

Expression of Bcl-2 and Bax genes in peripheral blood lymphocytes of depressed and nondepressed individuals

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Background: Involvement of the immune system is one of the issues raised in the pathophysiology of depression. BCL2 and BAX genes are related to immune system regulation. We investigated the BCL2 and BAX expression as a probable mechanism of immune system involvement in depression. **Materials and Methods:** This case-control study was conducted on 28 patients with major depression (case) and 28 nondepressed individuals (control) within the age range of 18–55 years in the Isfahan University of Medical Sciences. Clinical interviews, based on the Diagnostic and Statistical Manual of Mental Disorders, were conducted to detect depression, and Beck's Depression Inventory was used to measure the severity of depression in the individuals. In addition, a real-time polymerase chain reaction was employed to compare the level of Bax and Bcl-2 gene expression in peripheral blood lymphocytes. The multivariate covariance analysis was used to explore the correlation between BCL2 and BAX gene expression and to control the effect of duration and severity of depression. **Results:** The results showed that none of the variables including group membership, the duration of depression, and the severity of depression were not significantly correlated with the expression of BCL2 and BAX genes. Furthermore, there was no statistically significant relationship between the Bax and Bcl-2 genes expression in case and control groups ($P > 0.05$). **Conclusion:** Depression may have no impact on Bax and Bcl-2 gene expression in patients with major depression. Studies with larger sample size are recommended.

Key words: Bax gene, Bcl-2 gene, depression, gene expression

How to cite this article: Amidfar M, Karami Z, Kheirabadi GR, Afshar H, Esmaeili A. Expression of Bcl-2 and Bax genes in peripheral blood lymphocytes of depressed and nondepressed individuals. *J Res Med Sci* 2019;24:41.

INTRODUCTION

Major depression is one of the most common psychiatric disorders, with a global prevalence of 15%–20%. In addition, it is the fifth cause of disability in developed countries with high welfare and the leading cause of disability in countries with medium-to-low welfare.^[1,2]

Major depression disorder, which is defined by the fifth Diagnostic and Statistical Manual of Mental Disorders (DSM-V), is known as anhedonia (lack of desire to participate in activities that used to be joyful,

lack of energy, changes in sleep pattern and appetite, sadness, and suicidal thoughts).^[3]

It has been suggested that immune system impairment is affected by the pathogenesis of major depression, and there is an association between depression and activation of the innate inflammatory immune response, including changes in the ability of immune cells in expressing inflammatory cytokines.^[4]

In addition, there is an association between inflammatory markers and depression symptoms such as fatigue, poor cognitive function, and sleep disorders.^[5-7] Elevated total number of white blood cells and number and

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10.4103/jrms.JRMS_811_17

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Received: 10-10-2017; **Revised:** 08-05-2018; **Accepted:** 20-02-2019

percentage of neutrophils and lymphocytes are among the first immunological changes observed in depressed individuals.^[8]

Further evaluation of different types of lymphocytes demonstrated a negative relationship between depression and the number and percentage of lymphocytes; however, these differences did not constantly repeat.^[9] Meta-analysis approaches to this subject concluded that there is a significant reduction in instability of the responses of lymphocytes to mitogens of T cells in patients with depression.^[4,10] While there are some reports on the significant increase in the apoptosis of blood leukocytes in patients with depression, the type of affected blood cells and the mechanism of apoptosis are still inconspicuous.^[8]

The apoptosis process can mainly occur through two independent routes. The first route includes mechanisms of induced death by the receptor, which involves death receptor Fas (CD95).^[11] The second general process of cell death is conducted through mitochondria. This mitochondria-mediated apoptosis can be controlled by the protein family of Bcl-2 to some extent.^[12] Bcl-2 belongs to a family of apoptotic regulatory proteins, which can be divided into two groups of anti-apoptotic and pro-apoptotic.^[13]

An important member of the Bcl-2 family is the protein of Bcl-XL, which has anti-apoptotic activity.^[14] Pro-apoptotic members can be divided into two groups: Bax subset (Bax, Bak, and Bok proteins), components that contain BH1, BH2, and BH3 domains, and the group that only has BH3 domain (e.g., Bad, Bid, and Bim proteins).^[12]

The BH3 domain is essential for the cytotoxic activity of pro-apoptotic groups. Moreover, there is a functional antagonism between pro-apoptotic and anti-apoptotic components for intracellular balancing, which prevents the suppression of cell death.^[15]

Mechanisms that impair the proliferation of T cells in patients with major depression are still not recognized; however, a series of possibilities are evaluated. With this background in mind, this study was conducted to evaluate Bax and Bcl-2 gene expression on peripheral blood lymphocytes in patients with major depression and compare it with nondepressed individuals.

MATERIALS AND METHODS

This case-control study was conducted on 28 patients with major depression, who were selected randomly among the patients referred to the outpatient psychiatric clinic of AL Zahra Hospital, patients' consent form was obtained for

participation in the study, and then blood samples were taken from patients who were satisfied to participate and 28 nondepressed individuals who were selected from among the laboratory personnel, who were similar to patients in terms of age and gender and were eligible regarding inclusion and exclusion criteria.

