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Nrf2 Dysregulation in Major Depressive and Bipolar Disorders

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Abstract

Background: Major depressive disorder (MDD) and bipolar disorder (BPD) are two of the most important mental disorders that greatly impact different aspects of life. These conditions imply heavy health and economic burden and are heterogeneous in nature. Inflammation is reported as the etiology of mental disorders. Nrf2 transcription factor plays a key role in the defense mechanisms against inflammation and oxidative stress. So, this study aimed to evaluate the expression level of Nrf2 in MDD and BPD patients and compared it with healthy control subjects. **Materials and Methods:** In this study, real-time PCR was conducted to evaluate the expression level of Nrf2 in 100 MDD and 100 BPD patients compared to 100 healthy control subjects. Statistical analysis conducted on GraphPad Prism 8 and SPSS21 included ANOVA, Tukey's test, receiver operating characteristic (ROC), and odds ratio. **Results:** Results suggest a significant downregulation of Nrf2 in these conditions compared to the control group. ROC curve analysis demonstrates Nrf2 as a biomarker of these psychiatric disorders. **Conclusion:** The elevated levels of reactive oxygen species and downregulation of detoxifying enzymes were observed in MDD and BPD, which can be associated with the downregulation of Nrf2. Concerning its role in inflammatory response pathways, alternation of Nrf2 expression can be associated with the pathology of these conditions.

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Keywords: Major Depression Disorder; Bipolar Disorder; *Nrf2*

Introduction

Major depressive disorder (MDD) is a weakening condition. Relevant characteristics of depression include cognitive impairment, perturbations in sleep and appetite, and different chronic medical conditions. It also applies heavy health and economic burdens—12-month prevalence rates of 6% for MDD. Due to the nature of this disorder, its recurrence is estimated at about 80% [1]. Studies suggest that one in every six adults faces MDD at some point in their lifetime [1, 2]. It is more common in females than males, and genetic risk factors for MDD are reported at 35% [1, 2].

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Bipolar disorder (BPD) as a chronic mental condition consists of mood alternations between depression and mania or hypomania, which leads to functional impairment [3]. BPD affects more than 1-4% of the world's population.

Generally, this disorder manifests at the age of 20 years and rates as the fifth cause of disability globally [4]. BPD's 12-month prevalence and heritability are estimated at 1.5% and 85%, respectively. Although the etiology of BPD is yet to be discovered, the role of interaction between genetic, neurochemical, and environmental factors in the onset of this disorder has been reported [3, 4].

While the exact mechanisms of MDD and BD are not fully understood, inflammatory pathways have been reported to participate in the development of these disorders [5].

Dysregulations in neurotransmitter metabolism, endocrine system function, and brain activity are associated with the upregulation of cytokines in depression. In addition, cytokine exposure has been shown to affect behavior, including low mood, fatigue, anxiety, sleep disorders, loss of pleasure, and cognitive impairment, which are symptoms of MDD [6].

The bidirectional relationship between BPD and inflammation has recently come to the fore. However, it has been said that the inflammation etiology of BPD is limited to a subgroup of affected individuals, which is considered as a new treatment target [7].

The association of interleukin (IL)-11 and IL-6 with BPD have been revealed [8]. Also, dysregulation of inflammation-related genes can be considered as potential BPD biomarker. In addition, mood stabilizers affect this pathway [8].

Due to its participation in inflammation pathways during oxidative stress, the nuclear factor erythroid 2–related factor 2 (Nrf2) may play a role in the pathophysiology of depressive disorders [9]. Nrf2 is a vital transcription factor for oxidative stress response.

It binds to Antioxidant response elements in the promoter of phase II detoxifying or antioxidant enzymes and stress-response coding genes. As an adaptor protein of Nrf2, Kelch, like ECH-associated protein 1 (Keap1), represses Nrf2 in normal conditions [9, 10]. Nrf2 knocked out mice have shown depression symptoms and increased pro-inflammatory cytokine levels compared to normal controls [11].

In addition, reduction in Nrf2 and Keap1 levels leads to decreased brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB) signaling and synaptogenesis, which are also associated with depression [11].

In this study, regarding dysregulation of oxidative stress response observed in these psychiatric conditions, the expression level of Nrf2 as a key transcription factor in the inflammatory process in Iranians suffering from MDD and BD compared with healthy individuals has been evaluated.

Material and Methods

In this study, Nrf2 expression level was evaluated in 100 MDD patients (68 males and 32 females), 100 BD patients (68 males and 32 females), and 100 healthy controls (67 males and 33 females). Patients and healthy individuals were selected under the supervision of two experienced psychiatrists and were invited to participate if they met the Diagnostic and Statistical Manual version 5 (DSM-5) criteria for a diagnosis of MDD or BD. The study was compatible in terms of sex, age (MDD: 35.2±9.45 years; BPD: 27.1±5.89 years; normal: 31.9±8.65 years), race, physiological characteristics, familial and socioeconomic conditions, as well as their level of education.

