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Expression Changes of CX3CL1 and Interleukin-6 Genes During Remission Induction Therapy in Patients with Acute Myeloid Leukemia

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Abstract

Background: Acute myeloid leukemia (AML) syndrome is a hematologic malignancy due to the extensive clonal proliferation of leukemic precursor cells and is rapidly fatal unless treated or in response to chemotherapy. Cytogenetic findings have an important role in the prognosis and categorization of AML. This study aimed to investigate the expression changes in *CX3CL1* and *Interleukin-6* (*IL-6*) genes before and after chemotherapy as remission induction therapy in AML patients. **Materials and Methods:** In this study, 69 patients (36 males, 33 female) with AML were selected from tertiary medical health centers. A quantitative polymerase chain reaction was performed for mRNA expression of *CX3CL1* and *IL-6* genes before and after induction chemotherapy using the 2-ΔΔCT method. **Results:** The expression of *CX3CL1* and *IL-6* were significantly increased after induction chemotherapy. Also, the ΔCt mean of *CX3CL1* and *IL-6* mRNA was not significant between AML subtype groups. **Conclusion:** We showed that chemotherapy significantly increases the expression of *CX3CL1* and *IL-6*, which can be used as a prognostic factor of AML. **[GMJ.2021;10:e2288]** DOI:10.31661/gmj.v10i0.2288

Keywords: Acute Myeloid Leukemia; *CX3CL-1*; *IL-6*; Remission Therapy

Introduction

Acute myeloid leukemia (AML), as the most common type of acute leukemia, is an increased proliferation of precursor cells and acclamation of immature blast cells, which can result in the arrest of maturation.

The cure rate of AML is dependent on age, which is about 35% in younger than 60 years, and it is about 15% in those older than 60 years [1].

However, more than half of cured patients relapse after AML therapeutics, which may be due to the presence of minimal residual disease [2].

This residual presence may be due to chemotherapy. High-dose chemotherapy as a treatment for AML patients rapidly targeting dividing cells can lead to the acclamation of

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maturated proteins, poor efficacy, and high toxicity of this treatment option [3].

In recent studies, novel treatment strategies have been suggested based on small molecules and mediators involved in signal transduction pathways and cell cycle regulation in the prognosis of AML [4, 5].

In this regard, studies are underway to find non-invasive biomarkers that could be used for diagnosis, classification, and treatment progression [6]. Also, these potential markers were initial steps for the investigation of new targeted drug development [7, 8].

CX3CL1 (fractalkine) can act as an adhesion or chemical molecule for T-cells and natural killer cells. Except for *CX3CL1* and *CXCL12,* which are mainly expressed by neurons and astrocytes, most chemokines are not inherently expressed but can be simulated under various conditions of diseases [9]. *CX3CL1* expression has been reported in many types of hematopoietic and/or non-hematopoietic cells, such as endothelial and epithelial cells, lymphocytes, neurons, microglial cells, and osteoblasts. *CX3CL1* is involved in applying leukocytes associated with multiple inflammatory disorders and in the tumorigenic process and anti-tumor properties [10].

The role of *CX3CL1/CX3CR1* in the pathogenesis of cancer has long been discussed. The dual activity of *CX3CL1* as a chemoattractant for leukocytes and the adhesion molecule for tumor cells that express the receptor may provide various results regarding the tumorigenesis process [11]. The *CX3CL1/CX3CR1* axis is involved in B-cell chronic lymphocytic leukemia expression in the interaction between leukemia cells and the tumor microenvironment [12].

Interleukins (ILs) play an important role in the body's natural immune response and enhance the ability of the immune system to inhibit cancer. *IL-6* is involved in both innate and acquired immunity [13].

The active form of *IL-6* is a homodimer with each subunit being a spherical domain with four alpha-helices.

The *IL-6* receptor comprises a cytokine-binding subunit and a signal transduction subunit, which belong to the type I cytokine receptor family. AML often produces *IL-6* and other cytokines such as colonic stimulating factor (CSF; G-CSF, M-CSF, and GM-CSF), tumor necrosis factor (TNF)-α, and IL-1. AML blast cells that produce only *IL-6* could not be independent of in vitro colonies, whereas blast cells that express CSF in addition to *IL-6* were able to form such colonies [13, 14].

IL-6 can modify gene transcription through Janus kinase (JAK) and signal transducers and transcriptional activators (STAT) and could inhibit apoptosis in different types of tumor cells.

