ORIGINAL ARTICLE

Gene expression pattern of *CCL2*, *CCL3*, and *CXCL8* in patients with bipolar disorder

Mohsen Ghoryani[1,2], Farhad Faridhosseini[3,4], Ali Talaei[3,4], Reza Faridhosseini[5,6], Jalil Tavakkol-Afshari[1,6], Maliheh Dadgar Moghaddam[7], Parisa Azim[4], Zanireh Salimi[4], Hadi Zare Marzouni[5], Mojgan Mohammadi[1,5]

[1]Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, [2]Department of Laboratory Sciences, School of Para-Medical Sciences, Torbat Heydarieh University of Medical Sciences, Torbat Heydarieh, Iran, [3]Psychiatry and Behavioral Sciences Research Center, Mashhad University of Medical Sciences,Mashhad, Iran, [4]Department of Psychiatry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [5]Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, [6]Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [7]Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Background: Bipolar disorder (BD) is one of the most important psychiatric disorders in the world. There is evidence suggesting the role of inflammatory mediators such as chemokines in the etiology of BD. The objective of the current study was to evaluate the gene expression of *CCL2*, *CCL3*, and *CXCL8* in patients with BD and compare them to healthy controls. **Materials and Methods:** A total of 48 patients with confirmed BD and 48 healthy controls enrolled in this study. All patients were recruited from April to August 2016 at Ibn-Sina Psychiatric Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. RNA was extracted from the whole blood samples and then cDNA was synthesized. Gene expression of *CCL2*, *CCL3*, and *CXCL8* was measured using SYBR® Green real-time polymerase chain reaction. The difference of delta-CT values between patients and healthy controls was compared with the independent samples *t*-tests. **Results:** *CCL2* and *CXCL8* genes expressed at higher levels in patients with BD as compared to healthy controls, but not significant. On the contrary, we found lower expression levels for *CCL3* gene in our patients compared to healthy controls, but the difference was not statistically significant. **Conclusion:** Our findings do not show an association between the gene expression of *CCL2*, *CCL3* and *CXCL8* and BD. Increasing the sample size and evaluation on the gene expression of other chemokines in depression and mania phases of BD might be helpful to get a better conclusion.

Key words: Bipolar disorder, *CCL2* chemokine, *CCL3* chemokine, chemokines, *CXCL8* chemokine

How to cite this article: Ghoryani M, Faridhosseini F, Talaei A, Faridhosseini R, Tavakkol-Afshari J, Dadgar Moghaddam M, *et al*. Gene expression pattern of *CCL2*, *CCL3*, and *CXCL8* in patients with bipolar disorder. J Res Med Sci 2019;24:45.

INTRODUCTION

Psychiatric disorders are among the most common diseases in the world, they are a group of disorders associated with the lack of control over mood and mental experience of severe discomfort.[1,2] People with elevated mood (manic) show expansion, hypnosis, sleep deprivation and increased self-confidence and great thoughts. People who develop depression are characterized by reduced energy, feelings of guilt, anorexia, and suicidal ideation.[3,4] Other symptoms include alterations in the cognitive capabilities, level of activity, speech, and other functions such as appetite, sleep, sexual activity, and biological rhythms. These disorders often result in impairment of social and occupational functioning and put a lot of emotional and financial burden on the patient and the community.[5,6] BD is a severe chronic disease characterized by at least one episode of mania or hypomania, although episodes of depression are commonly seen during illness.[7,8] The main cause of BD is not yet known. Genetic, environmental factors, and biological disorders influence the incidence and severity of BD.[9-11]

In recent years, some studies have shown that immune and inflammatory biomarkers might have a role in the

Access this article online

Quick Response Code:

Website: www.jmsjournal.net

DOI: 10.4103/jrms.JRMS_763_18

Address for correspondence: Dr. Mojgan Mohammadi, Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. E-mail: mohammadimzh@mums.ac.ir