Subjects were evaluated by the Wechsler Abbreviated Intelligence Scale (WAIS) test to measure intellectual performance, and patients with an intelligence quotient (IQ) value of less than 80 (mean± SD; MDD: 98.72±9.1; BPD: 100.03±7.15; normal: 101.6±6.03) were excluded. None of the participants were suffering from serious medical issues, including diabetes, cancer, thyroid disorders, neurological disorders, or a history of trauma with loss of consciousness, and they had no addiction to psycho-stimulant, opioid drugs, alcohol, and nicotine.

The age of onset in MDD and BPD was 21±3

and 19±2 years, respectively. There also were no relative participants. After participants were explained the purpose and the procedure of the study, signed written consent was obtained from all individuals.

The whole blood sample (5ml) was collected before noon. According to the manufacturer's instructions, RNA was immediately extracted using the RNA Purification kit (GeneJET™,). In order to quantify RNA, its concentration and absorbance were evaluated by a Nanodrop spectrophotometer (Thermo scientific-Nanodrop 2000, Waltham, MA). The absorption ratio in 260/230 nm and 260/280 nm was assessed, and the ratio between 1.8 to 2.2 and 1.7 to 1.9 was considered as proper values, respectively.

A minimum value of 500 ng/µl RNA was considered a proper yield to perform cDNA synthesis in the next step. If necessary, an additional step was also conducted to remove DNA contamination from RNA preparations. Lastly, Samples were stored at 4°C until use.

Horizontal gel electrophoresis (1%) with 1x Tris-acetate-EDTA buffer (Sigma-Aldrich, Burlington, MA, United States) at pH 8.0 was conducted to determine the quality of RNA segments. Next, with ethidium bromide staining, RNA bands were visualized under ultraviolet light, and 18s rRNA and 28s rRNA were observed.

The following primer sequences were used:

Nrf2:

Forward: 5'CACATCCAGTCAGAAAC-CAGTGG3'

Reverse: 5'GGAATGTCTGCGC-CAAAAGCTG3'

GAPDH:

Forward: 5'AAATCCGTTGACTC-CGACCT3'

Reverse: 5'CACTAGGCGCTCACT-GTTCTC3'

The length of fragments was 112 and 90 base pairs for Nrf2 and GAPDH, respectively. Due to its stability in the peripheral blood of subjects, GAPDH was considered a suitable reference gene to normalize the expression of the target gene.

1000 ng RNA was used to synthesize the cDNA strand using Transcription First Strand cDNA Synthesis Kit (RevertAid Premium First Strand cDNA Synthesis Kit #K1652, Thermo scientific – Fermentas, Latvia). The products were used directly in a quantitative polymerase chain reaction (qPCR) or stored at -20°C. In the case of longer storage, cDNAs were stored at -70°C.

qPCR was performed on a CFX96 Touch Real-Time PCR Detection System (BIO-RAD, California, United States) using (Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X) #K0221, Thermo scientific-Fermentas, Latvia). Each reaction included 12.5µL of SYBR Green Master Mix (Latvia), 0.5 µL of each forward and reverse primers, and 2µL of template cDNA (100ng/µL) based on the user's manual. Lastly, water was added, and a total 25µL volume of real-time PCR mix was obtained. Reactions were performed in triplicates with a DNA-free reaction (NTC) for each gene to assess the contamination.

Ethical Issues

This study has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The first step to complying with the standards of medical ethics in research such as this study is to explain the purpose and the procedure of the study to the participants. Afterward written consent was obtained from all the individuals. Also, research on human beings can only be justified if the benefits outweigh the potential risks of research.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism8 (Graphpad Software, La Jolla, CA) and SPSS21 (SPSS Inc., Chicago, IL). The Shapiro-Wilk test and Kolmogorov-Smirnov test were applied to determine the Gaussian distribution of data in each group. The significance of mRNA expression level was obtained with the ANOVA test. The correlation between Nrf2 and demographic data was assessed by 2-way ANOVA.

The receiver operating characteristic (ROC) curve analysis was used to assess the diagnostic accuracy of the Nrf2 and define proper cut-off values. Considering the normal group as reference, the odds ratio (OR) was

Table 1. Tukey Test Results for the Difference of Means Between Groups.

