Multi-factorial Etiology of Bipolar Disorder and Schizophrenia in Iran: No Evidence of Borna Disease Virus Genome

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ABSTRACT

Background and Objectives: Psychiatric disorders such as schizophrenia and bipolar disorder pose a high burden among the general population. Etiological factor(s) of such disorders remain unknown. Borna disease virus (BDV) is a neurotropic virus that has been suggested as an etiological agent for psychiatric disorders. Therefore, we aimed to investigate the prevalence of BDV among patients with schizophrenia and bipolar disorder.

Methods: Peripheral blood mononuclear cells (PBMCs) of schizophrenic (n=75) and bipolar (n=55) patients and healthy blood donors (n=125) were extracted form whole blood samples. RNA was extracted from PBMCs and the presence of BDV P40 RNA was assessed by reverse transcription-polymerase chain reaction.

Results: The BDV genome was not detected in any of the subjects. Positive family history of disease was significantly more frequent among patients (P=0.0001). There was a significant association between contact with animals and psychiatric illnesses (P<0.05). Moreover, education level differed significantly between the two groups (P<0.05).

Conclusion: The results indicate no evidence of BDV genome among patients with psychiatric disorders. Serological examination for BDV antigens or antibodies could provide further information in this regard. In addition, contact with cats is significantly more prevalent among patients with mental illnesses, which might be due to infection with *Toxoplasma gondii*.

Keywords: Borna disease virus, Psychiatric disorder, Schizophrenia, Bipolar disorder, Risk factors.

INTRODUCTION

Schizophrenia and bipolar disorder are disabling mental illnesses with complex etiologies. Both genetic and environmental factors affect the development of these illnesses (1). These factors include genetics (2), family history of the disease (3), season and place of birth (4), and infectious agents (5).

Family studies have demonstrated that affected patients with familial history of psychosis have a higher risk of developing the disorders. In addition, various environmental factors have been evaluated as risk factors for psychosis (6). Among infectious agents considered as risk factors for the diseases, *Toxoplasma gondii* has been observed in patients with schizophrenia (7) and bipolar disorder (8). Additionally, there are several studies indicating the role of viral infections and human psychoses (9). Presence of Borna disease virus (BDV) infections have been investigated among various human populations with mental illnesses (10–14).

BDV is an enveloped, single-stranded, non-segmented RNA virus from the *Bornaviridae* family. The virus is highly neurotropic, non-cytolytic, and a pathogen of the central nervous system (CNS) in various vertebrate species (15). It has been suggested that the virus may be transmitted through the nose, saliva, and conjunctival secretions (16). BDV might reach the CNS through axonal migration via the oropharyngeal olfactory nerve, where it reaches the peripheral nerves (17). Depending on age, immunity status, and the host species, the virus can cause CNS disorders with diverse manifestations (18). For example, BDV infection can result in severe immune responses in brain cells of rats with motion abnormality, or induce persistent infection and cognitive disorders in newborn rats (19, 20).

Similar to animal models, infection of humans with BDV initially affects the brain, which is followed by psychiatric and neurological symptoms (21). Transmission of the virus to humans through infected animals is also possible (17). Behavioral health studies on animals infected with BDV have led to the idea that BDV infection in humans may be associated with psychiatric illnesses, such as mood and psychotic disorders (22). Epidemiological studies using peripheral-blood mononuclear cells (PBMCs) and brain tissues have shown that BDV can infect humans and may be associated with certain neuropsychiatric disorders (12).

In schizophrenia, the presence of antibodies against BDV antigens has been identified. Waltrip et al. found anti-BDV antibodies in 14.4% of schizophrenic patients (23). There may be a possible relationship between BDV and certain affective disorders such as unipolar and bipolar disorders, and major depression (19). Amsterdam and colleagues detected BDV antibodies in 4.5% of patients with affective disorders (24). Bode et al. found BDV antibodies in 20% of admitted patients with major depression (25).

In several studies, BDV genome (P24, P40) has been identified in brain tissue, cerebrospinal fluid (CSF) samples, and PBMCs using various polymerase chain reaction (PCR) methods including, reverse transcriptase–PCR (RT-PCR), nested PCR, quantitative fluorescent-PCR, and real time-PCR (26,27). Xu et al. detected BDV P24 sequence in 9.7% of patients with schizophrenia using RT-PCR (28). Borna virus infection is reported to be more frequent in young populations (17-30 years old) suffering from mental disorders (29).

