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Original Article

Low Prevalence of Borna Disease Virus RNA in Patients with Bipolar Major Depression and Schizophrenia in North of Iran

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Abstract

Background and Aims: Borna disease virus (BDV) is well known as a neurotropic virus, however, its role in human neurological diseases such as schizophrenia and bipolar disorder is still unclear. In this study, we aimed to investigate the BDV genome in such patients in Golestan province, North of Iran.

Materials and Methods: RNA was extracted from peripheral blood mononuclear cells (PBMCs) from 250 people (125 patients with bipolar disorder (94.5%) and schizophrenia (3.1%) and 125 sex and age-matched healthy blood donors). Demographic information was collected through a questionnaire. RNA-driven cDNA was used for further BDV P40 genome tracking by Polymerase Chain Reaction (PCR).

Results: Only one sample (1/125; 0.8%) of a female patient with bipolar disorder found to be positive for BDV P40. No significant family history of the disease found in both groups of patients. Seven patients with bipolar depression disorder (5.5%) had a history of animal contact.

Conclusions: No BDV genome has been detected in the blood samples of the patients admitted to the hospital section of psychiatry in Gorgan city. Despite other genetic and environmental factors involved in psychiatric disorders, the serological study will give us a better insight of BDV prevalence in the region.

Keywords: Borna disease virus, P40, Reverse Transcriptase Polymerase Chain Reaction, schizophrenia, bipolar depressive disorder.

Introduction

Borna disease virus (BDV) is a single-stranded, negative sense, non-segmented, enveloped, RNA virus, belonging to Bornaviridae family. BDV is a neurotropic virus, which causes non-cytolytic disease in central nervous system in vertebrates such as hours, sheep, cat, and etc. (1).

This virus transmission has been shown to be through the discharge and saliva (2, 3). Based on the different conditions of the immune system as well as the age of the host, BDV causes several central nervous system disorders (4). For example, BDV induces the immune response in rat's infected brain cells with dynamic abnormality. This virus can induce persistent infection causing cognitive disorder in newborn rats (5).

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Psychiatric patients show similar psychological symptoms as an animal model. Therefore, transmission of the virus from infected animal to humans is controversial (2). Observed behavioral disorders in the BDV-infected animals have led to the possibility of association of this virus in human with psychotic disorders (6). Moreover, previous studies on human brain and peripheral blood mononuclear cells have demonstrated BDV is infectious for human and can be associated with the neuropsychiatric disorder (7). Antibodies against BDV proteins have been detected in 14.4% of schizophrenic patients by Waltrip et, al. (8).

There is a possible relationship between BDV and affective disorder such as bipolar, and major depression (9). Amesterdam et. al showed the presence of anti-BDV antibody in 4.5 % of patients with affective disorder (10). Bode et, al. have shown 20% of the patients with major depression had a positive result using serologic measurement anti-BDV antibodies in hospitalized groups (11).

Considering the importance of mental disorders and their impacts on quality of life and possibility of association with BDV, there is a vacancy for an extensive research on such association and subsequent prevention and treatment. In the present study, we have examined peripheral blood mononuclear cells (PBMC) of the bipolar depression and schizophrenia patients for the BDV-p40 gene. We found no relation between the presence of BDV RNA and schizophrenia in the North of Iran.

Methods

125 patients diagnosed as a bipolar major depressive disorder or schizophrenia cases at the psychiatric department of the "5th Azar" Hospital, Gorgan were selected for the present study. 125 healthy blood donors were invited to the research from the Gorgan Blood Center, as the control group. The control group was matched regarding the sex and age parameters to the patient group. Self-concerns were obtained from both patients and control groups for voluntarily entrance to the research. Positive tests for viral hepatitis B and/or C infection were considered as exclusion criteria.

RNA Extraction from PBMC. Whole blood used for RNA extraction using RNxPlus kit (SinaGene, Iran) according to the kit protocol. RNA integrity has assessed by reading optical density (OD).

cDNA synthesis and PCR protocol. One microgram of extracted RNA was applied in BioNeer cDNA synthesis kit using random Hexamer primers according to manufactured protocols. cDNA products employed as a template for BDV p40 gene PCR amplification by using specific primers (table 1). A plasmid vector containing BDV-p40 region was benefited as a PCR positive control (Gifted from Dr. Majid Bouzari the University of Isfahan). Thermal cycles were as described previously (12).

Phylogenetic tree construction and polymorphism. IQTREE web server (13) was used for constructing the accurate phylogenic tree. BDV sequencing data was aligned with NCBI's GeneBank database (14). The result has been applied for further pairwise local alignment, in the ClustalW using CLC sequence viewer v7 (15). Ultrafast branch support analysis with bootstrap 1000 has performed on the aligned data. iTOL webserver has employed for tree visualization (16). P40 polymorphism (s) investigated by alignment of the sequenced gene with consensus P40 sequence of GenBank sequence.

