible to apoptosis compared to normal B cells, with varied expression of prognostic markers (Fiorcari et.al.2021). It has been postulated that IL-4 exerts its effects in CLL by paracrine interaction but not autocrine. Transcriptional gene analyses of follicular lymphoma have also shown IL-4-dependent cross-linking between T cells and neoplastic B cells that sustain tumor survival (Ruiz-Lafuente et.al.2014).

Chemokines are miniature signaling company that control the development, migration, and recruitment of leukocytes in the immune and inflammation reaction. They as well affect interleukin production, angiogenesis and tumor behavior by both autocrine and paracrine pathways (Li et.al.2022). One of them, the monocyte chemoattractant protein-1 (MCP-1/CCL2) is a noticeable 76-amino acid-long 8-helix- β -chemokine (Hamad and Mahmood.2022). The chemokines can either induce or inhibit tumor progression

based on the cellular setting (Yue et.al.2024). Tumor cells can produce CCL2 themselves, or cause CCL2 production by stromal and endothelial cells in the tumor microenvironment. When stimulated, mononuclear cells that have been stimulated with CCL2 release growth and survival factors, aiding tumor growth or causing angiogenesis by releasing factors like VEGF (Kadomoto et.al.2021). It has been demonstrated that, even though CCL2 promotes the recruitment of monocytes to leukemic cells, it does not stimulate the cytotoxic effect of monocytes. Notably, CCL2 expression has been reported to be high in CLL cells that live in the bone marrow niche (Dubois et.al.2020).

CCL3 is another inflammatory chemokine which is secreted by a broad spectrum of mature hematopoietic cells (Maurer and Vonstebut.2004). It plays a role in various physiological as well as pathological processes, such as osteoclast activation, wound healing, acute inflammation, hematologic and non-hematologic tumor development. Malignant B lymphocytes in CLL secrete large amounts of CCL3, which invites macrophages to the microenvironment of the leukemia. These recruited cells secrete inflammatory mediators, such as TNF- alpha that activate stromal cells to express VCAM-1. This adhesion molecule binds to CLL cells CD49d, and induces strong survival information. In this way, CCL3 is important in the development and preservation of the leukemia niche (Sivina et.al.2011).

The objectives of the current research were to determine plasma concentrations of IL-4, CCL2 and CCL3 among newly diagnosed patients with CLL compared to the healthy individuals, and to assess their relationships with clinical and hematological variables.

Materials and methods

Subjects

In this case-control study, subjects (n = 60) were selected as patients with Chronic Lymphocytic Leukemia (CLL) in Al-Karama Hospital / Oncology Center of the Wasit Health Department and at some of the private clinics in the city of Kut-Wasit Governorate / Iraq, between October 2024- April 2025. The Chronic Lymphocytic Leukemia causes considerable effects on immune mechanisms and chemokine concentration at this time. The diagnosis of CLL in patients was done by a special physician. Besides patients; 30 healthy people who came to the laboratory to check their health routinely were regarded as a control group that was compared with the patient group. both subjects were between the ages of 25 and 85 years. None of the two subjects had undergone therapies. The study excluded patients who had relapsing, prior history of other inflammatory diseases, autoimmune diseases, co-infection by pathogens, patients on immunosuppressive therapies and patients whose information was not complete. We further separated the confirmed CLL patients in 2 groups (≤ 5 years and > 5 years) based on the duration of the disease in this study..

Laboratory parameters affect the disease

Hematological profile

The hematological parameters included in this study were: the total white blood cell counts (WBCs), Lym (lymphocytes), neutrophils and monocyte these parameters were measured by CELL-DYN Ruby Hematology Analyzer (Abbott, Germany).