Received: 14-10-2018; **Revised:** 24-12-2018; **Accepted:** 21-02-2019

pathophysiology of BD.[12,13] Patients with BD have elevated peripheral levels of pro-inflammatory biomarkers such as interleukin 1 (IL-1) β, C-reactive protein, and tumor necrosis factor-α.[14,15] Chemoattractant cytokines known as chemokines are key immune mediators in leukocyte trafficking in inflammatory and normal conditions. Some evidence suggests that chemokines are involved in neurobiological processes like modulation of neurotransmitter system and neuroinflammatory responses, and may be good therapeutic targets in BD.[16,17] *CCL2* known as monocyte chemoattractant protein-1 has chemotactic effect on monocyte/macrophages, dendritic cells, and T lymphocytes. Neurons, microglia, and astrocytes up-regulate the expression of *CCL2* and CCR2 in inflammatory conditions.[18] *CCL3* also known as macrophage inflammatory protein 1-alpha (MIP-1-alpha) is considered as neutrophil chemoattractant. In the central nervous system (CNS), astrocytes express *CCL3* and its receptor (CCR1 and CCR5).[17,18] *CXCL8* (also IL-8) is a chemotactic factor for granulocytes, B, and T lymphocytes, dendritic cells, and natural killer cells. Astrocytes, neurons, microglia, and endothelial cells of the blood-brain barrier (BBB) express *CXCL8* receptors (CXCR1 and CXCR2), constitutively.[17,19]

Evaluation of inflammatory biomarkers such as chemokines in patient with BD and compare them with healthy individuals can provide valuable information for future studies to use chemokines for both therapeutic and diagnostic purposes in BD. In this study, we evaluated the gene expression of *CCL2*, *CCL3*, and *CXCL8* in whole blood samples of patients with BD and compared them with healthy individuals.

SUBJECTS AND METHODS

Participants

A total of 48 patients with BD and 48 age- and sex-matched healthy controls were enrolled in this study. All patients were included if they had no history of infection, inflammatory, autoimmune, and cancerous diseases. They were recruited from April to August 2016 at Ibn-Sina Psychiatric Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, and were assessed with the Structured Clinical Interview for DSM-IV-Axis I Disorders (SCID-I) to confirm BD. To characterize the severity of manic and depressive symptoms, patients were also examined with the Young Mania Rating Scale and the Hamilton Depression Rating Scale, respectively. The cutoff point for these scales was considered as 7. Healthy controls with no history of psychiatric disorders (evaluated through SCID-I) and also severe medical and inflammatory diseases from the local population were enrolled in this study. This study was approved by the Ethics Committee of Mashhad University of Medical Sciences, and an informed consent was obtained from each patient and healthy control. The ethical code is IR.MUMS.fm. REC.1394.494.

RNA extraction and cDNA synthesis

Total RNA was extracted from the whole blood samples using Total RNA Purification Mini kit for Blood/Cultured Cell/Tissue (Favorgen, Ping-Tung, Taiwan), and then cDNA was synthesized using cDNA Synthesis Kit (Favorgen), following the manufacturer's instructions.

SYBR® green real-time polymerase chain reaction

Nucleotide database at https://www.ncbi.nlm.nih.gov, and Beacon Designer software (version 7.0, Premier Biosoft, Palo Alto, CA, USA) were used to design specific primers for *CCL2*, *CCL3*, *CXCL8*, as target genes and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as housekeeping gene. Primers were designed and then blasted at https://www.ncbi.nlm.nih.gov/tools/primer-blast for use in the SYBR® Green real-time polymerase chain reaction (PCR). The primer sequences used in the present study are shown in Table 1. SYBR® Green real-time PCR was performed on the cDNA samples using SYBR® Premix EX Taq II (2X) (Takara Bio, Inc., Otsu, Japan) to evaluate *CCL2*, *CCL3*, *CXCL8*, and GAPDH gene expression levels. Real-time PCR was performed in a Rotor-Gene 6000 thermal cycler (QIAGEN, Hilden, Germany).

The SYBR® Green real-time PCR conditions were initial denaturation at 95°C for 10 min followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 20 s. To compare levels of mRNA expression between patients and healthy controls, delta-CT values were calculated.

