



## Effect of Chemotherapy on CXCL1 and CXCL10 Levels in Acute Myeloid Leukemia Patients with M4/M5 Subtype

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### ABSTRACT

**Background and objectives:** Acute myeloid leukemia (AML) is a heterogeneous malignancy caused by various pathological mechanisms. Chemokines are involved in the initiation, progression, migration, survival, treatment and complications of AML. CXCL1 has an indirect effect on the progression of cancer and CXCL10 produced by leukemia cells attracts natural killer cells toward tumor sites to eradicate cancer cells. The present study investigated effects of chemotherapy on serum levels of CXCL1 and CXCL10 in patients with AML.

**Methods:** Throughout this case-control study, blood samples were collected from AML patients with M4/M5 subtype (n=25) before and after the first stage of a chemotherapy regimen (7+3). Serum levels of the chemokines were determined using commercial ELISA kits. Data were analyzed using two-sample and paired T-test in SPSS 22 software.

**Results:** The level of CXCL10 was high in patients but decreased following chemotherapy. After the chemotherapy the patients attained partial remission. However, the level of CXCL1 did not change in the patients.

**Conclusion:** Although chemotherapy could decrease CXCL10 levels and induce partial remission, CXCL1 levels does not change in AML patients with M4/M5 subtype. Based on the results, the employment of CXCL1 and CXCL10 inhibitors in the chemotherapy regimen could prevent relapse in the later stages or even reduce the duration of treatment.

**Keywords:** Acute myeloid leukemia, chemotherapy, CXCL1, CXCL10.

## INTRODUCTION

Acute myeloid leukemia (AML) is cancer that affects the bone marrow and peripheral blood (1). Long-term disease-free survival is poor in AML patients despite sensitivity to chemotherapy. Minimal residual disease (MRD) usually incurs relapse in a large number of patients (2). Because of the adhesion of AML cells to the bone marrow components and the subsequent protection against chemotherapeutics, bone marrow is the main site of MRD (3). Chemokines and their equivalent receptors are highly engaged in the pathogenesis of AML (2). It has been elucidated that cytokine/receptor axes play a prominent role in leukemogenesis, the persistence of AML cells and treatment outcome (3-5). Scientists have stated that leukemic blasts most often secrete cytokines that potentially initiate or sustain paracrine or autocrine loops as well (5-7). CXCL1 (Gro $\alpha$ ) is a neutrophil recruiter involved in angiogenesis (8).

In many tumors, Bcl-2 is regulated as a pro-survival protein. It can induce CXCL1 expression in microvascular endothelia, which is involved in angiogenesis (9). Similarly, endothelia growth factor (EGF)-induced up-regulation of Bcl-XL induces sprouting angiogenesis in a CXCR2-dependent fashion, involving an ERK-dependent increase in VEGF and a subsequent rise in CXCL1 downstream of Bcl-2 (10).

IFN- $\gamma$  induces CXCL10 secretion by multiple cells including endothelial cells, fibroblasts and monocytes. CXCL10 can act as a chemoattractant for dendritic cells, T-cells, monocytes/macrophages, NK cells and an antitumor agent by inducing T-cell adhesion to endothelial cells, and angiogenesis (11). The antitumor effect of CXCL10 has been indicated through its immune-stimulating and angiostatic properties (12, 13).

Native human AML cells release chemokines such as CXCL1 and CXCL10 (14, 15). CXCL1 is important for the maintenance of leukemic cells in bone marrow (16). In addition, BM mature NK cells express CXCR3 responses to CXCL10/IP-10. On the other hand, CXCR3 is important for the exodus of NK cells from the BM into the blood circulation (17). Given the important roles of CXCL10 and CXCL1 in prognosis and pathogenesis of leukemia (16, 17), in the

present study, we investigated CXCL1 and CXCL10 levels in AML patients with M4/M5 subtype and healthy individuals following a standard regimen (the first stage).

## MATERIAL AND METHODS

The study was approved by the ethics committee of Kerman University of Medical Sciences (approval code =IR.KMU.REC.1395.598).

Samples were collected from 25 AML patients (M4 and M5) hospitalized at Shahid Bahonar Hospital in Kerman, Iran, during 2017-18. We classified AML patients into AML subtypes according to the French-American-British (FAB) classification. Then, FAB subtypes were further approved by immunophenotypic analysis (CD117, CD64, CD34, CD33, CD14, CD13 and HLA-DR) using commercial kits (BD Biosciences, USA) for immunophenotypic analysis. Written consent was obtained from all subjects. Throughout the study, all patients were given the same chemotherapy regimen (7+3: a 7-day continuous infusion of cytarabine at the dosage of 100 or 200 mg/m<sup>2</sup> per day on days 1 to 7 and daunorubicin at 60 mg/m<sup>2</sup> per day on days 1 to 3) and patients with different chemotherapy regimens were removed from the study.