Determination Interleukin-4 (IL-4), Monocyte Chemotactic Protein-1(CCL2) and Macrophage Inflammatory Protein-1(CCL3) by enzyme-linked immunosorbent assay

IL-4, CCL2 and CCL3 serum levels were measured using a human IL-4 ELISA kit (BT LAB, Catalog NO.: E0092Hu), human CCL2 ELISA kit (Reed Biotech, Catalog NO.: RE2731H), and human CCL3 ELISA kit (BT LAB, Catalogue NO.: E0139Hu) in serum samples from patients and healthy controls. The ELISA assay was performed according to the manufacturer's instructions.

Briefly, IL-4 and CCL3 assay was conducted by 50 μ L of standard and 40 μ L of sample were added along with the 10 μ L of either (Human IL-4 antibody or Human CCL3 antibody) respectively and 50 μ L of streptavidin-HRP. The contents were mixed well. The plate was covered with a sealer and incubated for 60 minutes at 37°C. The plate was then washed five times to ensure removal of unbound materials. 50 μ L of both substrate solutions A and B were added and incubated in the dark for 10 minutes at 37°C before stopping the reaction by 50 μ L of stop solution, which produced a color change from blue to yellow. Finally, the optical density was measured at 450 nm within ten minutes to ensure accurate and reliable results.

The CCL2 assay was conducted by adding 100 μ L of each standard dilution, blank, and sample into appropriate wells, followed by a 90-minute incubation at 37°C. After washing three times with 300 μ L of wash buffer, plates were blocked by adding 100 μ L of Biotinylated Detection Antibody to each well and incubating at room temperature for one hour. The next step was carried out by adding the working dilution of Streptavidin-HRP to each well. After washing, the working dilution of Streptavidin-HRP was added (100 μ L) to each well and incubated at room temperature for 30 min. Substrate solution was added (100 μ L) to each well, and plates were again incubated at 37°C for 15 min. In the final step, a stop solution was added (50 μ L) to each well, and the optical density of each well was read using a microplate reader at 450 nm with wavelength. Each standard or sample was assayed in duplicate.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 25.0 (IBM Corp., Armonk, New York, USA). A Chi-squared test was used to compare the associations between proportions. $P \leq 0.050$ was considered statistically significant.

Ethics Approval

This study was approved by the Ethics Committee of the Department of Biology, College of Science, University of Wasit, as did the Iraqi Ministry of Health. before they were included in the study tests. All participants in the study completed a written informed consent form before enrollment into this study.

Results

Characteristics of the study population

The study was conducted on a total of 60 CLL patients who were compared to 30 age- and sex-matched controls that were healthy. The demographic data of the two groups is presented in Table (1). Mean age of CLL group comprised 57.63 years with SD of 8.56 and the control group consisted of 54.73 years with SD of 9.96 with no statistically significant difference between both groups ($P = 0.229$). In terms of the gender composition, the CLL patients were 47 males (78.3) and 13 females (21.7) and the control group was 18 males (60.0), and 12 females (40.0). As in the present case, age showed no significant difference in the gender distribution of the patients and controls ($P = 0.067$).

Table (1): Characteristics of CLL patients and healthy control

Characteristic	CLL Patients (n=60)	Healthy Control (n=30)	P
Age (years)	57.63 ± 8.56	54.73 ± 9.96	0.813
Gender			
Male	47 (78.3 %)	18 (60.0 %)	0.067
Female	13 (21.7%)	12 (40.0%)	

Hematological parameters of patients and controls

Table (2) presents a comparison of the hematological parameters of CLL patients and healthy controls. No significant neutrophil count difference existed between the two groups ($p > 0.05$). Conversely, the patient sample had a high level of WBC, lymphocyte, and monocyte counts when compared to the control sample ($p < 0.05$). These results show that there is a typical rise in the total leukocytes, lymphocytes and monocytes of CLL patients.