Patients within the age range of 18–50 years with major depression diagnosis based on the DSM-V were selected, and their level of depression was assessed using the Beck Depression Scale. This scale is a self-report inventory, which can be completed in 5–10 min. This 21-item questionnaire is on different signs of depression rated using a 4-point Likert scale (from zero to three). Items of this questionnaire are related to sadness, pessimism, sense of failure, guilt, sleep disturbance, loss of appetite, and self-loathing. Therefore, this scale is able to determine different levels of depression from weak to extremely severe, with scores ranging from 0 to 63.^[16]

The exclusion criteria were history of other psychiatric disorders, consumption of antidepressants, and electroconvulsive therapy over the past 2 months, thyroid disorders, cardiovascular diseases, addiction to narcotics, neurological disorders (e.g., multiple sclerosis, cerebrovascular accident, and Alzheimer's disease), severe diseases such as cancer, and history of treatment with anti-inflammatory, and immunosuppressive medications.

Individuals of the control group were selected from among the laboratory personnel, who were similar to patients in terms of age and gender and above-mentioned exclusion criteria; none of them had depression based on the Beck Depression Scale.

After blood sampling, lymphocytes were extracted along with ethylenediaminetetraacetic acid anticoagulant using Ficoll solution. Afterward, RNA was extracted from lymphocytes using a RNA Extraction Kit made by Iranian Company of Yekta Tajhiz, according to the manufacturer's instructions. Thereafter, RevertAid First Strand cDNA Synthesis Kit (Fementas Co.) was applied to synthesize cDNA.

Ultimately, using the SyberGreen Master Mix (Ampliqon Co., Denmark) and applying real-time polymerase chain reaction (PCR), Bax and Bcl-2 gene expression were measured and optimized against ACTB gene expression. To do so, the primer of the desired gene was first designed and ordered for synthesis.

A real-time PCR reaction was conducted on all the synthesized cDNA samples using primers of Bax, Bcl-2, and β -actin genes (as an internal control) applying Syber Green dye in triplicate.

The standard curve (not shown) was drawn for quantitative assessment of Bax and Bcl-2 gene expression and comparative evaluation of relative gene expression. To draw the standard curve for each gene, serial dilutions were prepared from cDNA samples, and real time-PCR was carried out on the selected samples. Reaction efficiency was estimated for each target gene and β -actin gene using the equation of efficiency = $10^{(-1/\text{slope})} - 1$.

Statistical analysis

To analyze the data, SPSS version 20 (IBM, Armonk, NY, USA) was employed. *T*-test is used to compare the two groups in terms of age, duration, and severity of depression, and Chi-square or Fisher exact tests to compare sex distribution.

The multivariate covariance analysis was used to explore the correlation between BCL2 and BAX gene expression and to control the effect of duration and severity of depression.

RESULTS

Twenty-eight patients with major depression and 28 nondepressed individuals were compared in terms of Bax and Bcl-2 gene expression.

The comparison of the two groups in terms of age, duration and severity of depression (*t*-test), and sex (Chi-square) is shown in Table 1.

Table 2 and Figure 1 show descriptive indicators of expression of the two genes studied in the two groups of depressed and nondepressed.

Regarding the correlation between two variables of BCL2 and BAX gene expression and to control the effect of duration and severity of depression, multivariate covariance

Table 1: Demographic and clinical characteristics of the participants

Factor	Case group	Control group	P
Age (year), mean±SD	34.78±8.72	33.67±8.51	0.633
Gender (male), n (%)	22 (76.6)	22 (78.6)	-
Depression severity, mean±SD	28.17±2.76	-	-
Disease duration, mean±SD (month)	11.21±8.72	-	-

SD=Standard deviation

Table 2: Descriptive indices of two variables of expression of BCL2 and BAX genes in two groups

	Group	Mean±SD
BCL2	Case	0.94±0.17
	Control	1.00±0.00
BAX	Case	1.037±0.10
	Control	1.00±0.00

SD=Standard deviation

analysis was used. The results showed that none of the variables including group membership, the duration of depression, and the severity of depression were not significantly correlated with expression of BCL2 and BAX genes [Table 3 and Figure 2].

DISCUSSION

Several studies indicated that major depression is associated with the activation of the inflammatory system. In addition, there are some reports on increased blood levels of some inflammatory cytokines, such as interleukin-1 beta, interleukin-2, and interleukin-6, and reduced production of interferon-alpha in patients with major depression.^[17-19]

Some of the previous studies demonstrated that T cells might be involved in depression. Moreover, the recent advances in immunology have increasingly highlighted the significance of different types of T cells for the regulation of inflammatory responses.^[20]

One of the first observations in the impaired immune system in depression was damage to the proliferation of peripheral blood mononuclear cells in patients with depression as a response to mitogens of T cells.^[5-9] Significant increase of apoptosis in peripheral blood leukocytes in depression patients was previously reported.^[8] Flow cytometric findings revealed that CD4+ T cells in patients with depression are indicators of increased apoptosis and accelerated expression of Fas (CD95) receptor.^[18]

One of the general processes of cellular death, performed by mitochondria, is controlled by the Bcl-2 family.^[12] In the present study, we evaluated Bax and Bcl-2 gene expression to explain the mechanism of apoptosis of lymphocytes, which has been regarded by various studies to increase in patients with depression.