Smears of bone marrow and peripheral blood were prepared from patients and blast cell count was calculated at the time of diagnosis and after the first chemotherapy stage (at the end of the fourth week of complete chemotherapy when complete blood count indices were almost normal). First, 4 ml of blood were taken from patients before/after the chemotherapy (the first stage). Serum was separated and stored at -80 °C. In addition, 25 age- and gender-matched healthy individuals were enrolled from the healthy population in Kerman, Iran. The control subjects had normal CBC and were free from apparent illness or infection.

Serum levels of CXCL1 and CXCL10 were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits (R&D system, Minneapolis, USA) according to the manufacturer's guidelines.

Data were described as mean  $\pm$  standard deviation (SD). Analysis of data was done in SPSS (SPSS Inc., Chicago, IL, USA) using the Shapiro-Wilk- test as well as two-sample and

paired T-test. All statistical analyses were performed at significance level of 0.05.

## RESULTS

After the first stage of chemotherapy, partial remission ( $7.9 \pm 1.2\%$  blast cells in BM and absence of blast cells in PBS) was observed in the patients.

[Table 1](#) presents the demographic and clinical characteristics of patients and healthy control subjects.

The level of CXCL10 was very high before chemotherapy compared with controls. However, it was significantly decreased after the first stage of chemotherapy. There was a significant difference between CXCL10 levels

after the first stage of chemotherapy and the healthy control group (Figure 1) (Table 2). The level of CXCL1 before chemotherapy did not differ significantly with that of control individuals, and there was no significant difference between the level of CXCL1 before and after chemotherapy. However, there was significant difference between CXCL1 levels in the patients after the first stage of chemotherapy and the healthy control group ([Figure 1](#)) ([Table 2](#)).

The results of this study showed that after the first stage of a common (7+3) chemotherapy regimen, CXCL10 levels reduced significantly in AML patients diagnosed with monocytic differentiation.

**Table 1- Demographic and clinical characteristics of AML patients and healthy controls**

Variable	Healthy control	Patients
Gender	12 Male 13 Female	12 Male 13 Female
Age	40 ± 11 years	41.45 ± 15 years
Subtype		
M4	–	16
M5	–	9
% Blast cells in BM <sup>^</sup>	–	47 ± 15
% Blast cells in PB <sup>^</sup>	0	46.45 ± 13
WBC count in PB <sup>*</sup>	8050 ± 963	8360 ± 1230 <sup>#</sup>

Results are presented as mean ± standard deviation. BM: bone marrow, PB: peripheral blood.

<sup>^</sup> at the time of diagnosis

<sup>\*</sup>White blood cell (WBC) count in peripheral blood.

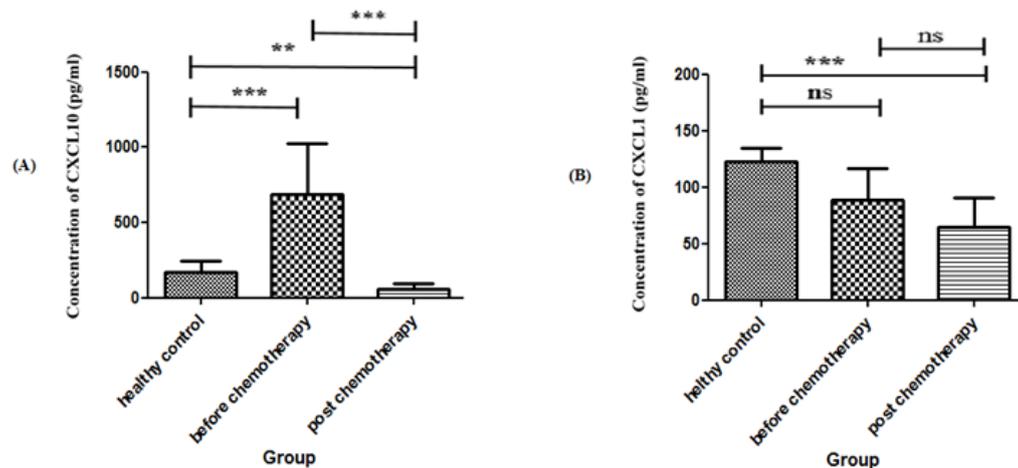
<sup>#</sup> After first stage of chemotherapy.

**Table 2- Serum levels of studied chemokines**

Chemokine	Serum levels of chemokines (pg/mL) <sup>*</sup>					
	AML Patients			p-values		
	Before chemotherapy	After chemotherapy	Healthy control subjects	Healthy control VS Before chemotherapy	Healthy control VS Post chemotherapy	Before chemotherapy VS After chemotherapy
CXCL1	89 ± 25	65 ± 20	123 ± 30	0.0648	P<0.0001	0.4412
CXCL10	690 ± 125	58 ± 15	169 ± 50	P < 0.0001	0.0002	0.0001

<sup>\*</sup>pg/mL as measured by ELISA.