Table 1: Primer sequences designed for SYBR® green real-time polymerase chain reaction

Gene name	Accession number	Sequence	Length (bp)
CCL2-F*	NM_002982.3	5'-AAACTGAAGCTCGCACTCTCG-3'	21
CCL2-R**		5'-TTGATTGCATCTGGCTGAGCG-3'	21
CCL3-F	NM_002983.2	5'-GGCTCTCTGCAACCAGTTCTC-3'	21
CCL3-R		5'-TCGCTTGGTTAGGAAGATGACAC-3'	23
CXCL8-F	NM_000584.3	5'-AGGACAAGAGCCAGGAAGAAAC-3'	22
CXCL8-R		5'-AAAACTGCACCTTCACACAGAG-3'	22
GAPDH-F	NM_001289746.1	5'-CACTAGGCGCTCACTGTTCTC-3'	21
GAPDH-R		5'-CCAATACGACCAAATCCGTTGAC-3'	23

*Forward primer, **Reverse primer

Statistical analysis

All statistical analyses were conducted using the IBM SPSS Statistics 21 (SPSS Inc., Chicago, IL, USA). Data were tested for normality of distribution using the Kolmogorov–Smirnov test and all data were normally distributed. The difference of delta-CT values between patients and healthy controls were compared with the independent samples *t*-tests. Statistical significance was defined as a value of $P < 0.05$.

RESULTS

The demographic characteristics of the patients and healthy controls are presented in Table 2. *CCL2* gene was expressed at higher levels in patients with BD (delta-CT; mean ± standard error of mean [SEM] −1.13 ± 0.26) as compared to healthy controls (delta-CT; Mean ± SEM −1.74 ± 0.25) but not significant ($P = 0.097$) [Figure 1a]. Patients with BD showed higher levels of *CXCL8* gene expression (delta-CT; mean ± SEM 3.93 ± 0.50) compared to healthy controls (delta-CT; mean ± SEM 3.40 ± 0.49), the difference was not statistically significant ($P = 0.450$) [Figure 1b]. On the contrary, the lower expression levels for *CCL3* gene in patients with BD (delta-CT; mean ± SEM −1.51 ± 0.80) was observed as compared to healthy controls (delta-CT; mean ± SEM −1.19 ± 0.30) but not statistically significant ($P = 0.714$) [Figure 1c].

Table 2: Demographic characteristics of patients with bipolar disorder and healthy controls

	Patients with BD	Healthy controls
Group size	48	48
Age (mean±SD)	35.95±12.32	34±12.37
Gender (%)		
Male	24 (50)	24 (50)
Female	24 (50)	24 (50)
Years of illness (mean±SD)	8.95±5.38	-
HDRS	6.54±2.04	-
YMRS	31.22±5.26	-

HDRS=Hamilton depression rating scale, YMRS=Young mania rating scale, BD=Bipolar disorder, SD=Standard deviation

DISCUSSION

Results of this study revealed that there was no significant difference in the gene expression levels of *CCL2*, *CCL3*, and *CXCL8* in patients with BD as compared to healthy controls. Previous studies on chemokines in patients with BD often focused on serum levels, and there is limited information available regarding the chemokine gene expression profiles in BD at different phases or stages of the disease. To our knowledge, there is just one report regarding the gene expression of *CCL2* in monocytes of patients with BD and their offspring with mood disorders that showed an increasing in the level of *CCL2*.[20] In addition, Nakatani

