Medical Laboratory Journal
Online ISSN: 2538-4449
Golestan University of Medical Sciences

Original Article

Effect of Chemotherapy on Serum Level of CCL2 in Acute Myeloid Leukemia Patients with Monocytic Differentiation

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Received: 2020/06/11
Revised: 2020/07/20
Accepted: 2020/07/30

DOI: 10.29252/mlj.15.4.9

ABSTRACT

Background and objectives: Acute myeloid leukemia (AML) is a malignancy that involves the bone marrow and peripheral blood. Some chemokines play a role in the progression, migration and tumor initiation and are therefore associated with poor prognosis. CCL2 promotes tumor growth and is associated with poor prognosis in AML patients. We investigated effects of chemotherapy on serum level of CCL2 in AML patients.

Methods: Throughout this case-control study, blood samples were collected from 25 healthy individuals and 25 AML (M4 and M5) patients before and after the first stage of the current chemotherapy regimen (7+3). Serum level of CCL2 was measured using commercial ELISA kits. Data were analyzed in SPSS 22 using the two-sample t-test and paired t-test.

Results: Before chemotherapy, serum level of CCL2 was significantly higher in the patients than in the healthy controls. Following chemotherapy, the serum level of CCL2 reduced significantly to a level comparable to that of the healthy controls.

Conclusion: The current chemotherapy (7+3) can effectively inhibit CCL2 in AML patients.

Keywords: Leukemia, Myeloid, Acute [MeSH], Chemokine CCL2 [MeSH], Chemokines [MeSH].

INTRODUCTION

Acute myeloid leukemia (AML) is an aggressive and heterogeneous cancer of the bone marrow (BM). Treatment of this cancer requires either intensive chemotherapy alone or in combination with allogeneic stem cell transplantation (1). Despite sensitivity to chemotherapy, survival rate is low in patients with AML. The standard chemotherapy for AML patients is the 7+3, with a 7-day continuous infusion of cytarabine (100 or 200 mg/m^2 per day) on days 1 to 7 and daunorubicin (60 mg/m^2 per day) on days 1 to 3 (2, 3).

Chemokines and their receptors are involved in the pathogenesis of AML (2). It has been indicated that cytokine/receptor signaling affects the treatment outcome, progression and persistence of AML (3-5). According to reports, leukemic blasts represent abnormal degrees of responsiveness to cytokine stimulation (5, 6).

CCL2 is a member of the ß-chemokines family that mainly initiates chemotaxis and transendothelial migration of monocytes. Several factors regulate CCL2 expression. Binding of CCL2 to its receptor activates several transduction pathways associated with survival, growth, proliferation, adhesion, cellular mobility and protein transduction (7, 8). CCL2 directly mediates angiogenesis, and interaction with CCR2 on the endothelial cell surface leads to elevated vessel sprout formation and tumor progression. Therefore, higher CCL2 levels relate to active angiogenesis, increased tumor proliferation and unfavorable prognosis in a large number of solid tumors (9, 10). Although the part played by CCL2 in hematological malignancies has not been ascertained yet, increased level of CCL2 in the BM environment of patients with acute lymphoblastic leukemia (ALL) and the serum of untreated AML patients has been observed (10). CCL2 is secreted by tumor cells, AML and B-ALL cells (11, 12). It promotes tumor growth by recruiting myeloid-derived suppressor cells and regulatory T-cells (13, 14). CCL2 induces proliferation and transmigration of AML cells but does not affect the susceptibility to apoptosis caused by Ara-C (15). The expression of CCL2 is negatively correlated with patient survival in breast cancer (16). In addition, CCL2 has a role in resistance to cytotoxic agents in some cancers (15). It can also inhibit proliferation of hematopoietic stem and progenitor cells (HSPCs). However, the inhibitory effects of CCL2 have not yet been evaluated extensively (17).

Because of the increased level of CCL2 and poor prognosis in AML patients as well as the role of CCL2 in tumor growth, we postulated that modulation of cytokine-dependent processes in AML could be included in standard chemotherapeutic regimens to improve overall treatment outcome. Thus, we investigated CCL2 levels before and after the first stage of current chemotherapy (7+3) regimen in AML patients and healthy controls.