Approval of the ethics committee. Golestan University of Medical Science's ethical committee has approved this study ethnically (approval code of 25181693102114).

Statistics analysis. The chi2 statistical analysis was performed using SPSS 16.0 software package. (95% CI).

Results

The case group consisted of 121 patients with a diagnosis of bipolar major depressive disorder and 4 patients with a diagnosis of schizophrenia confirmed by a psychiatrist, including 45 females and 80 males. Tow bipolar depression cases had psychiatric disease histories in their family.

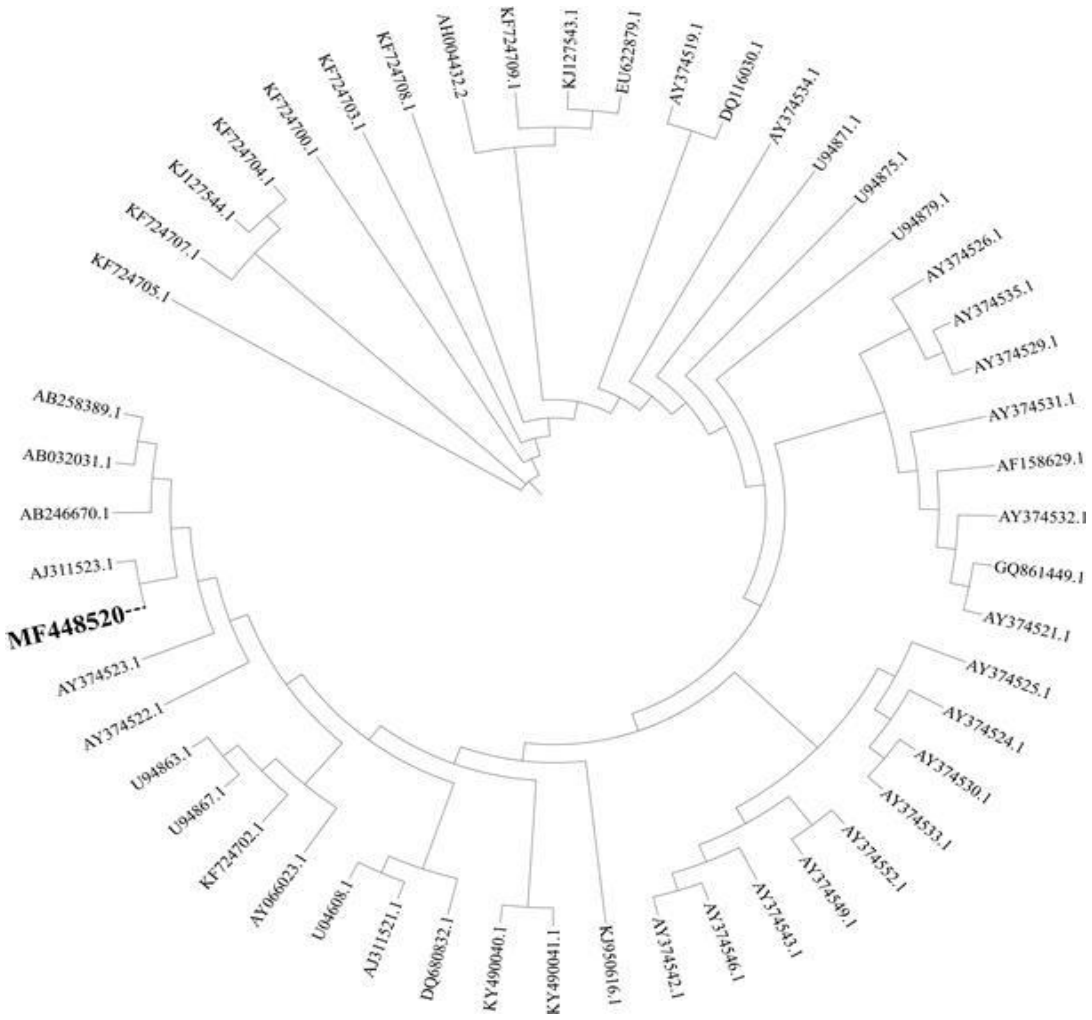

Fig. 1. Phylogenic tree of the p40 obtained here showing close similarity with other records of human isolated BDV genome.