Given the importance of mental disorders and their impact on quality of life of patients and their families, it is crucial to conduct extensive research on the cause(s) of mental disorders and subsequent prevention and treatment methods. In the present study, presence of the BDV P40 gene was evaluated among patients with psychiatric disorders and healthy blood donors using RT-PCR. Furthermore, we explored factors that have been suggested to be associated with mental illnesses in our cohort, including family history of the disease, education level, etc.

MATERIAL AND METHODS

Blood samples from 125 DSM-IV diagnosed psychiatric patients (75 patients with schizophrenia and 55 with bipolar disorder) and 125 age- and sex-matched healthy blood donors (57 female and 68 male) with no history of drug abuse and mental illnesses were collected from several mental health facilities in Isfahan, Iran. The patients were under antipsychotic treatment. The subjects were selected through randomized sampling and diagnosis was confirmed based on psychological tests by a physician.

Information such as age, sex, education level, occupation, family history of mental illness, and contact with animals were obtained. Depending on the subjects' physical and mental health status, data were collected via a questionnaire with the help of the patients or healthcare staff, and from patients' medical records. Exclusion criteria included having psychiatric disorders other than bipolar and schizophrenia, positive result for chronic viral diseases such as hepatitis B, C or HIV, not participating in sample collection, and incomplete filling of the questionnaire. The study received approval from the ethics committee of the Golestan University of Medical Sciences (approval code: 25181693102115). Informed consent was obtained from the patients or their families.

Whole blood samples were used to separate PBMCs. RNA extraction was done using RNX-Plus kit (Cat no. RN7713C; Cinnagen, Iran) according to the manufacture^r's instructions. Purity of the extracted RNA was assessed using a spectrophotometer (Eppendorf, Germany) by reading optical densities at 260/280 nm and 260/230 nm. One µg of the extracted RNA was used as template for cDNA synthesis using the RevertAid First Strand cDNA Synthesis Kit, according to manufacturer's instructions (Cat no. K1622; Thermo Fisher Scientific, Germany). GAPDH with the following primers was used as the control for cDNA synthesis: (Forward) 5'-GAAGGGGAAGGTCGGAGT-3', (Reverse):

5'-GAAGATGGTGATGGGATTTC-3'.

Presence of the BDV genome (p40 coding region) in the samples was analyzed by conventional PCR based on the manufacturer's instructions (Cat no. 100103; Ampliqon, China), and using the following primers encompassing nucleotides 259 to 829 within the BDV genome: (Forward) 5´-TTCATACAGTAACGCCCAGC-3´and (Reverse) 5´-GCAACTACAGGGATTGTAAGGG-3´. The amplification reaction conditions were as follows: 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 seconds, 58 °C for 45 second, and 72 °C for 1 min. A plasmid containing the BDV p40 gene (provided by Dr. Majid Bouzari, Isfahan University) was used as positive control.

Data analysis was performed with SPSS 16.0 using descriptive statistics and Chi-square test at 95% confidence interval (CI). P-values less than 0.05 were considered as significant.

RESULTS

Among total of 250 participants, 114 were female and 136 were male with a mean age of 36.82±10.854 and 37.90±13.632 years, respectively. As shown in table 1, 62.26% of bipolar patients were female, while 65.71% of schizophrenic patients were male. There was no significant difference between the schizophrenic and bipolar patients in terms of sex. BDV RNA was not found in any of the samples.

Table 1-Distribution of schizophrenic and bipolar patients based on sex and age

Groups	Number (%)	Age (mean ± standard deviation)
Healthy blood donors	125 (50%)	38±13.765
Male	68 (27.2%)	38.38±15.419
Female	57 (22.8%)	37.54±11.608
Schizophrenic	70 (28%)	36.47±10.614
Male	46 (18.4%)	37.07±11.765
Female	24 (9.6%)	35.33±8.069
Bipolar	53 (21.2%)	37.66±11.432
Male	20 (8%)	39.30±11.526
Female	33 (13.2%)	36.67±11.436
Both diseases	2 (0.8%)	26.50±7.778
Male	2 (0.8%)	26.50±7.778
Female	0 (0%)	-

Of 250 patients and healthy blood donors, only 73 (29.2%) had a positive family history of psychiatric disorders. Among patients with psychiatric disorders, 51.2% (64/125) had a family history of psychiatric disorders. More specifically, 30 (56.6%) patients with bipolar disorder, 33 (47.1%) patients with schizophrenia, and one (50%) patient with both illnesses had a family history of mental disorders.