Table (2): Comparison of some blood parameters (WBC, neutrophils, lymphocyte and monocyte) in CLL patients and healthy controls

Groups	WBC	Neutrophils	Lymphocyte	Monocyte
CLL patients	42.61 ± 5.72	5.39 ± 0.71	38.98 ± 5.79	1.32 ± 0.19
Control	7.45 ± 0.81	5.91 ± 0.65	6.31 ± 1.2	0.42 ± 0.04
p-value	0.001**	0.649	0.001**	0.002**

SD: standard deviation; †: Independent T test; **: significant at $P < 0.05$

Immunological parameters of patients and controls

Table (3) represents the comparison of immunological parameters of CLL patients and healthy controls. All the immunological markers determined in CLL patients such as IL-4, CCL2, and CCL3 were significantly higher in serum compared to the control group. The average IL-4 level of patients was 287.22 ± 38.9 ng/mL which was largely high as compared to 218.92 ± 22.6 ng/mL in controls ($P = 0.001$). On the same note, serum CCL2 levels were significantly higher in the group of patients (524.74/66.1 pg/mL) than in the controls (242.87/42.9 pg/mL; $P = 0.001$). Moreover, the levels of CCL3 were much higher in CLL patients (109.66 + 13.8 ng/L) when compared to the healthy individuals (75.33 + 10.1 ng/L; $P = 0.001$). These findings substantiate an intensive upsurge in the levels of cytokine and chemokine in CLL.

Table (3): Comparison of Immunological parameters (IL-4, CCL2 and CCL3) in CLL patients and healthy controls

Groups	IL-4 (ng/mL)	CCL2 (pg/mL)	CCL3 (ng/L)
CLL patients	287.22 ± 38.9	524.74 ± 66.1	109.66 ± 13.8
Control	218.92 ± 22.6	242.87 ± 42.9	75.33 ± 10.1

p-value	0.001**	0.001**	0.001**
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SD: standard deviation; †: Independent T test; **: significant at $P < 0.05$

Diagnostic performance of Immunological parameters (IL-4, CCL2 and CCL3)

Receiver operating characteristic (ROC) curve analysis (Table 4) revealed acceptable, excellent, and good diagnostic performance for IL-4, CCL2, and CCL3, respectively. IL-4 demonstrated an optimal cut-off value of >252.3 ng/mL, yielding 70.0% sensitivity, 70.0% specificity, 82.4% positive predictive value (PPV), and 53.8% negative predictive value (NPV). The area under the curve (AUC) was 0.707 (95% CI: 0.602–0.811; $P < 0.001$), indicating acceptable discriminative ability in distinguishing CLL patients from healthy controls.

In contrast, CCL2 displayed a cut-off value of >308.76 pg/mL, achieving 90.0% sensitivity, 90.0% specificity, 94.7% PPV, and 81.8% NPV, with an AUC of 0.905 (95% CI: 0.840–0.971; $P < 0.001$), reflecting excellent diagnostic accuracy for CLL.

Similarly, CCL3 demonstrated good diagnostic performance (details shown in Table 4), further supporting its usefulness as a biomarker for identifying CLL patients.

Table (4): Roc curve of Immunological parameters (IL-4, CCL2 and CCL3)

Characteristic	IL-4 (ng/mL)	CCL2 (pg/mL)	CCL3 (ng/L)
Cutoff value	> 252.3	> 308.76	> 104.85
P value	< 0.001	< 0.001	< 0.001
Sensitivity %	70.0 %	90.0 %	85.0 %
Specificity %	70.0 %	90.0 %	86.7 %
PPV %	82.4 %	94.7 %	92.7 %
NPV %	53.8 %	81.8 %	74.3 %
AUC (95% CI)	0.707 (0.602- 0.811)	0.905 (0.840- 0.971)	0.870 (0.807- 0.934)

CI: Confidence interval, AUC: Area under curve.