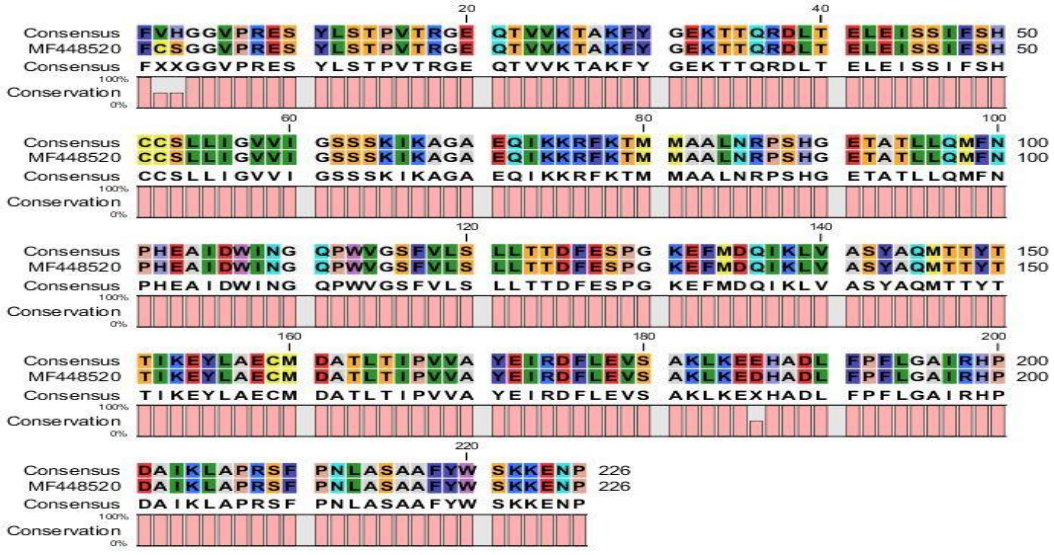

Fig. 2. Multiple Sequence Alignment of MF448520 with BDV p40 consensus sequence obtained from GenBank.

Furthermore,animal contact was reported in seven bipolar depression cases. 101 cases were urban and 24 rural area residents.

The control group consisted of the same number of sex and age-matched healthy individuals. The selected control group were interviewed and examined and had neither animal contact nor psychiatric disorders in their family. BDV RNA was detected in one 44 years old woman with bipolar depression only. There was no animal contact or psychiatric disorder history in her family. None of the control group samples showed BDV-RNA.

PCR product of the unique BDV positive case was then sequenced to draw the phylogenic tree (Figure 1). The P40 sequence has submitted to GenBank with the accession number of MF448520. The sequencing result has shown similarity with sequences recorded in the GenBank corresponding to human isolate BDV genome.

As demonstrated in figure 2, 21 variations have observed at the nucleotide level. Only 3 substitutions have found at the amino acid level. The substitutions were V91C, H92S, and E262D.

Discussion

Epidemiological studies have been shown that the Borna virus may be a possible etiology for a psychiatric disorder.

Several studies have conducted to examine the relationship between the BDV infection and human psychiatric disorders. The genome of this virus was isolated from psychiatric disorder patients in some countries. Therefore, the present study was designed to explore the presence of Borna virus infection in our country.

Several factors may affect the study of the Borna virus-induced psychiatric disorders including age, gender, type of disease and the viral genome.

Kubo et, al in Japan, conducted a research for BDV-p24 in 54 patients with psychiatric disorders and 27 healthy control. they have reported a prevalence of 44% of the Borna virus RNA presented in the patients that 24% in healthy control group (17). In Japan, Tusji et. al, have reported no positive BDV infection in 229 patients with psychiatric disorders, using RT-PCR and western blot(18). However, Fukuda et. al have found BDV-p24 and p40 RNA in 9% of patients with a mood disorder (4 out of 45) and 2% in control blood donors (1 out of 45) (19).Naks et. al (2004 -2007) have studied 198 mental disorder patients in Korea using the nested RT-PCR method. They have found no trace of viral p40 RNA in any samples (20).

Same as the present study, Miranda et. al in Brazil have tested BDV-p24 RNA in PBMC samples of 30 patients (19 with the behavioral disorder and 11 psychiatric disorder) and 30 controls. They have found the BDV-p24 RNA in 33% (10 out of 30) of patients and 13% of the control group (21).

Henrich et, al in Germany have evaluated titer of specific antibodies in three groups of psychiatric disorder, schizophrenia, and affective disorder. 43.5% of 94 patients were reported to be seropositive for BDV, where all of the control group samples were negative (22).

Conclusion

Our results could not conclude significant prevalence of BDV genome among psychiatric patients or healthy individuals. Therefore, further serological tests are required to confirm the results of this study.