According to the results of the current study, no significant difference was observed in the gene expression of Bax and

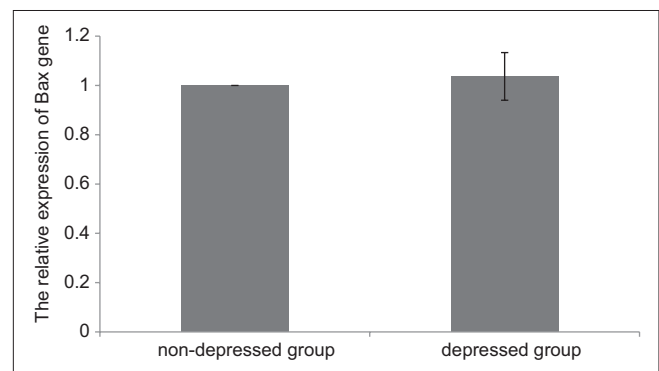


Figure 1: Level of Bax gene expression in patients (n = 28), compared to the controls (n = 28)

Table 3: Correlation between BCL2 and BAX gene expression and the group membership

	Group	Mean±SD	Mean difference	95% CI of the difference	t	df	P
BCL2	Case	0.94±0.17	-0.06	-0.12-0.01	-1.79	54.00	0.079
	Control	1.00±0.00					
BAX	Case	1.04±0.10	0.04	0.00-0.07	1.97	54.00	0.054
	Control	1.00±0.00					

SD=Standard deviation; CI=Confidence interval

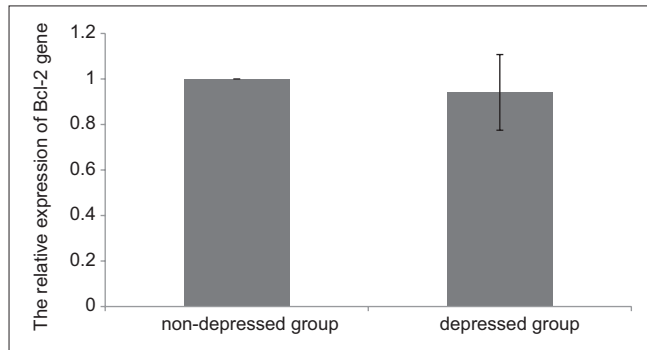


Figure 2: Level of Bcl-2 gene expression in depressed patients ($n = 28$), compared to the controls ($n = 28$)

Bcl-2 in depressed patients, compared to nondepressed individuals. Moreover, no significant association was found between gene expression and clinical variables, such as the severity of depression and duration of the disease.

Our findings indicated that expression of the considered genes could change on lymphocytes of patients with depression; however, these changes were not statistically different between the two groups, which might be due to our limited sample size.

Furthermore, different mechanisms were proposed to explain the increase of lymphocyte apoptosis in patients with depression. For instance, the proliferation of T cells in depressed patients might be affected by inflammatory cytokines, such as tumor necrosis factor (TNF) alpha, which were shown to increase in patients with depression.^[4] In fact, patients with major depression show increased production of inflammatory biomarkers, such as inflammatory cytokines, in the peripheral blood. It has been confirmed that these compounds enter the brain and cause depression through interfering with other neurobiological aspects that are involved in depression.^[4]

Therefore, the mitochondrial pathway might not play any role through Bax and Bcl-2 gene expression in apoptosis of lymphocytes in patients with depression. On the other hand, increased expression of Fas receptor or elevated level of inflammatory factors (e.g., TNF alpha) might play a role in accelerated apoptosis of lymphocytes.

Nevertheless, given the fact that lymphocytes were studied in the present study and various classes of lymphocytes were

not assessed separately, the obtained results cannot confirm the role of Bax and Bcl-2 genes in apoptosis of lymphocytes in patients with major depression. Accordingly, it is recommended that expression of the mentioned genes be assessed in a larger population of patients to recognize the role of mitochondrial pathway in apoptosis of lymphocytes in depressed patients. In addition, it is suggested to study various classes of lymphocytes, including CD4⁺ T cells, regulatory T cells, or B cells, to obtain more accurate results.

CONCLUSION

Bax and Bcl-2 gene expression had no impact on apoptosis of peripheral blood lymphocytes in patients with major depression. Bax and Bcl-2 gene expression were not different in patients with major depression and control group. Depression may have no impact on Bax and Bcl-2 gene expression in patients with major depression. Studies with larger sample size are recommended.

Limitations

The low sample size is the main limitation of this study.

Acknowledgments

Hereby, we appreciate the voluntary participation of the patients. In addition, the cooperation of the Al-Zahra Laboratory Personnel, Psychiatry Department of Al-Zahra Hospital, and Research Deputy of University of Medical Sciences of Isfahan, Iran, is greatly appreciated.

Financial support and sponsorship

This study was supported by a research grant from Vice Chancellor for Research of Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

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