RESEARCH ARTICLE

Impact of Brain derived Neurotrophic factor gene polymorphism on its peripheral levels in schizophrenic patients

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Abstract

Objective: To determine serum levels of brain-derived neurotrophic factor and its polymorphism rs12291063 in schizophrenic patients.

Method: The case-control study was conducted from January1, 2020, to May 15, 2021, at Dr Abdul Qadeer Khan Institute of Behavioural Sciences, Dow University of Health Sciences, Karachi, and comprised schizophrenia cases aged 14-60 years who were diagnosed using Diagnostic and Statistical Manual of Mental Disorders-V criteria, and healthy controls without any psychiatric illness. Positive and negative syndrome scale score was used to assess disease severity. The genomic deoxyribonucleic acid of the subjects was isolated from peripheral blood, followed by polymerase chain reaction, gel electrophoresis and sequencing of the amplicons. The sequences were analysed using MEGA X software for genotyping. Serum brain-derived neurotrophic factor levels were assessed using enzyme-linked immunosorbent assay. Data was analysed using SPSS 21.

Results: Of the 100 subjects, 50(50%) were cases; 36(72%) males and 14(28%) females ($p<0.05$) with mean age 34.34±10.32 years. There were 50(50%) controls; 32(64%) males and 18(36%) females ($p=0.391$) with mean age 30.886±8.88 years. Among the cases, the mean age at schizophrenia diagnosis was 25.14±9.54 years, and there was a significant association with positive family history for psychiatric disorders ($p<0.05$). Sequencing revealed no T>C substitution. Serum brain-derived neurotrophic factor levels were significantly higher in cases compared to controls ($p<0.001$). There was a weak negative correlation between brain-derived neurotrophic factor levels and positive and negative syndrome scale score ($p<0.05$).

Conclusion: Higher brain-derived neurotrophic factor levels were found to be associated with schizophrenia, while no association of rs12291063 T>C was found with schizophrenia.

Key Words: Brain derived neurotrphic factor, genotyping, Schizophrenia, Mental Disorders.

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Introduction

Schizophrenia is a serious, chronic and debilitating neurodevelopmental brain disorder characterised by hallucinations and delusions.[1] Schizophrenia affects 1% population worldwide, while the prevalence in Pakistan is 1-1.5%[2]. Schizophrenic patients are unable to differentiate between reality and imagination.[3] Studies have reported that 50% patients having schizophrenia attempt suicide[4]. Schizophrenia is among the main causes of disability in young adults.[5] As schizophrenia causes remarkable loss of physical and mental abilities, it leads to heavy economic burden for patients, families and societies.[4, 6]

Schizophrenia is a complex disorder with diverse aetiology and no specific known cause. A combination of physical, genetic, psychological and environmental factors can contribute to the development of schizophrenia.[2,4] Heritability of schizophrenia is 80%.[4]

Several genes are found to be involved with the complex aetiology of schizophrenia, and each gene has its effect on the disease in different populations due to ethnic variations.[4] Brain-derived neurotrophic factor (BDNF) is among the candidate genes for schizophrenia and is involved in the development of brain, neurogenesis, synaptogenesis, as well as differentiation, maturation and survival of neurons.[1,2,7] BDNF is also essential for neuronal plasticity, programmed cell death, and modulation of chemical messengers in adult brain.[7] BDNF has an important role in endurance of dopaminergic, cholinergic and serotonergic neurons that are important for memory

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and cognition in schizophrenia.[7] BDNF is a member of neurotrophin family[2] and is mostly found in hippocampus and cerebral cortex areas of the brain that control emotion, cognitive thinking and mood.[2,7] It is documented that BDNF crosses the blood-brain barrier, and its peripheral level has correlation with its concentration in the central nervous system (CNS).[2,8] BDNF is produced in endoplasmic reticulum as pre-pro-BDNF and is converted to pro-BDNF.[9] Pro-BDNF is then converted to mature BDNF and BDNF pro-peptide.[1,2] Mature BDNF affects trophic structure and physiologic functions through stimulating tyrosine receptor kinase B (TrkB).[1,2,7,9]

