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# A comparative study to investigate the behavioral traits and expression level of BDNF and DRD4 genes in bipolar patients referring to Tabriz Razi Hospital and healthy subjects

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## ABSTRACT

*Bipolar disorder is one of the major causes of disability causing cognitive and functional disorders and increased mortality and death, especially due to suicide. The first episode of bipolar disorder is mostly depression. Depression episodes last significantly longer than manic or hypomanic episodes for most people with bipolar I or bipolar II disorder. The estimated heritability of bipolar disorder is between 70 and 90%. Genetics, especially the effect of small genes on potential underlying neurobiological pathways of bipolar disorders, have been obtained from genome-related studies. The present comparative study investigated the expression of BDNF and DRD4 genes in samples with bipolar disorder and healthy subjects. For this purpose, 25 subjects with bipolar disorder with a history of at least two hospitalizations and with an MMPI score above 70 were randomly selected. Among the patients hospitalized in Tabriz Razi Hospital and 20 healthy subjects were randomly selected, 5 cc of their peripheral blood was extracted using Dena Zist and Sina Cologne kits, RNA was extracted and then synthesized After quality measurement of cDNA using Oligo dT. After synchronizing, their expression of genes was measured using specific primers and real-time PCR. The results revealed that the MMPI index in bipolar patients is significantly higher than in healthy subjects. However, the relative expression of BDNF and DRD4 genes in bipolar patients decreased significantly (P-value<0.05). If it is proven in more samples, it can be used as a molecular marker in early and accessible screening of people prone to bipolar disorder.*

***Keywords:*** *Bipolar disorder, Gene expression, BDNF, DRD4*

## INTRODUCTION

Bipolar disorder, or manic-depression, is a type of mental disorder characterized by episodes of depression, mania, and abnormal mood (Anderson and Haddad, 2012). If the patient's energy is very high or accompanied by psychosis, it will be called mania, while if the intensity is low, it will be called hypomania. Abnormal feelings and behaviors, irritability, grandiose delusions, concentration disorder, and over-optimism are symptoms of mania. During this episode, the patient mostly makes risky decisions without considering the consequences. The episode of mania is accompanied by a lack of sleep. During depression, the patient finds a negative view of his or her life (Goodwin, 2012). Sleep disorders, lack of self-confidence, fatigue, eating disorders, and lethargy are symptoms of depression. The risk of suicide is almost high in bipolar disorder patients, so during 20 years, 6% of patients died by suicide, and 30-40% reported

suicide ideation. Bipolar disorder is mostly accompanied by substance abuse disorder and anxiety disorder (Charney & Sklar, 2018).

Although the causes of bipolar disorder have not been clarified definitively, it is believed that environmental and genetic factors are involved in this disorder. Several low-functioning genes may be involved in this disorder. Genetic factors account for about 70-90% of the risk of developing bipolar disorder (Grandi et al., 2016). However, more scientific studies are needed regarding genetic factors. Genetics is one of the most probable and stable risk factors for bipolar disorders. Its risk is 10 times greater on average in adult relatives of the patient with bipolar I and bipolar II. The rate of risk increases with the degree of kinship. It seems that schizophrenia and bipolar disorder probably have a common genetic origin, which is reflected in patients with schizophrenia and bipolar disorder. Brain-Derived Neurotrophic Factor is coded by a gene called BDNF, which affects the nervous system. Neurotrophins are a family of growth factors regulating the survival, growth, differentiation, and maintenance of neurons in both the central nervous system and the PNS (Bibel & Barde, 2000). Four types of neurotrophins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), have been identified and their functions in the nervous system have been extensively investigated (IP et al., 1992). The multiple effects of different neurotrophins on neurons can be attributed to their selective binding to two classes of receptors including Trk (tyrosine kinase and receptor) and p75 neurotrophin (p75 NTR) family. NGF binding to Trka, BDNF, and NT-4/5 bonds to TrkB, NT-3 to TrkC, and all four neurotrophins bonds are associated with p75NTR (Barbacid, 1995). A few domestic and foreign studies have been conducted regarding the role of these genes in bipolar disorder.

