Research Article

Significance of Interleukin-22 and CD38 in Chronic Lymphocytic Leukemia

Esmat A. Al-Sharkawy Emad A. Abdelnaiem, Waleed M. Abdelhamed and Nancy A. Abdelhamed Youssef
Department of Clinical Pathology, El-Minia Faculty of Medicine

Abstract
Introduction: Chronic lymphocytic leukemia (CLL) is one of the most common leukemia in adults and elderly worldwide. It is a heterogeneous disease with variable clinical pattern and evolution. It is characterized by the clonal expansion and accumulation of neoplastic B lymphocytes expressing CD5, CD19, CD20 and CD23 in the bone marrow, peripheral blood and often the lymph nodes. **Aim of the work:** Assessment of expression of IL-22 in patients with chronic lymphocytic leukemia. Study expression of CD38 in patients with chronic lymphocytic leukemia. Correlate both parameters with each other and with clinical and laboratory data studied. **Subjects and Methods:** The present study was carried out at Clinical Pathology Department, Faculty of Medicine, Minia University. It was conducted on thirty five newly diagnosed B-CLL patients and thirty five apparently healthy individuals as control. **Results:** The present study included seventy subjects; thirty five newly diagnosed B-CLL patients and thirty five apparently healthy individuals as control. The patients were selected from Minia University Hospital Outpatient Oncology clinic and Minia Oncology Center from March 2018 to November 2018. **Summary:** This study was carried out at Clinical Pathology Department, Faculty of Medicine, Minia University.
Keywords: APRIL: A proliferation-inducing ligand, **CCR**: CC-chemokine receptor, **ECOG**: Eastern cooperative oncology group

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Introduction
Chronic lymphocytic leukemia (CLL) is one of the most common leukemia in adults and elderly worldwide. It is a heterogeneous disease with variable clinical pattern and evolution. It is characterized by the clonal expansion and accumulation of neoplastic B lymphocytes expressing CD5, CD19, CD20 and CD23 in the bone marrow, peripheral blood and often the lymph nodes[(1)].

Complex immune disorders, present even in patients in early clinical stages, are one of the characteristic features of CLL and are considered to play an important role in disease pathogenesis. It became clear that transformation and progression of tumors is not an independent process but it is controlled by their interactions with tumor microenvironment that sustains the malignant B cell clones, delays their apoptosis and may contribute to the pathogenesis and progression of the disease [(2)].

Cytokines has been indicated to be an associated factor with survival in B-CLL cells and may be correlated with disease progression by upregulating antiapoptotic proteins and by furnishing prosurvival signals[(3)].

Interleukin 22(IL-22), a member of the IL-10 family, is a cytokine secreted by several types of immune cells including IL-$22^{+}CD4^{+}$ T cells (Th22) and IL-22 expressing innate leukocytes (ILC22). The IL-22-R is a heterodimer complex of IL-22R1 and IL-10R2. It contains three main domains: extracellular, trans-membrane and an intracellular signaling region. IL-22 initially binds to the IL-22R1 subunit which undergoes a conformational change that allows binding of IL-10R2, leading to activation of STAT3 signaling cascades and mitogen-activated protein kinase pathways, which are both proliferative and anti-apoptotic, allowing for maintenance of epithelial barriers and tissue preservation[(4)].

IL-22 is involved in the regulation of cell cycle control, cell growth and proliferation; it is possible that IL-22 might play a role during tumor genesis. It is demonstrated that IL-22 expression and signaling is dysregulated in patients with many common cancers including skin, liver, gut and lung cancers[5]. Moreover, IL-22 is implicated to contribute in the pathogenesis of some leukemic disorders such as AML, ALL[6] and MDS[7].

CD38 is a trans-membrane glycoprotein that catalyzes the synthesis of cyclic Adenosine Diphosphate (ADP) ribose (cADPR), an important second messenger mobilizing Ca2+ from Ryanodine-sensitive intracellular stores. CD38 is expressed on a variety of cell types including immature B-lymphocytes and plasma cells. CD38 expression varies in CLL and there is evidence that its expression is induced in so-called pseudofollicles, the proliferative compartment of CLL[8].