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Author Declaration

The authors declare that there is no conflict of interest regarding the publication of this paper.

Authorship

Based on the ICMJE, all the authors have contributed in the following parts of the work:

1- The conception or design of the work; or the acquisition, analysis, or interpretation of data.
2- Drafting the work or revising it critically for important intellectual content.
3-Final approval of the version to be published.
4- All authors are agreed accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Tomonaga K, Kobayashi T, Ikuta K. [The neuropathogenesis of Borna disease virus infection]. Nihon rinsho. 2001;59(8):1605-13.
2. Ikuta K, Ibrahim MS, Kobayashi T, Tomonaga K. Borna disease virus and infection in humans. Front Biosci. 2002;7:d470-95.
3. Richt JA, Pfeuffer I, Christ M, Frese K, Bechter K, Herzog S. Borna disease virus infection in animals and humans. Emerg Infect Dis. 1997;3 (3):343-52.
4. Lipkin WI, Schneemann A, Solbrig MV. Borna disease virus: implications for human neuropsychiatric illness. Trends Microbiol. 1995;3(2):64-9.
5. Bautista JR, Rubin SA, Moran TH, Schwartz GJ, Carbone KM. Developmental injury to the cerebellum following perinatal Borna disease virus infection. Brain Res Dev Brain Res. 1995;90(1-2): 45-53.
6. Carbone KM. Borna disease virus and human disease. Clin Microbiol Rev. 2001;14(3):513-27.
7. Rybakowski F. Transmission of Borna disease virus as etiopathogenetic factor in schizophrenia and affective disorders. Psychiatr Pol. 1999;33(6): 947-58.
8. Waltrip RW 2nd, Buchanan RW, Summerfelt A, Breier A, Carpenter WT Jr, Bryant NL, et al. Borna disease virus and schizophrenia. Psychiatry Res. 1995;56(1):33-44.
9. Taieb O, Baleyte JM, Mazet P, Fillet AM. Borna disease virus and psychiatry. Eur Psychiatry. 2001;16(1):3-10.
10. Amsterdam JD, Winokur A, Dyson W, Herzog S, Gonzalez F, Rott R, et al. Borna disease virus. A possible etiologic factor in human affective disorders? Arch Gen Psychiatry. 1985;42(11): 1093-6.
11. Bode L, Ferszt R, Czech G. Borna disease virus infection and affective disorders in man. Arch Virol Suppl. 1993;7:159-67.
12. Planz O, Rentzsch C, Batra A, Batra A, Winkler T, Büttner M, et al. Pathogenesis of Borna Disease Virus: Granulocyte Fractions of Psychiatric Patients Harbor Infectious Virus in the Absence of Antiviral Antibodies. J Virol. 1999;73(8):6251-6.
13. Trifinopoulos J, Nguyen L-T, von Haeseler A, Minh BQ. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 2016;44(W1):W232-W5.
14. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10.

15. Knudsen B, Knudsen T, Flensborg M, Sandmann H, Heltzen M, Andersen A, et al. CLC Sequence Viewer. A/S Cb, version. 2011;6(2).
16. Letunic I, Bork P. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 2016;44(W1):W242-5.
17. Kubo K, Fujiyoshi T, Yokoyama MM, Kamei K, Richt JA, Kitze B, et al. Lack of association of Borna disease virus and human T-cell leukemia virus type 1 infections with psychiatric disorders among Japanese patients. Clin Diagn Lab Immunol. 1997;4(2):189-94.
18. Tsuji K, Toyomasu K, Imamura Y, Maeda H, Toyoda T. No association of borna disease virus with psychiatric disorders among patients in northern Kyushu, Japan. J Med Virol. 2000;61(3): 336-40.
19. Fukuda K, Takahashi K, Iwata Y, Mori N, Gonda K, Ogawa T, et al. Immunological and PCR analyses for Borna disease virus in psychiatric patients and blood donors in Japan. J Clin Microbiol. 2001;39(2):419-29.
20. Na K-S, Tae S-H, Song J-w, Kim Y-K. Failure to detect Borna disease virus antibody and RNA from peripheral blood mononuclear cells of psychiatric patients. Psychiatry Investig. 2009;6(4): 306-12.
21. Miranda HC, Nunes SO, Calvo ES, Suzart S, Itano EN, Watanabe MA. Detection of Borna disease virus p24 RNA in peripheral blood cells from Brazilian mood and psychotic disorder patients. J Affect Disord. 2006;90(1):43-7.
22. Heinrich A, Adamaszek M. Anti-Borna disease virus antibody responses in psychiatric patients: long-term follow up. Psychiatry Clin Neurosci. 2010;64(3):255-61.