Male Mir (2011) examined the gene expression of dopamine receptors in patients with schizophrenia. The expression of these genes on 17 blood samples of schizophrenic people was examined by real-time PCR technique and compared with the results of blood samples of healthy subjects. Moreover, a significant difference in the mRNA expression level of DRD 2, DRD 4, DRD5, and BDNF genes was shown in patients and healthy subjects. However, no difference was observed in mRNA expression levels of dat1 drd1 genes in healthy patients. The results revealed that DRD 2, DRD 4, DRD5, and BDNF genes were related to schizophrenia. Fakharian Zadeh and Shirvani Farsani (2020) investigated the level of expression of COX gene 2 in bipolar patients. For this purpose, 50 patients with bipolar disorder were selected. Fifty subjects were also considered as the control group, RNA was extracted from peripheral blood mononuclear cells (PBMC), and then cDNA was synthesized using oligo dT. The expression level of the desired genes was measured by real-time PCR and using specific primers for each gene. The expression level of COX2 in the peripheral blood of patients with bipolar disorder and healthy subjects was compared. They reported that COX2 expression decreased in patients with bipolar disorder.

Another study examined the expression of genes in bipolar disorders and DNA methylation in the relationship between prodynorphin and brain-derived neurotrophic factor. In this study, 99 bipolar patients and 42 healthy subjects were examined. Gene expression analysis revealed that prodynorphin (PDYN) mRNA levels were significantly reduced in subjects with BD-II. However, such a reduction was not observed in subjects with BD-I compared to healthy subjects. Other target genes (including catechol-O-methyltransferase (COMT), glutamate decarboxylase (GAD67), and serotonin transporter (SERT) mRNA levels did not change significantly. Also, an

increase in DNA methylation in the PDYN gene promoter was observed in BD-II patients compared to healthy subjects (Claudio et al. 2018).

Almeida et al. (2020) examined genetic differences between subtypes of bipolar disorder: a systematic review focusing on bipolar II disorder. A protein-protein interaction (PPI) network was further made to identify potential hub genes. Ten co-expression modules of the top 5000 genes were identified in 77 samples, and three modules were significantly associated with bipolar. Four genes (NOTCH1, POMC, NGF, and DRD2) were identified as candidate hub genes by PPI and CytoHubba analysis. Finally, NOTCH1 was reported as a hub gene and involved in several biological processes such as actin filament-based process and axon development. In the study by Kidnapillaei et al. (2021), microRNA putative target genes targeted the pathways they were identified as "neuronal projection development" and "axonogenesis" to examine the effect of the drugs used to treat bipolar disorder. Many of the target genes inhibited neurite growth and neurogenesis and were decreased after combined drug treatment of bipolar disorder ($P<0.05$). No study has been conducted to examine BDNF and DRD4 simultaneously in BMD subjects research. Hence, the present study was conducted to examine the level of expression of BDNF and DRD4 genes in bipolar subjects of Tabriz Razi Hospital and compare it with healthy subjects, and assess the feasibility of its use in bipolar subjects as a reliable molecular marker.

**Materials and Methods:**

In this study, 25 subjects with bipolar disorder with a history of hospitalization at least twice and with an MMPI score above 70 were randomly selected among the patients admitted to Razi Hospital in Tabriz, and 20 healthy subjects were randomly selected. Then, 5 ccs of blood were taken from their peripheral blood with prior consent using Dena Zist and Sina Cologne kit protocols and RNA messenger. After quality measurement (absorption at 260 and 280 nm), cDNA was synthesized from it using oligo dT and after synchronizing the expression rate of genes, they were measured with specific primers by Real-Time PCR.

**Results:**

The analysis of the demographic results of the samples (Table 1) revealed that in Razi Hospital in Tabriz, most of the patients were aged above 40 years and the same trend was observed in the healthy subjects. Sixty percent of bipolar subjects had under diploma and most of the healthy subjects had a university degree (Table 2).