Comparison of immunological parameters according to duration of disease

Table (5) shows a comparison of the immunological parameters (based on the duration of the disease) (IL-4, CCL2, and CCL3). The results show that the patients who had a disease period of over 5 years had a significant increase in CCL2 levels as compared to their counterparts diagnosed within 5 years (585.06 ± 61.5 vs. 445.86 ± 55.6 pg/mL; $P = 0.044$). Conversely, the IL-4 and CCL3 concentration did not find significant difference between the two duration groups ($P > 0.05$), which indicates that the two biomarkers are not affected by the duration of the disease progression.

Table (5): Comparison of Immunological parameters (IL-4, CCL2 and CCL3) according to duration of disease

Duration of disease	IL-4 (ng/mL)	CCL2 (pg/mL)	CCL3 (ng/L)
≤ 5 years	280.59 ± 34.19	445.86 ± 55.6	104.3 ± 11.1
> 5 years	292.25 ± 33.14	585.06 ± 61.5	113.2 ± 9.11

p-value	0.682	0.044**	0.134
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SD: standard deviation; †: Independent T test; **: significant at $P < 0.05$

Logestic regression correlations between Immunological parameters (IL-4, CCL2 and CCL3) parameters in CLL patients.

The logistic regression analysis showed that there was significant correlation between the measured immunological parameters in patients with CLL. Figure (1) and (2) demonstrate that IL-4 had a direct positive correlation with CCL2 and CCCL3. This observation implies that CLL microenvironment can encourage the higher production of IL-4, which consequently can correlate with the higher expression of CCL2 and CCL3. Besides, CCL2 was indirectly correlated with CCL3 (Figure 3), which demonstrates that the increased level of IL-4 production in CLL can also contribute to the increase in CCL3 due to mechanisms that are related to the expression of CCL2.

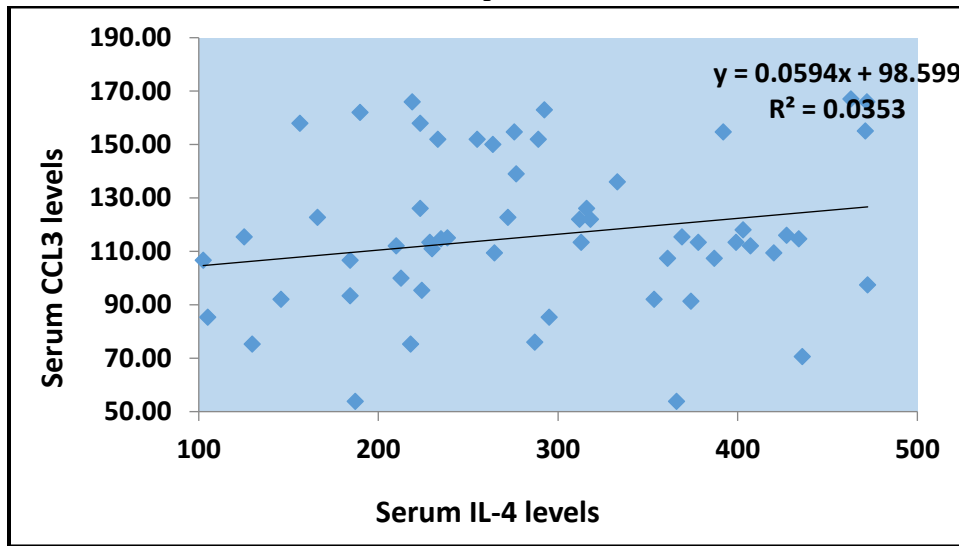


Figure (1): The Logestic scatter IL-4 and CCL3 levels among patients.