Abnormal functions of BDNF could alter the development of neurons, endurance, plasticity and synaptic connectivity. Several studies have reported changes in BDNF levels, and its association with schizophrenia in different populations.[10] There is significant evidence showing that serum BDNF levels are deregulated in brain and serum of schizophrenic patients.[6] Antipsychotic drugs have been reported to have varied effects on serum levels of BDNF. Zhao T et al. reported decreased levels of serum BDNF in 1st episode schizophrenic patients than controls.[11] Bakirhan et al. also reported significantly decreased levels of serum BDNF in patients with schizophrenia compared to controls.[6] Nieto et al. reported significantly lower serum BDNF levels in schizophrenia patients than controls.[12] Another study documented lower levels of BDNF in the serum of patients with 1st episode of schizophrenia compared to controls.[13] On the contrary, Lin et al. reported that BDNF levels were increased in schizophrenic patients with metabolic syndrome.[14] Serum BDNF levels were reported higher in Polish women with acute schizophrenia.[15]

Human BDNF gene is present on chromosome 11, having 11 exons and 9 functional promoters.[9] BDNF polymorphisms have been studied in various populations around the world and they were found to be associated with schizophrenia. There are several known single nucleotide polymorphisms (SNPs) in BDNF gene, including rs6265, C270T, rs7103411 and rs2030324.[9,16] Val66Met polymorphism is reported to be associated with schizophrenia in European and Asian populations, including the Chinese.[7]

A meta-analysis comprising 13 case-control studies of C270T polymorphism reported significant association of T allele with schizophrenia in East-Asian population, but not in Caucasians.[2,16] Notaras et al. found significant evidence that BDNF Val66Met polymorphism has regulatory effect on the age of onset of schizophrenia, clinical symptoms, intelligence, morphology of brain and response of schizophrenic patients to antipsychotics.[17] It has been reported that BDNF gene rs11030101/rs2030324/rs6265 AAC haplotype is associated with an increased risk of schizophrenia. Genotypes rs11030101 may affect negative symptoms, and rs6265 may affect the general symptoms of schizophrenic patients.[18] Further, rs12291063 is an intronic BDNF polymorphism. It is reported that major T allele at rs12291063 possesses a binding capacity for the transcriptional regulator and substituting C for T disrupts binding and transactivation functions, leading to decreased BDNF expression.[19]

The current study was planned to evaluate the association of BDNF levels and its polymorphism rs12291063 with schizophrenia, and to find correlation of BDNF levels with the severity of symptoms in schizophrenic patients.

Materials and Methods

The case-control study was conducted from January1, 2020, to May 15, 2021, at Dr Abdul Qadeer Khan Institute of Behavioural Sciences, Dow University of Health Sciences (DUHS), Karachi, after approval from the institutional ethics review board, the sample size was calculated using OpenEpi version 3.[20] The sample was raised using convenience sampling technique, from among hospitalised patients and those visiting the out-patient department (OPD). Those included were schizophrenia cases of either gender aged 14-60 years, and healthy controls with no psychiatric illness.

The cases were diagnosed and recruited by a psychiatrist in line with the Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) criteria.[21] Those excluded were cases with obesity or any diagnosed psychiatric and neurological disorder other than schizophrenia.

The controls were matched from age and gender, and were recruited from among visitors, students and staff at DUHS. Obese and those with any diagnosed psychiatric and neurological disorder were excluded.

Disease severity in schizophrenic patients was evaluated using the Positive and Negative Syndrome Scale (PANSS),[22] which was applied by a trained psychiatrist. Beck Depression Inventory II (BDI-II)[23] was applied to rule out depression in the control group.