Groups	Age, y	Intelligence quotient
MDD vs Normal	P=0.01 Mean diff =3.25	P=0.01 Mean diff =-2.88
BPD vs Normal	P<0.001 Mean diff =-4.8	P=0.3 Mean diff =-1.57
BPD vs MDD	P<0.001 Mean diff =-8.05	P =0.43 Mean diff =1.31

MDD: Major depressive disorder; **BPD:** Bipolar disorder

calculated. Also, the significant level was considered P=0.05.

Results

Real-time PCR was used to evaluate the expression level of Nrf2 in peripheral blood of MDD and BPT patients compared to healthy control subjects.

Results demonstrated significant downregulation of Nrf2 in MDD (fold change=0.3442; P<0.001) and BPD (fold change=0.2250, P<0.001, Figure-1).

There were no significant differences in regard to the gender of the individuals (P=0.95). ANOVA tests revealed a significant difference in age between MDD and BPD vs. normal (P<0.001).

ANOVA test for IQ evaluation was conducted, and only MDD vs. normal revealed a significant difference (P=0.02). Tukey test results are represented in Table-1.

The correlations between gender, age, age of onset, IQ, and gene expression via 2-way ANOVA were conducted. However, no significant association was reported between MDD, BPD, and control groups.

Furthermore, obtained results of ROC curve analysis suggests downregulation of Nrf2 gene as a biomarker for MDD (area=0.8748; P<0.001; 95% confidence interval [CI]=0.8269 to 0.9226) and BPD (area=0.8718, P<0.001, 95% CI=0.8240 to 0.9196). With regard to given sensitivity and specificity, ROC curve results for the cut-off values for MDD and BPD were 2.17-2.27 and 2.465-3.270, respectively (Figure-2).

Also, results reveal that upregulation of Nrf2 gene may have protective effects against MDD (adjusted OR=0.188; 95% CI=0.111-0.293; P<0.001) and bipolar disorder (adjusted OR=0.28, 95% CI=0.186-0.4, P<0.001).

Discussion

Although psychiatric disorders are highly heritable, they can be consequences of the interaction between numerous genes and the environment. This condition makes it challenging to define only one specific reason for the occurrence of a mental disorder. However, some shared gene expression patterns in five major psychiatric disorders have been reported. These data develop a framework that helps to identify more related cellular pathways in mental disorders [12].

MDD and BPD imply a heavy economic burden [13, 14], and based on the Global Health Data Exchange database, 1.36–2.38% and 0.21–0.48 of total disability-adjusted life years are assigned to MDD and BPD,

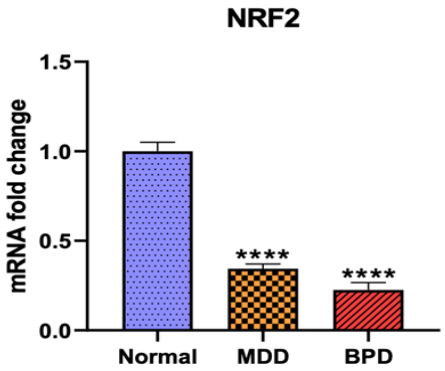

Figure 1. *Nrf2* Significant downregulation in MDD (ratio=0.3442) and BPD (ratio=0.225) in comparison to healthy control group. **MDD:** fold change=0.3442; P<0.001; **BPD:** fold change=0.2250, P<0.001.

respectively [15].

Nrf2 is a conserved transcription factor in vertebrates and a member of the cap'n'collar and leucine zipper family and is widely expressed in the human body [16].

It is reported that Nrf2 has a key role in the inflammation process, and dysfunction in this process may lead to the development of mental disorders such as anxiety, depression, neurodegenerative, cardiovascular, and pulmonary diseases as well as chronic inflammation [9,17].

In this study, the expression level of Nrf2 was evaluated. It was observed that the transcription level of the Nrf2 gene significantly decreases in both MDD and BPD patients. Results also suggested that Nrf2 could be considered as a potential biomarker for MDD and BPD. Furthermore, upregulation of Nrf2 may lower the risk of developing MDD and BPD.

Different studies have discussed the disruption of antioxidant enzymes and impairments in defense mechanisms against reactive oxygen species (ROS).

Hamed *et al*. reported significantly lower Nrf2 levels in the serum of MDD patients compared to healthy individuals. They concluded that oxidative stress was associated with a higher risk of MDD [18].

In a meta-analysis study, a reduction in serum total antioxidant capacity, paraoxonase, and antioxidants, in addition to elevated levels of ROS were reported, and exposure to antidepressants reversed this situation [19].

Concerning the dysregulation of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, catalase, malondialdehyde, and nitric oxide, Ozcan *et al*. suggest a disruption in oxidative defense mechanism was associated with episodes of psychiatric disorders [20].

In another metanalysis on oxidative stress biomarkers (8-OHdG and F2-isoprostanes) in MDD and BPD individuals, elevated levels of oxidative stress were reported [21].