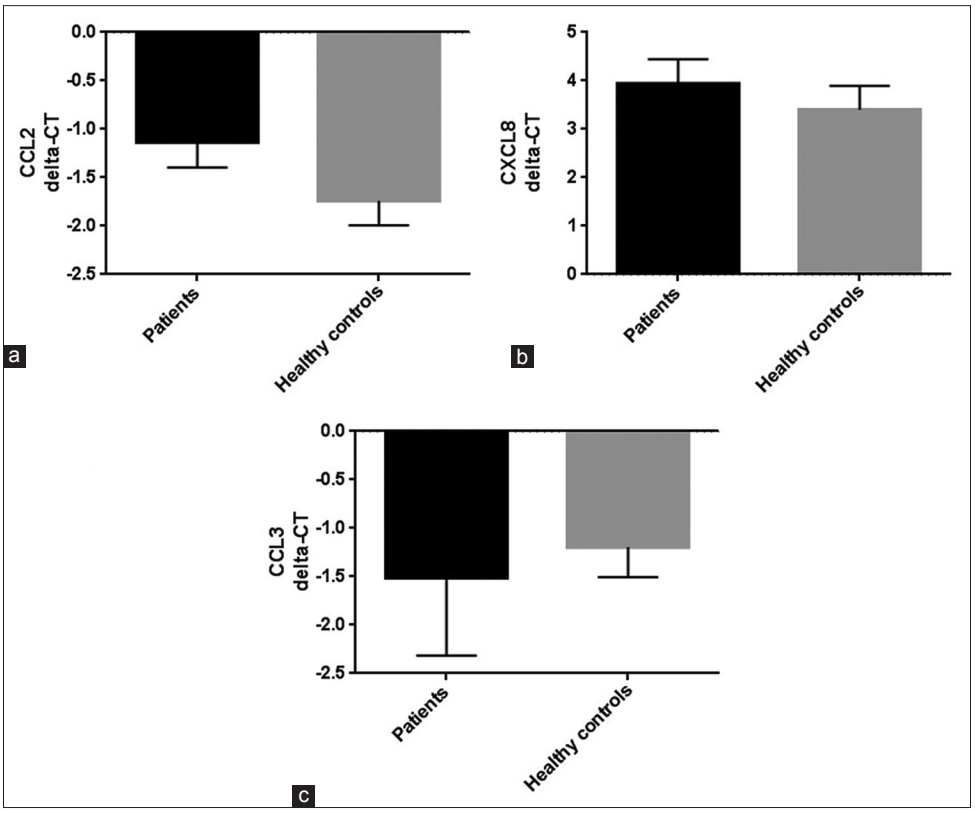

Figure 1: Delta-CT values for patients with bipolar disorder and healthy controls. (a) *CCL2*, $P = 0.097$. (b) *CXCL8*, $P = 0.450$. (c) *CCL3*, $P = 0.714$. Data are presented as mean ± standard error of the mean. The higher delta-CT value represents the higher expression of gene at mRNA level

et al. indicated that brain tissue from patients with BD had lower levels of *CCL3* gene expression in comparison to healthy controls.[21] There are no published data with regard to *CXCL8* gene expression in patients with BD. However, there are few studies evaluating the serum levels of *CCL2*, *CCL3*, and *CXCL8* in patients with BD, Alzheimer's disease (AD) and schizophrenia. Brietzke *et al*. showed no statistically significant difference in the serum levels of *CCL2*, *CCL3*, and *CXCL8* between patients with BD and healthy controls.[22] Drexhage *et al*. demonstrated that patients with BD had elevated level of *CCL2* in serum in comparison to healthy controls.[23] O'Brien *et al*. and Barbosa *et al*. reported that patients with BD had increased serum levels of *CXCL8* compared to healthy controls.[24,25] Expression of *CCL2*, *CCL3*, and *CXCL8* and their receptors by neurons, astrocytes, and endothelial cells of the BBB may indicate the role of neuroinflammatory processes in the etiology of BD.[17-19] Neuroinflammation is considered as one of the potential mechanisms contributing to the pathogenesis of other neuropsychiatric diseases such as AD and schizophrenia.[17,26] High levels of *CCL2* and *CXCL8* in the serum of patients with AD was reported.[27] Results about the association of serum levels of *CCL2* and *CXCL8* in patients with schizophrenia are controversial. In some studies, higher serum levels of *CCL2* and *CXCL8* in patients with schizophrenia compared to healthy controls was reported, whereas other studies showed no significant differences between serum levels of mentioned chemokines in patients with schizophrenia and healthy controls.[17,28]