Also, *IL-6* has been known as an inflammatory mediator involved in the biology of carcinoma and can serve as both mitogen and survival factors [15].

The aim of this study was to investigate the expression changes of *CX3CL1* and *IL-6* genes as two important factors during remission induction therapy as a possible prognostic factor in AML patients.

Materials and Methods

In this study, 69 patients diagnosed with AML were selected from the hematology-oncology ward of Namazi hospital affiliated to Shiraz University of Medical Sciences (SUMS) as a tertiary medical health center using the census selection method. Diagnosis of AML was considered based on the World Health Organization (WHO) system.

All the procedures were approved by the Ethics Committee of SUMS (code: IR.SUMS.REC.1394-01-01-11234). All subjects signed an informed contest at the beginning of the study. Bone marrow sample was evaluated in the case of morphology and cytochemistry, and classified according to the French–American–British (FAB) classification system. Also, 5 ml of venous blood was sampled for evaluating complete blood count, blast percentage, and hemoglobin (HB) levels. All patients received standard chemotherapy, which consisted of daunorubicin and cytarabine, as well as, for AML-M3 patients, arsenic trioxide and all-trans-retinoic acid (ATRA) in two divided doses in addition to standard remission induction chemotherapy strategy as previously described [16-20].

Table 1. Primer Sequence for RT-PCR

Gene	Primer sequences (5'->3')
IL-6	Forward: AGAGGCACTGGCAGAAAACA
	Reverse: CAGCTCTGGCTTGTTCCTCA
CX3CL-1	Forward: CCACTCCCTGCAACCTGATT
	Reverse: ACACCACACTAGCCTCATGC
GAPDH	Forward: GGACTCATGACCACAGTCCA
	Reverse: CCAGTAGAGGCAGGGATGAT

Table 2. Clinical and Laboratory Variable of AML Patients Before Induction Chemotherapy

Variable	n (%) or Mean±SD
Sex	
Male	36 (52.2)
Female	33 (47.8)
Age (year)	47.42±16.54
FAB classification	
M0	11 (15.9)
M2	30 (43.5)
M3	7 (10.1)
M4	10 (14.5)
M5	8 (11.6)
M6	2 (2.9)
M7	0 (0)
Blood count	
WBC (10^3/µL)	42175.54±65163.05
HB (g/dl)	8.08±2.27
PLT (10^3/µL)	56168.59 ± 64373.26
LDH (U/L)	1307.88 ± 994.78
Response rate after treatment	
CR	34 (49.3)
NCR	35 (50.7)

FAB: French-American-British; **WBC:** White blood cell; **HB:** Hemoglobin; **PLT:** Platelets; **LDH:** Lactate dehydrogenase; **CR:** Complete remission; **NCR:** Not complete remission.

RNA Isolation and cDNA Synthesiss

Before and after chemotherapy, 5 ml of venous blood was sampled and was collected in ethylenediaminetetraacetic acid (EDTA). The peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient centrifugation. Total RNA was isolated by Trizol (Invitrogen, Carlsbad, CA, USA) using the RNX-Plus solution (CinnaGen, Tehran, Iran) according to the protocol supplied by the manufacturer as previously described [16-18, 21].

Real-Time Polymerase Chain Reaction (RT-PCR)

The relative mRNA expression of *CX3CL1* and *IL-6* genes were evaluated by the SYBR Premix Ex Taq II (Tli RNaseH Plus) Master Mix (Takara, Japan). Internal control for minor fluctuations was the glyceraldehyde 3-phosphate dehydrogenase gene, and the expressions of *CX3CL1* and *IL-6* mRNAs were normalized. The RT-PCR method was performed using SYBR Premix Ex TaqTM II (Tli RNaseH Plus, Takara, Japan) and designed specific primers for each gene in an iQ5 thermocycler (BioRad Laboratories, USA) according to the manufacturer's instructions, as previously described [16-18]. Primer sequences are presented in Table-1.

Statistical Analysis

The differences in the mean expression levels of *CX3CL1* and *IL-6* before and after chemotherapy were compared using the paired t-test. To determine significant differences in gene expression levels among patients with a different type of AML (FAB subtypes), we used the one-way ANOVA. Furthermore, a Chi-square test was used to analyze the mean expression levels of *CX3CL1* and *IL-6* and other laboratory data. The results were analyzed by using SPSS software version 15 (IBM Corp., Armonk, NY, USA). The P-value less than 0.05 was considered significant.