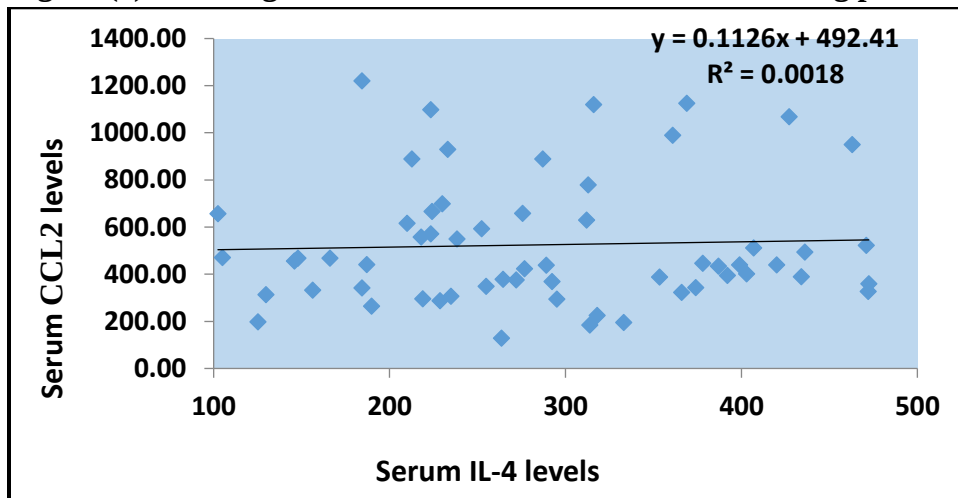


Figure (2): The Logestic scatter IL-4 and CCL2 levels among patients.

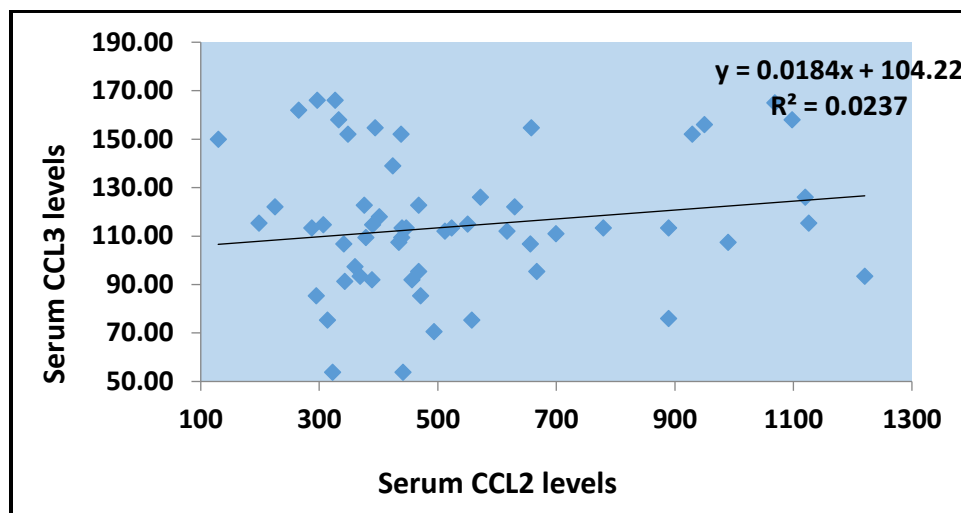


Figure (3): The Logistic scatter CCL2 and CCL3 levels among patients.

Discussion

Chronic lymphocytic leukemia (CLL) is the most common leukemia that is met in adults and is commonly diagnosed in people aged 30 years and above with a definite excess in males (Eichhorst et.al.2021). Age is regarded as a significant risk factor of CLL. In the current study, the average age of the patients was $57.63/1.56 = 57.63$ years, which was equivalent to the average age of patients in the control group $54.73/1.96 = 54.73$ years, with a significant difference of 0.23. These results can be compared with those of Nasif and Al-Mahdi (Nasif and Al-Mahdi.2024) who reported a mean age of 62.67 12.07 years at the time of diagnosis (35-87 years old). Median age has been described as similar in various parts as well; e.g. Erbil/Kurdistan-Iraq 65 years (Rashid et.al.2021), Turkey 65 years (Even et.al.2019) and the Kingdom of Saudi Arabia 62 years (range 3689) (Rawas et.al.2018). In foreign countries, the diagnosis of CLL is usually made at the age of approximately 72 years, and only in approximately one out of ten cases, the diagnosis is made prior to the age of 55. It is 30 cases per 100, 000 individuals per year in persons aged 80 years and older (Waiuman et.al.2023). Such differences might be related to genetic, biological, and environmental disparities between populations including the one seen in Iraqi patients.