MATERIALS AND METHODS

This case-control study was approved by the ethics committee of Kerman University of Medical Sciences, Iran. Written consent was obtained from all participants. Subjects included 25 healthy individuals and 25 AML patients (M4 and M5, 15 females, mean age: 40 ± 15 years) in Shahid Bahonar Hospital (Kerman, Iran) during 2017-18.The subjects had no clinical sign or symptom, and individuals who had abnormal complete blood count (CBC) were excluded from the study. The case and control subjects were matched in terms of gender and age. All patients received the same chemotherapy regimen (7+3) and patients that received different chemotherapy regimens were excluded. In addition, BM and peripheral blood smears (PBS) were prepared for the patients at the time of diagnosis and after the first stage of chemotherapy (at the end of the fourth week of complete chemotherapy when CBC indices reached almost normal) and percentage of blast cells was computed. A pathologist evaluated peripheral blood and BM smears. Then, based on the type of cell in which leukemia developed and the level of maturity of the cells, AML patients were categorized into AML subtypes according to the FAB standard criteria. Next, the FAB subtypes were further approved by immunophenotypic analysis (CD13, CD14, CD33, CD34, CD64, CD117 and HLA-DR) using flow cytometry.

Blood samples (5 ml) were taken from each patient before initiation of chemotherapy and after the first stage of chemotherapy (following the fourth week of complete chemotherapy when CBC was approximately

and CCL3 chemokines were high in AML patients before chemotherapy. CCL5
normal). Serum specimens were immediately frozen and stored at −80 °C.

The serum levels of CCL2 were measured by a commercial ELISA kit (R&D system, Minneapolis, USA) according to the manufacturer's guidelines. The sensitivity of the kit was 6.6 pg/mL.

Data were analyzed using SPSS 22 software (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± standard deviation (SD). The two-sample and paired t-tests were employed for comparison of the underlying variables after verifying normality of data by the Shapiro-Wilk test. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Partial remission was observed in the patients after the first stage of chemotherapy (7.9 ± 1.2% blast cells in BM and absence of blast cells in PBS).

Table 1 presents the clinical and demographic characteristics of the patients and healthy controls.

The level of CCL2 was very high before chemotherapy. However, after the first stage of chemotherapy, CCL2 decreased significantly to a level comparable with that of the healthy control group (Figure 1).

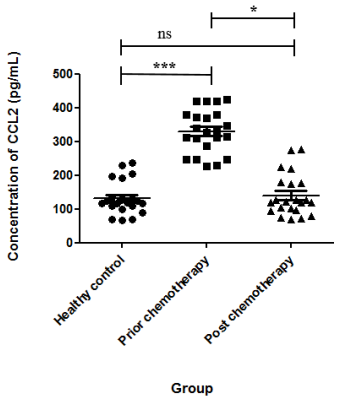

Figure 1- Demonstrates the serum level of CCL2 in AML patients and healthy control. The chemokine serum level was analyzed by ELISA. There was significant difference in patients prior chemotherapy with healthy controls (*P<0.0001). There was not significant difference in patients post chemotherapy with healthy control group (P>0.05). Also there was significant difference in patients prior and post chemotherapy (*P<0.05).**

Table 1- Clinical and demographic characteristics of AML patients and healthy controls

Group	Age (years) (Mean±SEM)	Gender	M4/M5	% Blast cells in BM$^{\Delta}$ (Mean±SEM)	% Blast cells in PB$^{\Delta}$ (Mean±SEM)	WBC count in PB* (Mean±SEM)		Extramedullary involvement
						Before chemotherapy	After chemotherapy	
Patients	41.45 ± 11	12 males 13 females	9/16	47 ± 15	46.45 ± 13	18000±10600	8360 ± 1230$^{\#}$	No
Healthy controls	40 ± 15	10 males 15 females	_	_	_	8050 ± 963		_

Data are presented as mean ± standard deviation.
$^{\Delta}$ at the time of diagnosis
*White blood cell (WBC) count in peripheral blood.
$^{\#}$ post first stage of chemotherapy.

DISCUSSION

Some studies indicated that cancer cells can alter the chemokine system. The altered cytokine network overcomes resistance and improves treatment outcome in AML patients (3). Therefore, we aimed to investigate the effects of current chemotherapy (7+3) on CCL2 in patients suffering from AML with monocytic differentiation. The first stage of chemotherapy was chosen to evaluate the response of AML patients to chemotherapy based on the expression of these chemokines and to help determine whether to increase or decrease the dose of chemotherapy reagents in the next stages. In other words, the level of these chemokines and their associated receptors can serve as biological markers of response to chemotherapy in most patients.