The ratio of males to females was almost equal in individuals with bipolar disorder, while most of the healthy subjects were male (Table 3). The mean age of the patients was about 45 years and that of healthy subjects it was 46 years. Selected samples from Razi Hospital with bipolar disorder o had at least 2 hospitalizations, had an MMPI score above 80 in the medical records, and were significantly different from healthy subjects (P value < 0.01). Among the bipolar subjects, 20% had major depression, manic, and psychosis in addition to bipolar.

Table (1)- Frequency distribution of age of patients and healthy subjects

| Group<br>Age range | % (patients) | % (healthy) |
|---|---|---|
| Below 25 years | 12 | 15 |
| 25-40 | 24 | 25 |
| 40-60 | 64 | 60 |

| Group<br>Age range | % (patients) | % (healthy) |
|---|---|---|
| Total | 100 | 100 |

Table (2) frequency distribution of education level of patients and healthy subjects

| Group<br>Education level | % (patients) | % (healthy) |
|---|---|---|
| Under diploma | 60 | 10 |
| Diploma | 28 | 30 |
| University | 12 | 60 |
| Total | 100 | 100 |

Table (3)- Frequency distribution of gender in patients and healthy subjects

| Group<br>Gender | % (patients) | % (healthy) |
|---|---|---|
| Male | 45.16 | 75 |
| Female | 54.84 | 25 |
| Total | 100 | 100 |

Table (4)- Mean and standard deviation of age in patients and healthy subjects

| Group | Mean | SD |
|---|---|---|
| Patients | 45.2 | 6.25 |
| Healthy | 46.55 | 5.656 |

DRD4 gene relative expression

The quality of cDNA synthesized from the mRNA extracted from the blood was measured using nanodrop, and their values varied from 1.78 to 2.1, indicating the appropriate quality. Moreover, the quality of the PCR of cDNA product was tested in gel electrophoresis, as shown in Figure 1.

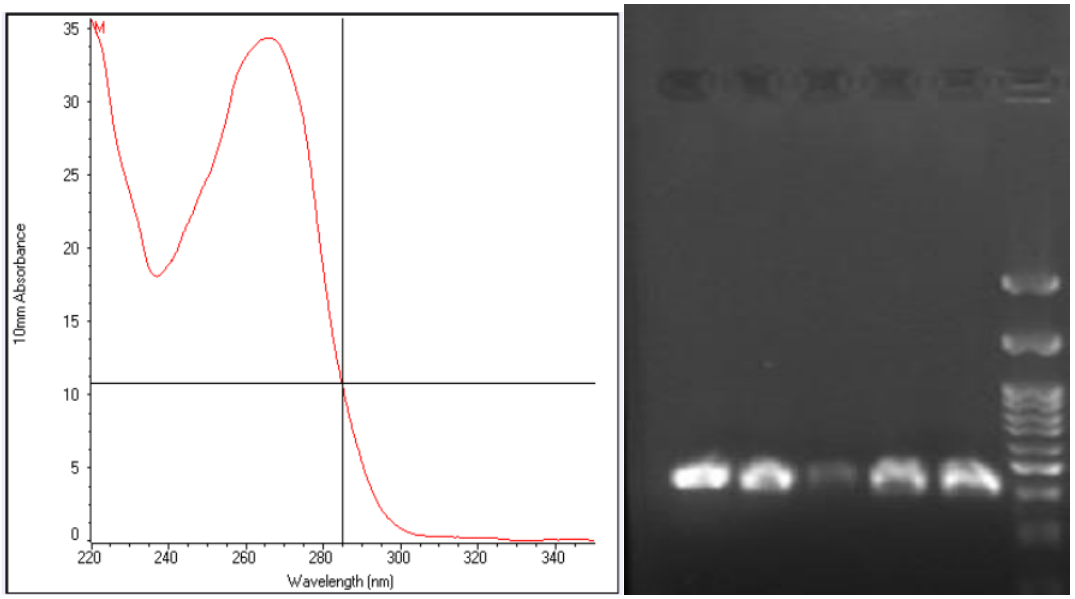
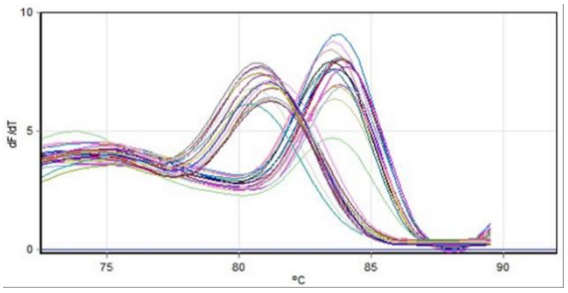