Regarding the distribution of genders, there were no important differences found between the patients and the controls ($P = 0.067$). In the present study, 78.3 percent (47/60) CLL cases were in male, and 21.7 percent (13/60) CLL cases were in female. This male dominance is in line with many reports (Nassif and Al-Mahdi.2024, Mustafa et.al.2021, Alwan et.al.2021) which indicate that male to female ratios are nearly 2:1 (Hasan.2018). A number of lifestyle and biological attributes have been suggested to account the excess occurrence of CLL in males and these include smoking, occupational exposures, dietary habits, and hormonal differences.

WBC, lymphocytes, neutrophils and monocytes were also analyzed as hematological parameters. In line with the established pathophysiology of CLL, the current findings demonstrated a statistically significant change in the WBC, lymphocytes, and monocytes in CLL patients as compared to their healthy counterparts, which indicated significant changes in the immune cell composition. Leukocytosis in CLL is much more caused by a significant lymphocytosis and rapid lymphocytic doubling time (less than 12 months) represents an outcome of the disease progression. Genetic instability among growing subclones

is also an additional factor that leads to disease progression (Morabito et.al.2021). We obtained similar results with Nasif and Al-Mahdi (18) who obtained substantially higher WBC counts in CLL (69.44 ± 52.8) than of the controls (7.85 ± 1.82 , $P < 0.001$). The common clinical manifestations related to these abnormalities are lymphadenopathy, hepatosplenomegaly, and bone marrow infiltration (Vitale et.al.2020). There are however a few contradictory reports including Ali et al. (Ali et.al.2017) who had reported lower WBC counts in their CLL cohort possibly because of sample size, disease stage, or geographic.

The count of lymphocytes and monocytes was found to be greatly increased in the patient group, which is in line with the previous finding that the absolute lymphocyte count can be used to determine the recovery of bone marrow and the degree of immune surveillance (Menetrier-Caux et.al.2019). The elevated numbers of lymphocytes ($>100 \times 10^9/L$) have been generally accepted as an adverse prognostic factor in CLL (Korubo et.al.2021).

The immune responses are majorly controlled by cytokines which play a major role in the disease behavior and management of CLL (Cao and Yan.2020). The IL-4 is a critical survival factor derived under microenvironment that shields CLL cells against spontaneous and drug-induced cell apoptosis especially those of fludarabine and chlorambucil (Guo et.al.2016). Earlier researchers have established that CLL cells are highly vulnerable to apoptosis as compared to normal B cells and high expression of prognostic markers with desirable outcome (Fiorcari et.al.2021). The levels of IL-4 were significantly higher in the CLL patients than controls in the present study, which indicates a change in immune profile as Th1-dominant to Th2-dominant response. This result can promote gradual yet steady development of CLL (Kholoud et.al.2024). B-CLL pathogenesis has also been linked to increased IL-4 expression (Allegra et.al.2020) and the same has been observed with ELISA by Ali et al. (Ali et.al.2020), who show significantly high IL-4 levels in CLL patients. IL-4 stimulates the differentiation of B-cells, the switching of immunoglobulin classes (especially to IgE), antigen presentation, and cell growth (Hui et.al.2022). IL-4-IL-4R binding triggers JAK1/JAK3 phosphorylation that triggers anti-apoptotic signaling via PI3K/AKT, Ras/MAPK, and NF-kB pathways triggered by the activation of STAT6 (Leslie et.al.2019). In the current study the proportion of CLL patients who were above the IL-4 cut-off value (>252.3 ng/mL) were 70/30 of CLL patients and the healthy controls respectively ($P < 0.001$). The analysis of ROC indicated that IL-4 had 70 percent sensitivity, 70 percent specificity, and AUC value of 0.707, which may indicate the potential effect of IL-4 as a useful biomarker to calculate disease activity. Such results comply with Ali et al. (Ali et.al.2020) who also found the same diagnostic significance of IL-4 in CLL.