CCL2 is a member of the CC chemokine family that plays various roles in cancers. Cancer cells and different types of stromal cells including monocytes, endothelial cells and fibroblasts can produce CCL2. CCL2-stimulated mononuclear cells produce growth and survival factors, which contribute to tumor progression and angiogenesis (18-21). In a previous study, serum levels of CCL5, CCL4 and CCL3 chemokines were high in AML patients before chemotherapy. CCL5 and CCL4 did not return to the basal level after chemotherapy and were high compared to healthy controls, while CCL3 reached the baseline level after chemotherapy (22).

Wu et al. showed that the expression of CCL2 differs among various leukemia cell lines, suggesting heterogeneity of the leukemia cell lines. Moreover, they showed overexpression of CCL2 in THP-1 cells, a monocytic leukemia cell line driven by the MLL-AF9 fusion gene. They also demonstrated that knocking down CCL2 in HL-60 cells reduce the proliferation rate and leads to G1 growth arrest (10). Elevated plasma level of CCL-2 in untreated AML patients has been reported (18). Our findings showed that the serum level of CCL2 was significantly increased in AML patients with monocytic differentiation compared with healthy individuals, suggesting an inflammatory state. It has been claimed that CCL2 inhibits normal HSPCs but not chronic myelogenous leukemia (CML) progenitor cells, whereas transforming growth factor inhibits both normal HSPCs and CML cells. Thus, CCL2 can contribute to CML development (17). It can be concluded that the high level of CCL2 can indicate poor prognosis in AML patients. Since CCL2 attracts regulatory T cells and macrophages to the tumor environment and contributes to tumor growth (23), we evaluated the response of this chemokine to first stage of chemotherapy. In this regard, a previous study reported that inhibiting CCL2/CCR2 signaling in mice receiving anthracycline-based chemotherapy could induce immune responses against cancer and improve the treatment process (23). In our study, after the first stage of chemotherapy, CCL2 level reduced significantly to the baseline state that was comparable with healthy individuals. Chemotherapy-induced secretion of cytokines potently affects host tissues and subsequently the treatment response and prognosis. In one study, chemotherapy for ALL functionally deregulates stromal cells of BM and reduces CCL3 due to BM damage (24). In other words, chemotherapy increases secretion of various inflammatory cytokines including CCL5 and reduced CCL3 levels (25). Thus, chemotherapy may be the cause of CCL2 depletion in our cases. Therefore, it may not be necessary to control CCL2 in the following stages of chemotherapy. Nevertheless, the role of chemokines in etiology, pathogenesis and treatment of AML is quite intricate, and more studies are needed to further elucidate the part played by the chemokine network in the treatment of AML. It is crucial to consider the expression of these chemokines and their down/upstream gene targets both before and after chemotherapy to gain a deeper understanding of its function.

CONCLUSION

Our results specified that the first stage of chemotherapy could inhibit CCL2 in AML patients with monocytic differentiation. It is suggested to identify other chemokines associated with poor prognosis in AML and investigate how they respond to chemotherapy.

ACKNOWLEDGMENTS

This study was supported by the Rafsanjan University of Medical Sciences, Iran. The authors are grateful to all those who participated in this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Brenner AK, Reikvam H, Bruserud O. *A Subset of Patients with Acute Myeloid Leukemia Has Leukemia Cells Characterized by Chemokine Responsiveness and Altered Expression of Transcriptional as well as Angiogenic Regulators.* Frontiers in immunology. 2016; 7: 205. [DOI:10.3389/fimmu.2016.00205] [PubMed] [Google Scholar]

2. Khorramdelazad H, Mortazavi Y, Momeni M, Arababadi MK, Khandany BK, Moogooei M, et al. *Lack of Correlation Between the CCR5-Delta32 Mutation and Acute Myeloid Leukemia in Iranian Patients.* Indian journal of hematology & blood transfusion : an official journal of Indian Society of Hematology and Blood Transfusion. 2015; 31(1): 29-31. [DOI:10.1007/s12288-014-0408-y] [PubMed] [Google Scholar]