After written informed consent was obtained from all the participants in both groups, peripheral blood was drawn, and genomic deoxyribonucleic acid (DNA) was extracted using an extraction kit (Promega (USA)- Catalogue number: A1125). Quantity and purity of all DNA samples were evaluated using a spectrophotometer Thermo Nano Drop Lite UV visible spectrophotometer (Thermofisher

scientific, USA). Amplification of the region of BDNF gene encompassing polymorphism rs12291063 T/C was done in a thermal cycler (ThermoFisher SimpliAmp Thermal Cycler Scientific, USA). The forward primer used was 5′ TCCCTGATCCCTGAACATAGCC3′ And the reverse primer used was 5′ TTGGGTTACCTCCTGGGAACTT 3′. Negative control containing all reagents except DNA was also run to confirm that there was no contamination in the reaction mixture. Gel electrophoresis was performed to confirm the amplification of the desired area. Further, 2µl of PCR product from each sample was run on 1% agarose gel to confirm amplification and band size. PCR products were stored at -20C till sequencing.

PCR amplicons of all the samples were sent (Macrogen, Inc. South Korea) for sequencing through Sanger Sequencing[24] for the detection of polymorphism. Electropherogram was analysed to locate homozygous and heterozygous variants.

Analysis for nucleotide sequence was done using MEGA X software.[25] The first 80-100bp at 5′ end, and the last 100bp at 3′ end were terminated because of uncertainty and poor electropherogram. For the identification of allelic variants, sequences of all the participants were matched with BDNF reference sequence (GenBank accession no:GRCh38.p14 chr 11 NC_000011.10:g.27672554T>C) from the National Centre for Biotechnology Information (NCBI) nucleotide data bank using CLUSTAL W25 tool of the software.

BDNF level in serum was assessed by performing sandwich enzyme-linked immunosorbent assay (ELISA) kit (Cloud-Clone,China Catalogue No: SEA011Mi 96T). All the components were allowed to reach room temperature (18-25ºC) before use, and stepwise protocol was followed for all samples as per the manufacturer's guidelines.

Data was analysed using SPSS 21. Quantitative and qualitative variables were expressed as mean ± standard deviation, and frequencies and percentages, respectively. Pearson chi-square test was used to check associations among the onset, family history of schizophrenia, and genotype variables. Independent samples t-test was used to check the mean difference of anthropometric measurements, BDNF levels and PANNS scores between the groups. Mean differences in BDNF levels and PANNS measurements were assessed using one-way analysis of variance (ANOVA). Correlation between serum levels of BDNF and PANSS was evaluated by using Pearson's correlation test. P<0.05 was considered significant.

Results

Of the 100 subjects, 50(50%) were cases; 36(72%) males and 14(28%) females (p<0.05) with mean age 34.34±10.32 years. There were 50(50%) controls; 32(64%) males and 18(36%) females (p=0.391) with mean age 30.886±8.88 years. Among the cases, the mean age at schizophrenia diagnosis was 25.14±9.54 years, and there was a significant association with positive family history for psychiatric disorders (p<0.05). There was also a significant variation (p=0.008) between the cases and the controls regarding ethnicity, with more Urdu-speaking individuals among the cases compared to Sindhis and Punjabis among the controls. Positive family history for psychiatric disorder was in 18(36%) of the cases compared to none among the controls (p=0.001). There were broken marriages among the cases than the controls, and the association between high divorce ratio and schizophrenia was significant (p=0.007) (Table).

Table : Demographic characteristics of the participants.

Characteristics	n (%)	n (%)	*P*-Value
Age at Onset(year)	-	25.14±9.54	
Gender			**0.391**
Female	18(36.0%)	14(28.0%)	
Male	32(64.0%)	36(72.0%)	
Education			**0.148**
Nil	11(22.0%)	11(22.0%)	
School	10(20.0%)	19(38.0%)	
College	9(18.0%)	8(16.0%)	
Graduate	14(28.0%)	11(22.0%)	
Postgraduate	6(12.0%)	1(2.0%)	
Ethnicity			**0.008**
Baloch	0(0.0%)	2(4.0%)	
Punjabi	11(22.0%)	3(6.0%)	
Pashto	1(2.0%)	3(6.0%)	
Sindhi	18(36.0%)	9(18.0%)	
Urdu speaking	20(40.0%)	33(66.0%)	
Marital status			**0.001**
Divorced	1(2.0%)	7(14.0%)	
Married	13(26.0%)	25(50.0%)	
Unmarried	36(72.0%)	18(36.0%)	

Sequencing revealed no T>C substitution in wither group. There was no difference in genotype frequencies of rs12291063 between the cases and controls because all the 100(100%) subjects were found to have same TT genotype. There was no difference in allelic distribution for SNP rs12291063 between the groups (p>0.05).