The percentage of CD38 (CD38+ cells) is an indicator of the potential and actual degree of cellular activation of the clone. High levels of CD38 in CLL cells are generally associated with advanced disease stage, higher incidence of lymphadenopathy, high-risk cytogenetics, shorter lymphocyte doubling time, shorter time to first treatment and poorer response to therapy. Besides being a prognostic marker, CD38 is a component of a molecular network which delivers growth and survival signals to CLL cells. The aggressiveness of $CD38^+$ cells appears to rely upon their ability to migrate and take advantage of interactions with the microenvironment[9].

Aim of the work

- Assessment of expression of IL-22 in patients with chronic lymphocytic leukemia.
- Study expression of CD38 in patients with chronic lymphocytic leukemia.
- Correlate both parameters with each other and with clinical and laboratory data studied.

Subjects and Methods

The present study was carried out at Clinical Pathology Department, Faculty of Medicine, Minia University. It was conducted on thirty five newly diagnosed B-CLL patients and thirty five apparently healthy individuals as control.

Patients were attending Minia University Hospital outpatient oncology clinic and Minia Oncology Center and from March 2018 to November 2018.

The selected subjects included in the study were divided into two groups.

- **Group I (patients group):** It included thirty five newly diagnosed patients with B-CLL; 22 males and 13 females, their ages ranged from 48 to 71 years old.
- **Group II** (**control group):** It included thirty five apparently healthy individuals matched for age and sex, 23 males and 12 females, their ages ranged from 48 to 61 years old

Exclusion criteria:

1. Acute or chronic infection
2. Acute or chronic inflammatory diseases
3. Acute or chronic kidney diseases.

Patients were diagnosed in accordance with the guidelines outlined by the International Work shop on CLL for Diagnosis[10] after being subjected to full assessment of the following;

Results

The present study included seventy subjects; thirty five newly diagnosed B-CLL patients and thirty five apparently healthy individuals as control. The patients were selected from Minia University Hospital Outpatient Oncology clinic and Minia Oncology Center from March 2018 to November 2018.

The selected subjects included in the study were divided into two groups.

- **Group I (patients group):** It included thirty five newly diagnosed patients with B-CLL, 22 males and 13 females, their ages ranged from 48 to 71 years old with Mean ± SD (61.6±5.3**).**
- **Group II** (**control group):** It included thirty five apparently healthy individuals matched for age and sex, 23 males and 12 females, their ages ranged from 48 to 61 years old with Mean ± SD (59.1±6.1).

Table (1): Comparison of the studied hematological laboratory data between both groups.

		Patients (group I)	Control (group II)	P value
		N=35	N=35	
HB (g/dl)	Range Mean ± SD	(7.3-11.2) 10±1.2	(12-15.1) 12.9±0.9	**<0.001***
Platelets (x10^3 /µl)	Range Mean ± SD Median	(34.5-254) 130±62.6 133	(227-393) 284.5±50.4 268	**<0.001***
WBCs (x10^3 /µl)	Range Mean ± SD Median	(10.2-420) 71±84.9 42	(4-10.9) 6.7±1.9 6.7	**<0.001***
Percentage of lymphocytes (%)	Range Mean ± SD	(66-94) 78±7.8	(20-44) 32.8±7	**<0.001***
Absolute lymphocyte count (x10^3/µl)	Range Mean ± SD Median	(7.6-336) 50.1±60 29.8	(1.6-3.4) 2.1±0.6 2.1	**<0.001***
ESR 1st hour (mm/hr)	Range Mean ± SD	(30-100) 69±17	(8-18) 12.1±2.7	**<0.001***

- **Independent samples T test for parametric quantitative data between the two groups**
- **Mann Whitney test for non-parametric quantitative data (expressed by median) between the two groups**
- ***: Significant difference at P value < 0.05**

Table 1 shows comparison between group I and group II as regard Hemoglobin level, Platelet count, WBC count, Lymphocyte percentage, Absolute lymphocytic count and ESR.