3. Kupsa T, Horacek JM, Jebavy L. *The role of cytokines in acute myeloid leukemia: a systematic review.* Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia. 2012;156(4):291-301. [DOI:10.5507/bp.2012.108] [PubMed] [Google Scholar]

4. Faaij CM, Willemze AJ, Revesz T, Balzarolo M, Tensen CP, Hoogeboom M, et al. *Chemokine/chemokine receptor interactions in extramedullary leukaemia of the skin in childhood AML: differential roles for CCR2, CCR5, CXCR4 and CXCR7.* Pediatric blood & cancer. 2010;55(2):344-8. [DOI:10.1002/pbc.22500] [PubMed] [Google Scholar]

5. Ma Y, Adjemian S, Galluzzi L, Zitvogel L, Kroemer G. *Chemokines and chemokine receptors required for optimal responses to anticancer chemotherapy. Oncoimmunology.* 2014; 3(1): e27663. [DOI:10.4161/onci.27663] [PubMed] [Google Scholar]

6. Kornblau SM, McCue D, Singh N, Chen W, Estrov Z, Coombes KR. *Recurrent expression signatures of cytokines and chemokines are present and are independently prognostic in acute myelogenous leukemia and myelodysplasia.* Blood. 2010;116(20):4251-61. [DOI:10.1182/blood-2010-01-262071] [PubMed] [Google Scholar]

7. Liu Y, Pan J, Pan X, Wu L, Bian J, Lin Z, et al. *Klotho-mediated targeting of CCL2 suppresses the induction of colorectal cancer progression by stromal cell senescent microenvironments*. Molecular oncology. 2019. [View at Publisher] [DOI:10.1002/1878-0261.12577] [PubMed] [Google Scholar]

8. Macanas-Pirard P, Quezada T, Navarrete L, Broekhuizen R, Leisewitz A, Nervi B, et al. *The CCL2/CCR2 Axis Affects Transmigration and Proliferation but Not Resistance to Chemotherapy of Acute Myeloid Leukemia Cells. PloS one.* 2017;12(1):e0168888. [DOI:10.1371/journal.pone.0168888] [PubMed] [Google Scholar]

9. Ravindran D, Cartland SP, Bursill CA, Kavurma MM. *Broad-spectrum chemokine inhibition blocks inflammation-induced angiogenesis, but preserves ischemia-driven angiogenesis.* FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2019:fj201900232RR. [DOI:10.1096/fj.201900232RR]

10. Wu SY, Yang J, Hong D, Xiao PF, Lu J, Gao L, Hu YX, Wang M, Shao XJ, Zhou CY, Li JQ, Pan J, Ling J, Gu WY, Chen RH, Hu SY. *Suppressed CCL2 expression inhibits the proliferation of leukemia cells via the cell cycle protein Cyclin D1: preliminary in vitro data*. Eur Rev Med Pharmacol Sci. 2018; 22(17): 5588-5596. [View at Publisher] [DOI] [PubMed] [Google Scholar]

11. Kitamura T, Qian BZ, Soong D, Cassetta L, Noy R, Sugano G, et al. *CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages*. The Journal of experimental medicine. 2015;212(7):1043-59. [DOI:10.1084/jem.20141836] [PubMed] [Google Scholar]

12. Nagarsheth N, Wicha MS, Zou W. *Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy*. Nature reviews Immunology. 2017;17(9):559-72. [DOI:10.1038/nri.2017.49] [PubMed] [Google Scholar]

13. Chang AL, Miska J, Wainwright DA, Dey M, Rivetta CV, Yu D, et al. *CCL2 Produced by the Glioma Microenvironment Is Essential for the Recruitment of Regulatory T Cells and Myeloid-Derived Suppressor Cells. Cancer research*. 2016;76(19):5671-82. [DOI:10.1158/0008-5472.CAN-16-0144] [PubMed] [Google Scholar]

14. Loyher PL, Rochefort J, Baudesson de Chanville C, Hamon P, Lescaille G, Bertolus C, et al. *CCR2 Influences T Regulatory Cell Migration to Tumors and Serves as a Biomarker of Cyclophosphamide Sensitivity*. Cancer research. 2016;76(22):6483-94. [DOI:10.1158/0008-5472.CAN-16-0984] [PubMed] [Google Scholar]