In this study, we extracted total RNA from leukocytes after lysis of erythrocytes and evaluated the expression of *CCL2*, *CCL3*, and *CXCL8* genes. As previously mentioned, these chemokines in addition to some leukocytes, are also expressed in neurons, astrocytes, and endothelial cells of the BBB.[17-19] Chemokine gene expression in CNS-associated cells may show the different patterns from that of leukocytes, and hence we suggest evaluating and comparison of chemokines gene expression in CNS-associated cells with leukocytes in patients with BD and comparison with healthy individuals in future studies. Access to brain tissue may be associated with some limitations. Emerging technologies such as reprogramming and differentiation of somatic peripheral cells of patients into neurons[29] might provide an alternative source of neurons to study of biological and immunological effects of chemokines gene expression in patients with BD.

Limitations

One of the limitations of our study was the loss of follow-up due to the lack of cooperation of the patients. In addition, lack of measurement of the chemokines in the serum levels and comparison with gene expression data not only in mania but also in depression phase of BD made us unable to have a better conclusion regarding the effect of the chemokines on BD.

CONCLUSION

We found no significant differences in the expression levels of *CCL2*, *CCL3*, and *CXCL8* genes in peripheral blood cells of patients with BD as compared to healthy controls. Our suggestions for future studies are an increase in the sample size, following up the patients in various time points and sample collections from patients with BD in mania and also in depression phases to have a more accurate conclusion regarding the effects of chemokines in BD. To have serum levels of the chemokines of patients with BD and comparing with the gene expression levels would be so valuable too.

Acknowledgments

This study was financially supported by the Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran (Grant Code: 940695), approved by the Ethics Committee of Mashhad University of Medical Sciences, and an informed consent was obtained from each patient and healthy control. The ethical code is IR.MUMS.fm.REC.1394.494. Presented data were extracted from the thesis of Parisa Azim, medical student at Mashhad University of Medical Sciences, Mashhad, Iran. We thank patients with BD and healthy controls for their participation in our study.

Financial support and sponsorship

This study was financially supported by the Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Krystal JH, State MW. Psychiatric disorders: Diagnosis to therapy. Cell 2014;157:201-14.
2. Angst J, Ajdacic-Gross V, Rössler W. Classification of mood disorders. Psychiatr Pol 2015;49:663-71.
3. Cerimele JM, Chwastiak LA, Chan YF, Harrison DA, Unützer J. The presentation, recognition and management of bipolar depression in primary care. J Gen Intern Med 2013;28:1648-56.
4. De Crescenzo F, Serra G, Maisto F, Uchida M, Woodworth H, Casini MP, *et al.* Suicide attempts in juvenile bipolar versus major depressive disorders: Systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2017;56:825-31000.
5. Lima IM, Peckham AD, Johnson SL. Cognitive deficits in bipolar disorders: Implications for emotion. Clin Psychol Rev 2018;59:126-36.
6. Waldinger MD. Psychiatric disorders and sexual dysfunction. Handb Clin Neurol 2015;130:469-89.
7. Fenchel D, Levkovitz Y, Kotler M. Bipolar disorder as a multi-system illness. Harefuah 2017;156:772-4.