Figure 1- Serum levels of CXCL1 and CXCL10 in AML patients and healthy controls.



(A) shows serum levels of CXCL10 in AML patients prior and post chemotherapy alongside with healthy controls. There was a significant difference in CXCL10 levels between patients before chemotherapy and healthy controls ( $***P<0.0001$ ) and between post chemotherapy and healthy controls ( $**P=0.0002$ ). There was also a significant difference in CXCL10 levels between patients before and after chemotherapy ( $***P=0.0001$ ). (B) shows serum levels of CXCL1 in AML patients before and after chemotherapy and healthy controls. There was no significant difference in CXCL1 levels in patients before chemotherapy and healthy controls ( $P=0.06$ ). There was no significant difference in CXCL1 between patients before and after chemotherapy ( $P=0.4$ ). In addition, there was a significant difference in CXCL1 levels between patients after chemotherapy and healthy controls ( $***P<0.0001$ ).

## DISCUSSION

A bulk of evidence is suggesting that cancer cells can alter the chemokine system. The altered cytokine network interrupts signaling pathways, induces resistance, decreases treatment-associated side effects and improves AML treatment outcomes (3). Therefore we aimed to investigate the effects of a chemotherapy regimen (7+3) on CXCL1 and CXCL10 levels in patients suffering from AML with M4/M5 subtype. Most previous studies indicated that CXCL10/CXCR3 signaling does not play a role in metastasis and tumorigenesis. However, Wightman et al. showed that CXCL10/CXCR3 signaling could be considered as a predictor of metastasis using in vivo and in vitro models of B16F1

melanoma (18). In our study, none of the patients had metastasis and extramedullary AML.

In a study on advanced pancreatic ductal adenocarcinoma, longer overall survival of patients was significantly correlated with the high level of CXCL10. In addition, the high levels of CXCL10 were associated with longer time to progression in patients under chemotherapy. Therefore, scientists and physicians can use plasma CXCL10 as a predictor of survival in advanced pancreatic ductal adenocarcinoma patients receiving chemotherapy (19).

It has been demonstrated that AML cells can produce CXCL10 (15). We also observed that the plasma level of CXCL10 was significantly increased in AML patients with M4/M5 subtype. Activated natural killer (NK) cells express the CXCR3 receptor, which regulates chemotaxis of lymphocytes also because of the migration of NK cells toward gradients of the chemokine CXCL10. Recently, natural killer (NK) cells have been investigated for treatment of patients suffering from hematological malignancies (20). In addition, alloreactive donor NK cells can exert anti-tumor effects in patients suffering from AML that received hematopoietic cell transplantation (20).

In our study, the level of CXCL10 was very high in untreated AML patients. The patients attained partial remission on the (7+3) chemotherapy regimen. It can be inferred that the high level of CXCL10 may attract NK cells, thereby inducing anti-tumor effects resulting in a partial remission. However, CXCL10 was significantly reduced compared with healthy control. Increased release of

several T-cell chemotactic chemokines, such as CXCL10 together with a protein kinase C agonist induce apoptosis in AML cells. A combination of immunostimulation through increased local T-cell recruitment and direct anti-leukemic effects may result in synergistic anti-leukemic effects (21).

According to another study, the concept of chemokine-dependent immune angiostasis proposes that interferon-inducible ELR-chemokines in combination with Th1-type immune cells may synergistically induce tumor regression. Thus, inhibition of CXCL10 may induce metastasis and growth in non-small cell lung cancer (NSCLC) in SCID mice (22).

It has been also demonstrated that CXCL1 can attract CD11b+Gr1+ myeloid cells to the tumor site, resulting in a release some chemokines that enhance the survival of cancer cells (23). It has been suggested that inhibition of CXCL1 can increase efficacy of chemotherapy in breast cancer (23).

We found no significant difference between the level of CXCL1 in the patients before chemotherapy and healthy control group. However, the CXCL1 level decreased following the chemotherapy regimen, suggesting a good prognosis for AML patients. Earlier studies suggested that inhibiting the expression of chemokines associated with poor prognosis along with chemotherapy in AML with monocytic differentiation may shorten the

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treatment period and lowering the dose of chemotherapeutics or impeded relapse. In a previous study, we found that the serum levels of CCL3, CCL4 and CCL5 chemokines were elevated in AML patients before chemotherapy. Chemotherapy inhibited CCL3 but not CCL4 and CCL5, which could be associated with a poor prognosis in AML patients (24).

#### CONCLUSION

This study was the first to evaluate effect of chemotherapy on the level of CXCL1 and CXCL10 chemokines in AML patients. Our results specified that the level of CXCL10 is increased in AML patients. Although chemotherapy could decrease CXCL10 levels and induce partial remission, CXCL1 levels does not change in AML patients with M4/M5 subtype. Based on the results, the employment of CXCL1 inhibitors in the chemotherapy regimen could prevent relapse in the later stages or even reduce the duration of treatment.

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#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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