Results

Thirty-six men and 33 women with a mean age of more than 40 years old were evaluated.

Table 3. Change in *CX3CL1* and *IL-6* Expression According to Clinical and Other Laboratory Variable of Patients

Variable	CX3CL1		IL-6	
	Mean ± SD	P-value	Mean ± SD	P-value
Sex				
Male	28±2.59	0.007	26.31±3.73	0.168
Female	29.43±1.58		27.38±2.43	
Age (year)	-0.178*	0.142	0.101*	0.407
FAB classification				
M0	28.13±0.82		26.57±2.32	
M2	29.27±1.95		27.36±2.78	
M3	28.48±2.63		24.44±2.61	
M4	28.19±3.34	0.068	26.89±4.19	0.101
M5	28.87±1.87		27.94±2.97	
M6	24.48±3.34		22.66±7.58	
M7	------		-----	
Blood count				
WBC (10^3/µL)	-0.261*	0.03	-.096*	0.433
HB (g/dl)	0.163*	0.18	0.011*	0.93
PLT (10^3/µL)	0.219*	0.07	0.059*	0.632
LDH (U/L)	-0.2*	0.327	-0.294*	0.145
Response rate after treatment				
CR	28.71±2.6	0.932	27.12±3.63	0.459
NCR	28.64±1.94		26.5±2.74	

* Pearson correlation
FAB: French-American-British; **WBC:** White blood cell; **HB:** Hemoglobin; **PLT:** Platelets; **LDH:** Lactate dehydrogenase; **CR:** Complete remission; **NCR:** Not complete remission.

AML-M2 was the most frequent (43.5%) subgroup among AML patients. More descriptive variables are presented in Table-2. AML patients present with increased white blood cell (WBC) count (called leukocytosis); however, the platelet (PLT) count and HB were critically low. Before chemotherapy, the expression of the *CX3CL1* was markedly different between both genders (P=0.007), while expression of the *IL-6* was not significant (P=0.161). Also, before chemotherapy, there was no significant correlation between the expression of *CX3CL1* and *IL-6* with age, PLT, and HB levels of AML patients (P>0.05). It was the same with *IL-6* expression and WBC count. While, there was a significant correlation between *CX3CL1* expression and WBC count (r=-0.261, P=0.03). Also, the expression of *CX3CL1* and *IL-6* before chemotherapy showed no differences among AML subgroups (P=0.068 and P=0.101, respectively, Table-2). The ΔCt mean of *CX3CL1* and *IL-6* expressions was 6.48±4.68 and 4.62±5.43, respectively. The ΔCt mean of *CX3CL1* had a significant difference according to gender (P=0.013), whereas in the case of

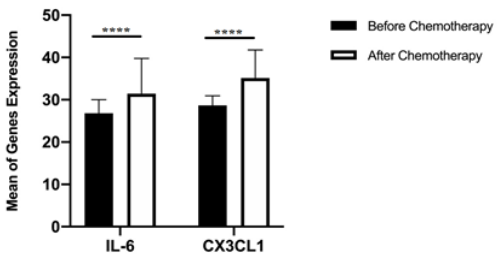

Figure 1. Expression change in *CX3CL1* and *IL-6* following chemotherapy in AML patients.
**** P<0.0001.

Table 4. Change in the ΔCt Mean of *CX3CL1* and *IL-6* Expression According to Clinical and Laboratory Variable of Patients

Variable	ΔCt CX3CL1		ΔCt IL-6	
	Mean ± SD	P-value	Mean ± SD	P-value
Sex				
Male	5.16±4.73	0.013	3.47±5.81	0.065
Female	7.91±4.23		5.86±4.76	
Age (year)	-0.262	0.03	-0.091	0.457
FAB classification				
M0	4.41±2.78	0.51	2.85±3.28	0.11
M2	7.98±4.47		6.07±5.07	
M3	5.81±3.2		1.77±3.79	
M4	6.7±6.24		5.41±7.53	
M5	5.12±4.76		4.19±5.21	
M6	-0.062±2.13		-2.44±8.78	
M7	-------		------	
Blood count				
WBC (10^3/µL)	0.13	0.018	-0.192	0.114
HB (g/dl)	0.13	0.286	0.05	0.681
PLT (10^3/µL)	0.1	0.414	0.029	0.813
LDH (U/L)	-0.235	0.249	0.289	0.152
Response rate after treatment				
CR	6.77±5.07	0.397	5.18± 6.06	0.605
NCR	6.18±4.32		4.06±4.77	

FAB: French-American-British; **WBC:** White blood cell; **HB:** Hemoglobin; **PLT:** Platelets; **LDH:** Lactate dehydrogenase; **CR:** Complete remission; **NCR:** Not complete remission.