8. Manji HK, Henter ID, Zarate CA Jr. Bipolar disorder: A neurobiological synthesis. Curr Top Behav Neurosci 2011;5:331-40.
9. Marangoni C, Faedda GL, Baldessarini RJ. Clinical and environmental risk factors for bipolar disorder: Review of prospective studies. Harv Rev Psychiatry 2018;26:1-7.
10. Budde M, Forstner AJ, Adorjan K, Schaupp SK, Nöthen MM, Schulze TG, *et al*. Genetics of bipolar disorder. Nervenarzt 2017;88:755-9.
11. Mousavizadegan S, Maroufi M. Comparison of salivary testosterone levels in different phases of bipolar I disorder and control group. J Res Med Sci 2018;23:31.
12. Colpo GD, Leboyer M, Dantzer R, Trivedi MH, Teixeira AL. Immune-based strategies for mood disorders: Facts and challenges. Expert Rev Neurother 2018;18:139-52.
13. Rosenblat JD, McIntyre RS. Bipolar disorder and immune dysfunction: Epidemiological findings, proposed pathophysiology and clinical implications. Brain Sci 2017;7. pii: E144.
14. Bai YM, Su TP, Li CT, Tsai SJ, Chen MH, Tu PC, *et al.* Comparison of pro-inflammatory cytokines among patients with bipolar disorder and unipolar depression and normal controls. Bipolar Disord 2015;17:269-77.
15. Muneer A. Bipolar disorder: Role of inflammation and the development of disease biomarkers. Psychiatry Investig 2016;13:18-33.
16. Stuart MJ, Singhal G, Baune BT. Systematic review of the neurobiological relevance of chemokines to psychiatric disorders. Front Cell Neurosci 2015;9:357.
17. Stuart MJ, Baune BT. Chemokines and chemokine receptors in mood disorders, schizophrenia, and cognitive impairment: A systematic review of biomarker studies. Neurosci Biobehav Rev 2014;42:93-115.
18. Reichel CA, Rehberg M, Lerchenberger M, Berberich N, Bihari P, Khandoga AG, *et al.* Ccl2 and Ccl3 mediate neutrophil recruitment via induction of protein synthesis and generation of lipid mediators. Arterioscler Thromb Vasc Biol 2009;29:1787-93.
19. Russo RC, Garcia CC, Teixeira MM, Amaral FA. The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. Expert Rev Clin Immunol 2014;10:593-619.
20. Padmos RC, Hillegers MH, Knijff EM, Vonk R, Bouvy A, Staal FJ, *et al.* A discriminating messenger RNA signature for bipolar disorder formed by an aberrant expression of inflammatory genes in monocytes. Arch Gen Psychiatry 2008;65:395-407.
21. Nakatani N, Hattori E, Ohnishi T, Dean B, Iwayama Y, Matsumoto I, *et al.* Genome-wide expression analysis detects eight genes with robust alterations specific to bipolar I disorder: Relevance to neuronal network perturbation. Hum Mol Genet 2006;15:1949-62.
22. Brietzke E, Kauer-Sant'Anna M, Teixeira AL, Kapczinski F. Abnormalities in serum chemokine levels in euthymic patients with bipolar disorder. Brain Behav Immun 2009;23:1079-82.
23. Drexhage RC, Hoogenboezem TH, Versnel MA, Berghout A, Nolen WA, Drexhage HA, *et al.* The activation of monocyte and T cell networks in patients with bipolar disorder. Brain Behav Immun 2011;25:1206-13.
24. O'Brien SM, Scully P, Scott LV, Dinan TG. Cytokine profiles in bipolar affective disorder: Focus on acutely ill patients. J Affect Disord 2006;90:263-7.
25. Barbosa IG, Rocha NP, Bauer ME, de Miranda AS, Huguet RB, Reis HJ, *et al.* Chemokines in bipolar disorder: Trait or state? Eur Arch Psychiatry Clin Neurosci 2013;263:159-65.
26. Azizi G, Khannazer N, Mirshafiey A. The potential role of chemokines in Alzheimer's disease pathogenesis. Am J Alzheimers Dis Other Demen 2014;29:415-25.
27. Liu C, Cui G, Zhu M, Kang X, Guo H. Neuroinflammation in Alzheimer's disease: Chemokines produced by astrocytes and chemokine receptors. Int J Clin Exp Pathol 2014;7:8342-55.
28. Hong S, Lee EE, Martin AS, Soontornniyomkij B, Soontornniyomkij V, Achim CL, *et al.* Abnormalities in chemokine levels in schizophrenia and their clinical correlates. Schizophr Res 2017;181:63-9.
29. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, *et al.* Modelling schizophrenia using human induced pluripotent stem cells. Nature 2011;473:221-5.