The range of HB in group I was 7.3 – 11.2 g/dl with mean ± SD (10±1.2) whereas in group II was 12 – 15.1 g/dl with mean ± SD was (12.9 ± 0.9). There was a statistically significant decrease in HB level in group I when compared to group II (P<0.001).

The range of platelet count in group I was 34.5-254 x10^3/µl with mean ± SD (130±62.6), while in group II, it was 227-393 x10^3/µl with mean ± SD (284.5±50.4). There was a statistically significant reduction in platelet count in group I when compared to group II (P< 0.001).

In group I, WBC count ranged from 10.2-420 (x10^3/ µl) with mean ± SD (71±84.9) while in group II, it ranged from 4 – 10.9 (×10^3/µl) with mean ± SD (6.7± 1.9). There was a statistically significant increase found in WBCs when comparing group I to group II (P<0.001).

In group I, the range of lymphocyte percentage was 66-94**%** with mean ± SD (78±7.8) whereas in group II, it was 20-44**%** with mean ± SD (32.8±7).There was a statistically significant increase in lymphocyte percentage in group I when compared to group II (P< 0.001).

There was a statistically significant increase in absolute lymphocytic count in group I when compared to group II with mean ± SD (50.1±60) versus (2.1±0.6) respectively (P< 0.001)..

The range of ESR in group I was 30-100 mm/hr with mean ± SD (69±17) while in group II, it was 8-18mm/hr with mean ± SD (12.1±2.7). There was a statistically significant increase found in ESR when comparing group I to group II (P<0.001).

Discussion

Chronic lymphocytic leukemia (CLL) is the most common type of adult leukemia in the world[11]. B-CLL is a malignancy of CD5$^+$ B cells that is characterized by accumulation of small, mature-appearing neoplastic lymphocytes in the blood, marrow and secondary lymphoid tissues, resulting in lymphocytosis,

leukemia cell infiltration of the marrow, lymphadenopathy and splenomegaly[12].

CLL can be divided into two main subsets, which differ in their clinical behavior. These subsets are distinguished by whether CLL cells express an unmutated or mutated immune-globulin heavy-chain variable region gene (IGHV), reflecting the stage of normal B cell differentiation from which they originate. Patients with CLL cells that express an unmutated IGHV typically have moreaggr-essive disease than patients with CLL cells that express a mutated IGHV[13].

The diagnosis of CLL requires the presence of $\geq 5 \times 10^9$/L B lymphocytes in the peripheral blood, sustained for at least 3 months. The clonality of these B lymphocytes needs to be confirmed by demonstrating immunoglobulin light chain restriction using flow cytometry[14].

Flow cytometric analysis of the mononuclear cells in the blood, marrow or lymph nodes can help to reach definitive diagnosis. CLL B cells typically express CD5, CD19 and CD23 and have low levels of CD20, but lack expression of CD10 and stain poorly, if at all, with the FMC7 monoclonal antibody [15].

CLL is a dynamic malignancy that not only depends on intrinsic genetic defects, but is maintained by microenvironmental interactions with stromal cells through different cytokine and chemokine signals that exert a prosurvival effect and contribute toward disease progression[16]

Conclusions & Recommendations

1. B-CLL patients have elevated IL-22 %.
2. IL-22 expression is associated with CD38 expression which is a dependable marker of poor prognosis in patients with CLL.

Recommendations

1. Larger scale studies with long follow-up periods are needed to assess the prognostic impact of the IL-22 signaling pathway.
2. Further large studies are required to unveil the biological importance of this pathway in the pathophysiology of CLL.
3. Further large scale studies are needed to investigate its possible use as a marker of an unfavorable prognosis.
4. Further studies are needed to decide whether its inhibition could have any therapeutic implications in the treatment of the disease.

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