IL-6 expression, there was no significant difference based on gender (P=0.065).

Concerning remission of patients, the ΔCt mean of *CX3CL1* and *IL-6* mRNA revealed a significant difference in patients with complete remission compared to patients without complete remission (P>0.05). The ΔCt mean of *CX3CL1* and *IL-6* mRNAs had no significant difference among AML subtype groups (P=0.51 and P=0.11, respectively).

There was an inverse correlation between the *CX3CL1* expression and age (r=-0.262, P=0.03). Also, regarding laboratory data before chemotherapy, WBC positively correlated with *CX3CL1* expression. However, there was no significant correlation between *IL-6* expression and age and laboratory data (Table-3). The expression rate of *CX3CL1* and *IL-6* were significantly increased after chemotherapy (P<0.0001, Figure-1 and Table-4).

Discussion

In this study, the expression changes of *CX3CL1* and *IL-6* were investigated during remission induction therapy in patients with AML to find out the effect of expression changes of these genes on the prognosis of AML. Based on our data, *CX3CL1* and *IL-6* expression were increased after chemotherapy. Also, our data showed that the *CX3CL1* expression at the baseline was related to WBC count, and it had a significant difference between gender.

Also, there was a negative correlation between the ΔCt mean of *CX3CL1* and the age of patients. On the other hand, the ΔCt mean of *CX3CL1* had a considerable difference

between both genders, while this difference was not observed in the ΔCt mean of *IL-6*.

As the regulators of the immune system, cytokines play a role in immune response and inflammation. Therefore, they are effective in transplant success [22]. The *CX3CL1* and *IL-6* play an important role in regulating the expression of pro- and anti-inflammatory cytokines [9, 13]. Allogeneic hematopoietic cell transplantation is a curative treatment of AML, especially for young patients, but this method is complicated by the occurrence of graft-versus-host disease (GVHD) [23, 24]. Brissot *et al*. reported that the *CX3CL1* pathway participates in acute GVHD pathogenesis [25]. Furthermore, Li *et al*. showed that the low level of *IL-6* was related to treatment failure of AML [26]. Devemy *et al*. demonstrated that changes of the leukemia cells during the remission phase were associated with an increased level of *IL-6* [27].

Erreni *et al*. showed that glioblastoma cancer stem cells and precursor cells express *CX3CL1* and CX3CR1, which can occur early in the tumorigenesis process [28]. In other studies, expression of the *CX3CL1* in neuroblastoma cell lines elicited an effective anti-neuroblastoma immune response through natural killer cells and T-cells [29]. Zeng *et al*. studied the expression of *IL-6* and its receptor in 39 patients AML, 23 patients with acute lymphoblastic leukemia (ALL), and seven patients with acute mixed lineage leukemia (AMLL). They showed high levels of *IL-6* were expressed in 21% of AML patients and 29% of AMLL patients, whereas in ALL, the expression of *IL-6* was almost negligible [29]. Also, Sugiyama *et al*. reported similar results. They reported that the expression of *IL-6* and its associated genes in acute leukemia was common in AML but rare in ALL [13]. However, the roles of *IL-6* and *CXCL3* in the development and prognosis of AML are unclear. So, it seems the *CX3L1* and *IL-6* have important roles in response to the treatment of AML.

Conclusion

Based on the results of our study, increased expression of *CX3CL1* and *IL-6* after induction chemotherapy might indicate a possible role of these two chemokines in AML. So, *CX3L1* and *IL-6* can be used as markers to repose the induction chemotherapy. Further large-scale studies are needed to confirm the specific role of *CX3CL1* and *IL-6* in the prognosis of AML.

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Conflict of Interest

One of the authors of the article (A. Karimi) is the "editor in chief" of the journal. Based on the journal policy, this author was completely excluded from any review process of this article and the review process of this article. Other authors declare that there are no conflicts of interest.

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