Figure 1- Electrophoresis results of real-time PCR product and cDNA

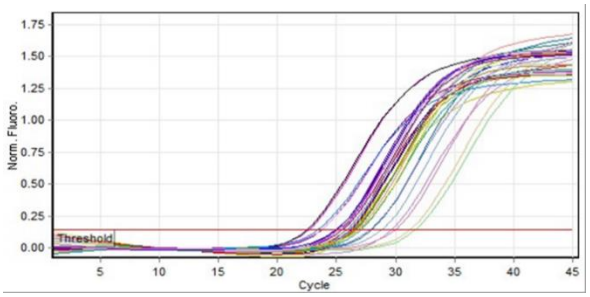
Figure 2-a and Figure 2-b show the graphical results of the relative expression of genes related to DRD4 in the logarithmic phase of MT. The value of CT varied from 22 to 33. The lower value of CT indicates the higher expression of the gene and vice versa.

The sharpness of the TM curve indicates the specificity of the respective gene amplification in Figure 2-a did not show the amplification specificity of the corresponding gene in Figure 2-a, and peak indicates the expression of the DRD4 gene and the housekeeping gene GAPDH2.

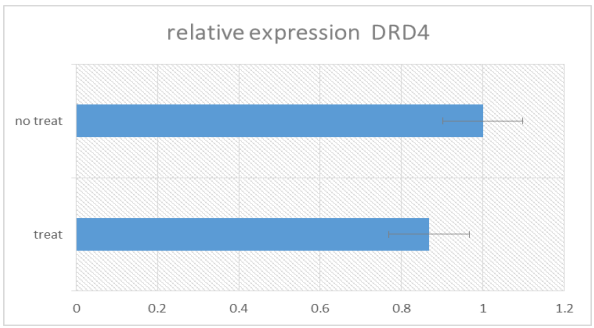
B




A




C




A:
CT in the samples as an amplification marker

B:
TM for the specificity of gene amplification in real-time PCR

C:
Relative expression of the DRD4 gene in two control and bipolar groups

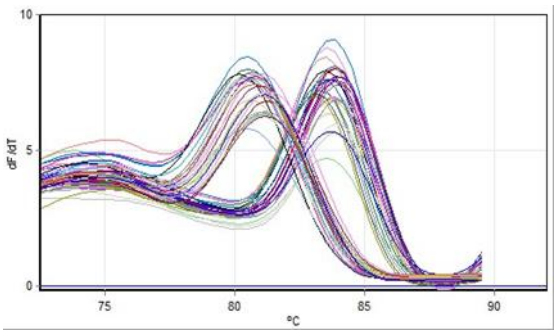
Figure 2 presents the CT and MT, and DRD4 relative expression in two groups of patients and healthy subjects

Figure 2 presents the relative expression of the DRD4 gene in two groups of people with bipolar disorder and healthy subjects. DRD4 gene expression was significantly decreased (P-value<.05).