Positive family history of mental illness was significantly more prevalent among patients compared with the controls (Pearson's Chi-square, 64:9, X^2 (1) =58.529, p=0.0001). Table 2 shows the demographic information of patients and healthy controls.

Results indicated a significant association between contact with pets or farm animals and psychiatric diseases.

The number of patients (21, 16.8%) with history of contact with cats was significantly higher than the controls (3, 0.24%) (Pearson's, X^2 (1) = 14.934, P< 0.001). The ratio of patients to controls in terms of contact with animals was 11:5. Positive history of contact with horses was found to be more common in patients than in the controls (Pearson's, P= 0.098).

There was no significant difference in terms of contact with other animals including rabbit, sheep, fowl, cattle, dog, donkey, camel, and hamster between schizophrenic and bipolar patients. The subjects were divided into three categories based on their education level: high (Bachelor's degree, Master's degree and PhD), moderate (Diploma and Associated degree) and low (Illiterate, Elementary, Middle school, and High school). Based on the results, 30.8% of all participants (77/250) were in the low education group, 37.6% (94/250) were in the moderate education group, and 31.6% were in the high education group. As illustrated in figure 1, the level of education was significantly higher in the control subjects compared with the patients (Pearson's Chi-square, X^2 (2) = 33.779, P< 0.0001). In addition, female patients had a significantly higher education level compared with male patients (Pearson's Chi-square, P <0.05). There was no significant difference between the schizophrenic and bipolar patients in terms of education level.

Table 2- Demographics of patients and healthy blood donors

Groups	Place of residence		Traveling history			Household size											
	Countryside	Isfahan city	None	Domestic	Abroad & Domestic	1	2	3	4	5	6	7	8	9	10	11	12
Healthy Controls																	
Male	30	38	2	44	22	0	15	20	27	5	1	0	0	0	0	0	0
Female	15	42	0	40	17	1	6	21	21	6	2	0	0	0	0	0	0
Schizophrenic																	
Male	16	30	14	31	1	5	6	10	10	9	2	1	2	0	0	0	1
Female	7	17	4	14	6	1	3	3	7	5	3	0	0	1	1	0	0
Bipolar																	
Male	4	16	5	15	0	0	5	4	3	4	1	3	0	0	0	0	0
Female	14	19	5	25	3	1	3	8	13	7	1	0	0	0	0	0	0
Both																	
Male	0	2	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0
Total	88	164	31	170	49	8	38	66	83	36	10	4	2	1	1	0	1

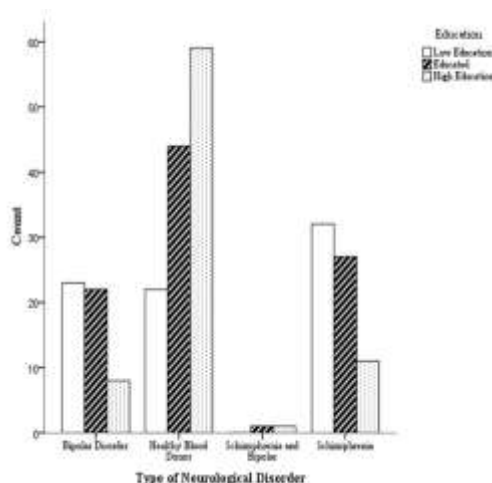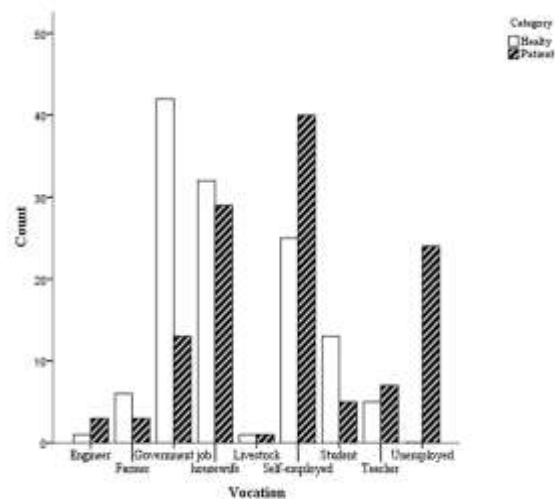**Figure 1 -Education level of patients and healthy controls**

Figure 2- Distribution of patients and control subjects based on occupation

DISCUSSION

The epidemiology and clinical complications of human BDV infection remain controversial. Although most reports of the association between infection and mental disorders have focused on unipolar depression, bipolar disorder and schizophrenia (30), BDV has also been implicated in a wide range of disorders including, chronic fatigue syndrome and multiple sclerosis (31,32). The vast majority of these studies have been based on molecular or serological methods. Limited number of studies have attempted to isolate an infectious virus from humans with psychiatric illnesses (15,18). Most investigations have used nested RT-PCR, a method prone to amplification artifacts due to inadvertent introduction of template from laboratory isolates or cross-contamination of samples (33). Methods used for serological diagnosis of infections include indirect immunofluorescence of infected cells, immunoblotting, enzyme-linked immunosorbent assay of infected cell extracts or recombinant proteins, and circulating immune complex (CIC) assay (11,13,26).