15 Macanas-Pirard P, Quezada T, Navarrete L, Broekhuizen R, Leisewitz A, Nervi B, et al. *The CCL2/CCR2 Axis Affects Transmigration and Proliferation but Not Resistance to Chemotherapy of Acute Myeloid Leukemia Cells.* PloS one. 2017;12(1):e0168888. [DOI:10.1371/journal.pone.0168888] [PubMed] [Google Scholar]

16. Fang WB, Yao M, Brummer G, Acevedo D, Alhakamy N, Berkland C, et al. *Targeted gene silencing of CCL2 inhibits triple negative breast cancer progression by blocking cancer stem cell renewal and M2 macrophage recruitment.* Oncotarget. 2016;7(31):49349-67. [View at Publisher] [DOI:10.18632/oncotarget.9885] [PubMed] [Google Scholar]

17. Mukaida N, Tanabe Y, Baba T. *Chemokines as a Conductor of Bone Marrow Microenvironment in Chronic Myeloid Leukemia*. International journal of molecular sciences. 2017;18(8). [DOI:10.3390/ijms18081824] [PubMed] [Google Scholar]

18. Mazur G, Wrobel T, Butrym A, Kapelko-Slowik K, Poreba R, Kuliczkowski K. *Increased monocyte chemoattractant protein 1 (MCP-1/CCL-2) serum level in acute myeloid leukemia.* Neoplasma. 2007;54(4):285-9 [PubMed] [Google Scholar]

19. Cho HR, Kumari N, Thi Vu H, Kim H, Park CK, Choi SH. *Increased Antiangiogenic Effect by Blocking CCL2-dependent Macrophages in a Rodent Glioblastoma Model: Correlation Study with Dynamic Susceptibility Contrast Perfusion MRI*. Scientific reports. 2019;9(1):11085. [DOI:10.1038/s41598-019-47438-4] [PubMed] [Google Scholar]

20. Merle M, Fischbacher D, Liepert A, Grabrucker C, Kroell T, Kremser A, et al. *Serum Chemokine-release Profiles in AML-patients Might Contribute to Predict the Clinical Course of the Disease*. Immunological investigations. 2019:1-21. [DOI:10.1080/08820139.2019.1661429] [Google Scholar]

21. Li D, Ji H, Niu X, Yin L, Wang Y, Gu Y, et al. *Tumor-associated macrophages secrete CCL2 and induce tamoxifen resistance by activating PI3K/Akt/mTOR in breast cancer*. Cancer science. 2019. [DOI:10.1111/cas.14230] [PubMed] [Google Scholar]

22. Yazdani Z, Mousavi Z, Ghasemimehr N, Kalantary Khandany B, Nikbakht R, Jafari E, et al. *Differential regulatory effects of chemotherapeutic protocol on CCL3_CCL4_CCL5/CCR5 axes in acute myeloid leukemia patients with monocytic lineage*. Life sciences. 2019;240:117071. [DOI:10.1016/j.lfs.2019.117071] [PubMed]

23. Ma Y, Adjemian S, Galluzzi L, Zitvogel L, Kroemer G. *Chemokines and chemokine receptors required for optimal responses to anticancer chemotherapy*. Oncoimmunology. 2014;3(1):e27663 [DOI:10.4161/onci.27663] [PubMed] [Google Scholar]

24. Corazza F, Hermans C, Ferster A, Fondu P, Demulder A, Sariban E. *Bone marrow stroma damage induced by chemotherapy for acute lymphoblastic leukemia in children*. Pediatric research. 2004;55(1):152-8. [DOI:10.1203/01.PDR.0000099773.71438.91] [PubMed] [Google Scholar]

25. Edwardson DW, Parissenti AM, Kovala AT. *Chemotherapy and Inflammatory Cytokine Signalling in Cancer Cells and the Tumour Microenvironment*. Adv Exp Med Biol. 2019;1152:173-215. [DOI:10.1007/978-3-030-20301-6_9] [PubMed] [Google Scholar]

How to Cite:
Yazdani B, Anani H, Baluchi I, Kalantary Khandany B, Hassanshahi GH [Effect of Chemotherapy on Serum Level of CCL2 in Acute Myeloid Leukemia Patients with Monocytic Differentiation]. mljgoums. 2021; 15(4):9-14 DOI: 10.29252/mlj.15.4.9