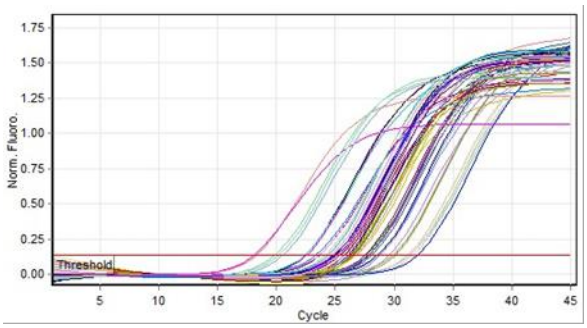
BDNF gene relative expression

The CT and MT diagrams of the relative expression of genes of BDNF have been shown Figure 3-a and Figure 3-b show. The sigmoid shape of CT indicates the amplification of gene and housekeeping gene, and the range of CT varied between 17 and 33. Each CT value represents a 2-fold change in expression. The sharpness of the TM diagram indicated the specificity of the studied gene amplification using the primers of housekeeping genes and BDNF.

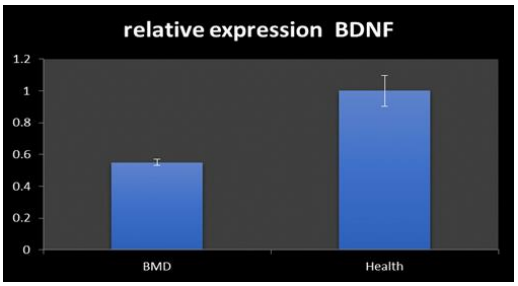
B




A




C




A:
CT in the samples as an amplification marker
B:
TM for the specificity of gene amplification in real-time PCR
C:
Relative expression of the DRD4 gene in two control and bipolar groups

Figure (3)- CT concentration diagram in samples and threshold line (a), TM diagram (b), and BDNF relative expression in two groups of patients and healthy subjects

Based on Figure 3-c, which shows the BDNF relative gene expression in two groups of sick and healthy subjects, the decrease in BDNF relative gene expression in subjects with bipolar was significantly higher than in healthy subjects (P value<0.01), so this value was almost half of the value in the healthy subjects. Pearson correlation between age, history, BDNF, DRD4, and MMPI variables is presented in the table below.

Table 4- Pearson correlation between the studied variables

| Variable | Age | History | BDNF | MMPI |
|---|---|---|---|---|
| Age | 1 | | | |
| History | 0.164 | 1 | | |
| BDNF | -0.551* | 0.671** | 1 | |

| Variable | Age | History | BDNF | MMPI |
|---|---|---|---|---|
| MMPI | 0.351 | 0.771** | 0.769**- | 1 |
| DRD4 | 0.351- | 0.771** | 0.769** | 0.86**- |

**Significance at the 0.01 level * Significance at the 0.05 level

Based on Table 4, there was no direct and significant correlation between age and MMPI ($p>0.05$). However, there was an inverse correlation between age with BNDF($r=-0.551$) and DRD 4 ($r=-0.353$). MMPI was significantly correlated with BDNF (-0.769), history (0.771), and DRD4 (-0.86) ($p<0.05$; $p<0.01$).

Table (5) The relationship between the studied variables and the expression of the studied genes

| Row | Variable | Mean expression of DRD4 | Mean expression of BDNF | Sig |
|---|---|---|---|---|
| Gender | Female<br>Male<br>T value | 0.733<br>.65<br>1.79 | 0.65<br>.69<br>1.08 | >0.05<br>Ns |
| Marital status | Single<br>Married<br>Divorced<br>F-value | 0.71<br>0.61<br>.63<br><br>1.43 | 0.66<br>0.69<br>0.64<br><br>.98 | >.05<br>Ns |
| Literacy | Illiterate<br>Higher than the secondary level of education<br>T value | 0.78<br>.65<br>.69 | .66<br>.68<br>.68 | |

Statistical tests revealed no significant relationship between gender, marital status, literacy, and the expression of studied genes (Table 5).