Mean serum level of BDNF in cases was 34.18±12.44ng/ml compared to 22.21±9.065ng/ml in controls (p<0.001) (Figure 1). Mean BDNF level in males was 27.111±11.431ng/ml compared to 30.496±14.13ng/ml in

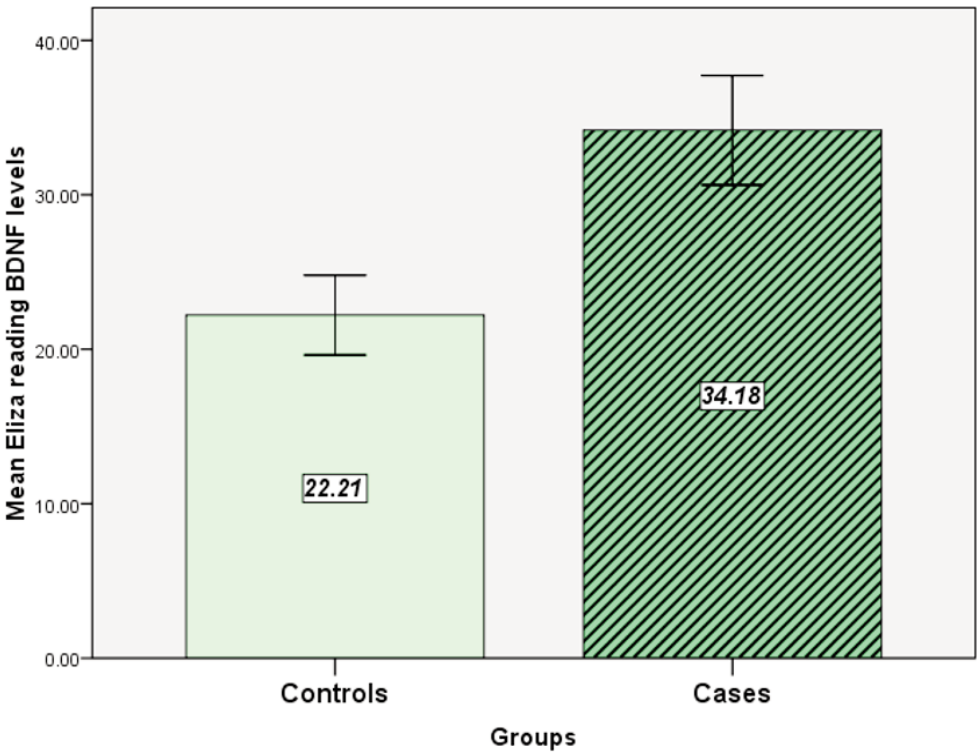

Figure-1: Mean serum brain-derived neurotrophic factor (BDNF) levels between cases and controls.