The patients with CLL also had a significant elevation in serum levels of MCP-1 (CCL2). These observations are in agreement with other literature findings in malignancies, such as mesothelioma (Kishimoto et.al.2019), Hodgkin disease (Hao et.al.2020) and various solid tumours. High CCL2 levels have been linked in the past with bad prognostic outcome in CLL and augmented tumor-supportive signaling (Gandhi and Balakrishnan.2013). It was shown by Burgess and co-authors (Burgess et.al.2012) that CLL displayed a high level of CCL2, particularly in the bone marrow microenvironment, which supports the survival of leukemic cells. This is consistent with a set of findings that predict a more aggressive disease following CCL3 and CCL4 expression (Nasif and Al-Mahdi.2016). It is speculated that elevated chemokine signaling leads to the stimulation of many anti-apoptotic signals, and therefore the targets of new therapeutic methods are potential candidates.

CCL2 levels in the current study were considerably greater in patients with a disease duration of more than 5 years than in patients with a disease duration of 5 years or less (585.06 $\pm$ 61.5 vs. 445.86 $\pm$ 55.6; $P = 0.044$), which suggests that CCL2 may play a role in long-term disease progression.

On the same note, serum MIP-1 α (CCL3) in CLL patients (109.66 $\pm$ 13.8) was significantly high compared to controls (75.33 $\pm$ 10.1, $P < 0.001$). Nonetheless, the level of CCL3 was no longer significantly different by the duration of the disease ($P = 0.134$). CCL3 is an important mediator of B-cell receptor (BCR)-independent microenvironmental interactions. The BCR stimulation results in the release of CCL3 that magnetizes the arrival of accessory cells to the leukemia niche, and subsequently, enhances the survival of the CLL cells. BCR signaling is highly inhibited, which demonstrates that it is reliant on CCL3 expression (Tolbah et.al.2016). In line with our results, Nasif and Al-Mahdi (Nasif and Al-Mahdi.2024) and Hartmann et al. (Hartmann et.al.2016) documented a statistically high level of CCL3 amongst CLL patients. High levels of CCL3 are possible evidence of active BCR signaling and continuous clonal growth (Blanco et.al.2021).

The increasing attention to prognostic biomarkers has demonstrated the necessity of parameters that would anticipate disease progression and help when making treatment decisions due to the heterogeneity of CLL (Chen et.al,2022). Eighty-five percent of the patients in this study had a CCL3 cut-off value (>104.85 ng/L) that was above the cut-off of healthy controls (13.3%). The ROC analysis established a sensitivity of 85.0, specificity of 86.7, PPV of 92.7, NPV of 74.3, and an AUC of 0.870 indicating that CCL3 is sensitive and the imagination of the prognosis of CLL.

Conclusion

This study demonstrates that the immunological profile of patients with CLL is markedly altered, as evidenced by significant elevations in serum IL-4, CCL2, and CCL3 levels compared with healthy individuals. Among the evaluated biomarkers, CCL2 exhibited excellent diagnostic performance, highlighting their potential utility in distinguishing CLL patients from healthy controls. Furthermore, the association between higher CCL2 levels and longer disease duration suggests that this chemokine may also serve as an indicator of disease advancement. Collectively, these findings underscore the importance of immune-mediated mechanisms in CLL and point to IL-4, CCL2, and CCL3 as promising biomarkers for early detection, disease monitoring, and potentially therapeutic targeting.

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