A study investigated Nrf2 in a rat model of social defeat, and due to the downregulation of BDNF as a regulator of Nrf2, a 40% increase in susceptibility to developing depression was observed, and using antioxidant treatment reversed the situation [9]. The subcellular localization of Nrf2 is dependent on BDNF. Low levels of BDNF can prevent Nrf2 from translocation, which in turn results in lower expression of protective cellular enzymes and accumulation of oxidative stress [9].

In another investigation on the hippocampus of mice with depression behavior, lower expressions of Keap1 and Nrf2 were reported [10].

In addition, higher cytokine levels in Nrf2 knock-out mice were observed [10]. Furthermore, in an animal model of Senescence-Accelerated Mouse Prone 8, significant downregulation of Nrf2 was associated with neuroinflammation and cognitive impairments [22].

In addition, reduced organelles and synapses were observed. They also reported that neural inflammation and synaptic plasticity are

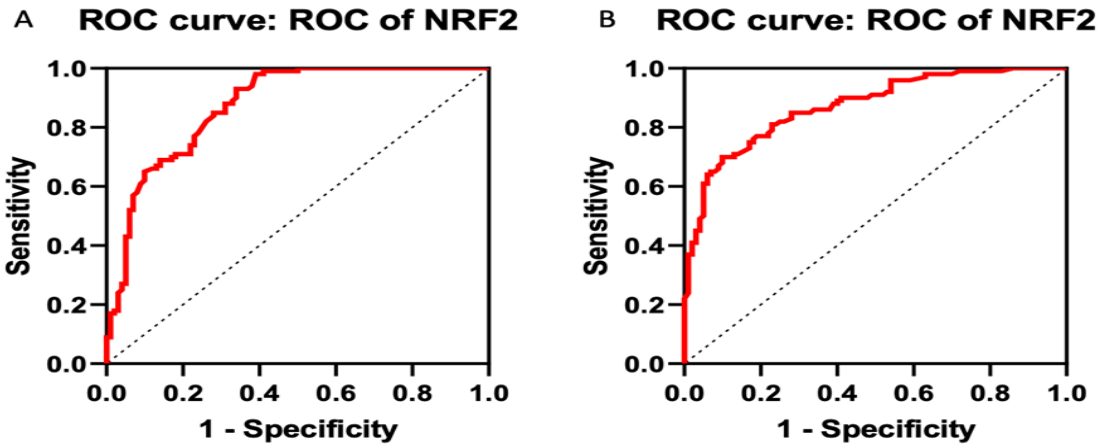

Figure 2. Results of ROC curve analysis for Nrf2 in MDD (A) and BPD (B) groups.

associated with the aging process [22].

In comparison between alcohol-addicted patients and healthy individuals, elevated expressions of Nrf2 and Hmox1 were observed [23]. This study also reported a significant reduction in Nrf2 and Hmox1 levels in schizophrenic patients [23]. Martín-Hernández *et al*. [24] investigation on the brain of MDD individuals also showed a reduction in Nrf2 level [24].

In another study performed by Zhang *et al*., [25] downregulation of Nrf2-Keap1 was reported in MDD, schizophrenia, and BPD. They concluded that Nrf2 and Keap1 reduced expression may be correlated with mental disorders [25].

Furthermore, the role of inflammation in many neurodegenerative diseases has also been studied. In Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis, downregulation of Nrf2 has been reported [26]. In turn, activation of Nrf2 in these conditions reduces the symptoms of these diseases and can be considered as a potential therapeutic target [26].

In another study on patients suffering from AD, a downregulation of Nrf2 target genes was observed, which indicates a disruption in antioxidant defense mechanism due to dysregulation in nuclear localization or transcription activity of Nrf2 [27].

This situation leads to a dysfunction in neural activity observed in AD patients [27]. In the study conducted by Otter in 2010, eight single nucleotide polymorphisms in Nrf2 were investigated for a possible association with AD and cataracts [28].

They reported that these polymorphisms could not be defined as biomarkers of these conditions; however, they could be associated with different progression levels of these disorders [28].

Conclusion

In line with previous findings, our results show a decrease in Nrf2 expression level in the peripheral blood of patients affected by MDD and BPD compared with healthy control subjects. Since the Nrf2 transcription factor has a protective role against inflammation, this study provides evidence for the inflammation etiology of these mental conditions.

It can be concluded that Nrf2 can be a potential biomarker of MDD and BPD, and targeting this transcription factor could be considered as a promising therapeutic approach.

However, understanding the precise neuro-inflammation mechanisms requires more comprehensive studies and different approaches to clarifying the molecular interactions underlying MDD and BPD.

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Conflict of Interest

The authors declare no conflict of interest.

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