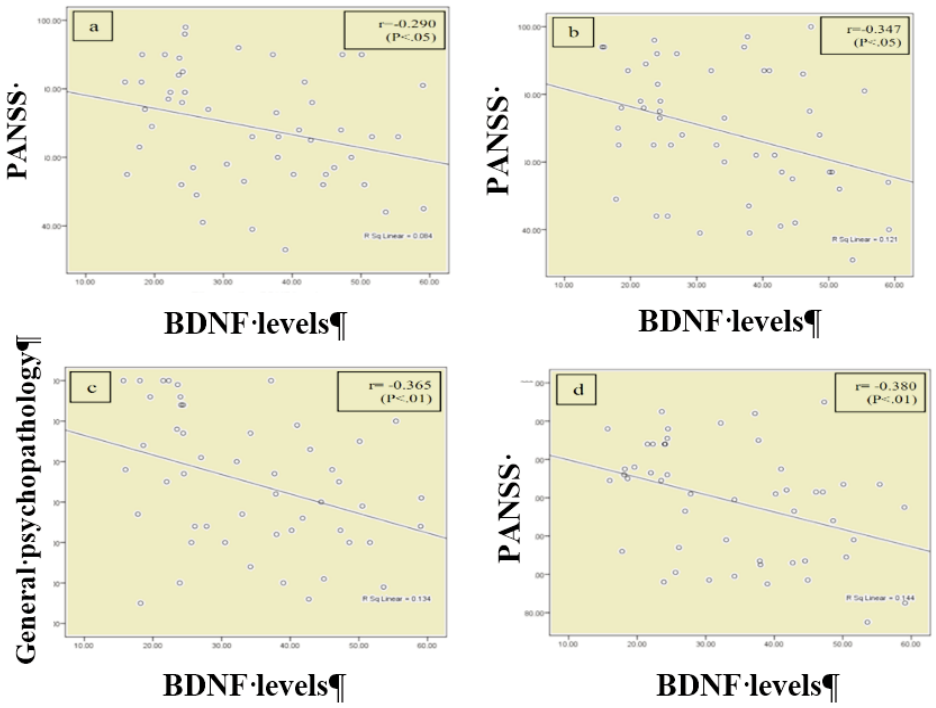

Figure-2: Correlation of brain-derived neurotrophic factor (BDNF) levels with Positive and negative syndrome (PANSS) score (a-b), and correlation between BDNF levels and PANSS general psychopathology and PANSS total scores (c-d).

females. Overall mean BDNF level across the sample was 128.195±12.391ng/ml.

There was a weak, negative correlation of BDNF level with PANSS positive

(r= -0.290) and negative (r= -0.347) scores (p<0.05) (Figure 2A-B). There was a weak, negative correlation of BDNF levels with general psychopathology (r= -0.365) and PANSS total score (r= -0.380) (p<0.01) (Figure 2C-D).

Discussion

The current present study explored polymorphism rs12291063 and its association with serum BDNF and disease severity. To the best of our knowledge, no prior data was available regarding the association of rs12291063 with schizophrenia in any population.

The study found that age of onset of schizophrenia was 25.14±9.54 years. Previous studies reported age 15-35 years at onset of schizophrenia.[3,4] In the present study, males were affected more than females, which was in line with previous studies.[26] This may be due to the protective effect of oestrogen in females

through the effect of estradiol on gene encoding for neurotrophic factor and by a direct effect on neurotransmitter, specially dopamine.[27] There was a strong association between ethnicity and schizophrenia in the current study, which was in line with previous studies.[16,18] Significant association of positive family history of psychiatric disorder with schizophrenia was also found, which was also reported earlier.[4] Strong association was observed regarding marital status in relation to schizophrenia with increased ratio of divorce in patients compared to the controls. The finding reflected that social and cognitive abilities were compromised in schizophrenia, leading to bad and broken marriages.[28] The present study reported compromised educational milestones in schizophrenic patients. Escot et al. also reported the same findings.[29]

The current study observed significantly higher levels of serum BDNF in cases compared to controls, which agreed with literature.[30] Several studies have reported significant decrease in BDNF levels in schizophrenia.[6,11-13] While few studies have reported increased BDNF levels in cases.[14,15]

With respect to PANSS score, a weak, negative correlation between BDNF levels and PANSS in schizophrenia was found in the present study. Hendriati et al. reported a negative correlation between serum BDNF levels and negative symptoms and total subscale score in males with schizophrenia.[31]

The current study did not find any association of BDNF polymorphism rs12291063 with schizophrenia, and the lack of association may be due to genetic variations and ethnicity among the study participants.[18] The other reason could be the small sample size of the study. The effect of polymorphism rs12291063 on BDNF level could not be analysed as no sample with rs12291063 polymorphism was detected.

The current study has limitations as it was conducted at a single centre with a small sample size and explored only 1 polymorphism. Multicentre, experimental studies with larger and multi-ethnic sample sizes exploring other SNPs of BDNF are recommended.

Conclusion

Higher BDNF levels were found to be associated with schizophrenia, while there was no association of SNP rs1229106 with schizophrenia. Males were affected more with schizophrenia than females. Positive family history of psychiatric disorders, ethnicity and higher divorce ratio were strongly associated with schizophrenia

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