**Discussion**

This present study was conducted to compare the relative expression levels of BDNF1 and DRD4 genes in patients with bipolar disorder and healthy subjects. Evaluating the difference in behavioral traits and expression levels of BDNF and DRD4 genes in bipolar patients of Razi Tabriz hospital and comparing it with healthy subjects by t-test method indicated that the MMPI index in bipolar subjects was more than 70 and it was significantly more than healthy subjects, which was not unexpected. The subjects with this disorder have different traits, including abnormal feelings and behaviors, elated, grandiose delusions, concentration disorder, over-optimism, risky decisions without considering the consequences, lack of sleep (Goodwin, 2012), lack of self-confidence, sleep and eating disorders, fatigue. The suicide risk is high among bipolar disorder patients (Charney and Sklar, 2018).

Bipolar disorder is a mental disorder characterized by episodes of depression, mania, and abnormal mood (Anderson and Haddad, 2012). A patient with very high energy or with psychosis is called mania. However, when its severity is low, it is called hypomania. The difference between healthy subjects and bipolar patients in terms of BDNF gene expression was examined by t-test and the results showed the level of BDNF gene expression was significantly reduced in the bipolar patients. The causes of bipolar disorder might vary between individuals and the exact mechanism of this disorder is unclear (Nierenberg et al., 2014).

Studies have referred to the role of genetic (Kerner, 2014) and environmental factors (Geddes & Miklowitz, 2013; Young & Dulcis, 2015). Moreover, it is believed that genetic effects account for 73-93% of the risk of developing the disorder, indicating a strong hereditary component (Bobo, 2017). The overall heritability of the bipolar spectrum is estimated to be 0.71 (Edvardson et al., 2008). Behavioral genetic studies have indicated that many chromosomal regions and candidate genes are related to bipolar disorder susceptibility and each gene has a mild to moderate effect (Kerner, 2014). Although the first finding of genetic linkage for mania was in 1969, the linkage studies have provided conflicting and inconsistent results (Barnett JH, & Smoller, 2000). In other words, the findings strongly refer to heterogeneity. However, strong and reproducible genome-wide associations have indicated that several common single nucleotide polymorphisms (SNPs) are associated with bipolar disorder, including different types of CACNA1C, ODZ4, and NCAN genes (Craddock & Sklar, 2013; Kerner, 2014).

Moreover, the most recent genome-wide association study regarding the role of genes reinforced the idea that no single gene is responsible for bipolar disorder in most cases (Craddock & Sklar, 2013). BDNF is a neurotrophic transcription factor whose expression has been reported to decrease in some mental disorders, including Alzheimer's, mania, and bipolar (Abassi et al., 2020). Additionally, polymorphisms in BDNF, DRD4, DAO, and TPH1 were reported to be associated with bipolar disorder (Seifuddin et al., 2012). Also, it was found that two polymorphisms in TPH2 are associated with bipolar disorder (Young et al., 2019). A study by Cheng et al. (2013) examined the expression of BDNF and DRD3 genes in bipolar disorder with anxiety disorder (AD). The results revealed that DRD3 Ser9Gly polymorphism is associated with comorbidity of BP-II with AD (BPII) +AD and BDNF Val66Met polymorphism was associated with comorbidity of BP-I with AD (BPI+AD).

The results of the study by Claudio et al. (2018) revealed that prodynorphin (PDYN) mRNA levels were significantly reduced in BD-II subjects, but not in BD-I subjects compared to healthy subjects. Lee et al. (2021) found 14 conditionally-independent genes in this study, 10 of which were not previously involved with BD. There was a difference between DRD4 gene expression levels in bipolar patients of Razi Hospital in Tabriz and healthy subjects. Based on the real-time PCR test results in Figure (4) and the significant t-test result, the expression level of DRD4 was reduced at the probability level of 5%. DRD4 is an energetic dopamine receptor subunit 4. In this study, its expression was reduced in bipolar subjects. A reduction in its expression in schizophrenia patients due to epigenetic factors and methylation of the promoter of the DRD1-5 gene had been already reported (Male Mir 2011). Also, a change in its expression in major depression has been confirmed (Aghazadeh et al. 2021). Since one pole in bipolar has a depression phase, this result was in line with the results in depression.

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