In contrast with our findings, several studies have found RNA of the BDV virus in patients with psychiatric disorders (34,35). However, considering reports of presence of the virus in healthy blood donors, the etiological role of the virus remains controversial (36). Some studies reported no sign of BDV genome and specific antibodies in patients with cognitive disorders (37–39). In addition to RT-PCR, the CIC method can be used for investigating the presence of virus because of its ability to uncover circulating anti-BDV immune complexes in a host (10,40,41). Different methods such as nested RT-PCR and CIC have been applied in studies performed in Iran (10,14,27). Based on these studies, prevalence of BDV RNA among psychiatric patients and blood donors was 6% and 2.2%, respectively. Mazaheri et al. showed the presence of CICs of BDV in more than 40% (46/114) of psychiatric patients and in 22.5% (59/200) of healthy blood donors (10). Disparities in the results of these studies may reflect differences in the subject recruitment and sampling procedures.

The role of BDV in psychiatric disorders is controversial. However, other factors including gender, genetics and environment are involved in these diseases. Sex has been reported as a risk factor for psychosis (42). We also found that a higher percentage of schizophrenic patients were male, while most patients with bipolar disorder were female. However, this difference was not statistically significant and may be due to the difference in the number of female and male patients in each group. The role of genetics in development of bipolar disorder and schizophrenia has been well-established (43). There are some genetic loci which are believed to be associated with both diseases (44). Accordingly, we found that 51.2% of patients had a family history of mental illness. This findings was more evident in schizophrenic patients (56.6%, 30/53) compared with patients with bipolar disorder (47.1%, 33/70).

In a cohort study performed in Denmark, it was demonstrated that first degree family history of mental illness is a risk factor for schizophrenia and bipolar disorder (45).

The subjects were also asked to report any previous contact with animals. Interestingly, cognitive disorders were significantly more prevalent in those having contact with horses and cats. BDV was first identified in horses and then in sheep, cattle, birds, rats, shrews, cats, and dogs (46–48). While this may suggest a possible role for BDV transmission from animals to human, we found no evidence of BDV genome sequences in any of the patients in contact with animals. This could be due to limitations of the RT-PCR in detection of possible BDV infections; however, we consider it unlikely. Meanwhile, it is well known that cats might become infected with *T. gondii* (49). Furthermore, psychiatric patients have high frequency of *Toxoplasma* antibodies and develop psychotic-like symptoms (5). Therefore, the more frequent contact with cats among psychiatric patients in this study may also be related to *T. gondii* infection, which requires further screening.

Education level is regarded as a critical factor for occupation and welfare in developing countries (50). Schizophrenic and bipolar disorder patients were found to be less educated compared to healthy blood donors. Therefore, unemployment and occupations with lower incomes were more common among psychiatric patients. A cohort study has reported that unemployment and low income are associated with psychiatric disorders in young people (51). Moreover, major depression is more frequent among educationally unqualified and unemployed individuals with lower incomes (52). We found that psychiatric disorders are frequent (85.7%) among female teachers. In Iran, teachers face two general and professional problems; relatively low salary and occupational stress. Lack of psychological counseling for teachers might be another contributing factor to the high prevalence of psychiatric disorders among teachers in Iran. However, the sample size for this group of individuals was small.

CONCLUSION

We found no evidence of BDV RNA among Iranian schizophrenic, bipolar and healthy blood donors. Contact with farm animals or cats as well as positive family history of mental illnesses could indicate a previous BDV infection. Therefore, animals in this region should be also screened for BDV infections. The present study also revealed that factors such as education level and occupation are associated with development of psychiatric disorders. Overall, absence of BDV genome could be a proof for absence of current infection. However, it is necessary to investigate previous viral infections using serological methods.

ACKNOWLEDGMENTS

CONFLICTS OF INTEREST

There is no conflict of interest to declare.

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