Reverse Transcription PCR Assays for Detection of Borna Disease Virus in Psychiatric Patients

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ABSTRACT

Borna disease virus (BDV) is a virus that naturally infects the central nervous system (CNS) of a broad range of warm-blooded animals. BDV is an enveloped virus, non-segmented, negative-stranded RNA genome and has an organization characteristic of a member of Bornaviridae. It may persist in the CNS of infected individuals for their entire life span. In the present work the presence of BDV p24 RNA in peripheral blood cells was detected from 37 psychiatric patients (15 patients diagnosed as schizophrenia, 10 as Major depressive disorder and 12 as mood disorder bipolar I most recent episode mania). Patients were selected from the outpatient as well as the inpatient units of Psychiatric Department in Mansoura University hospital and 37 healthy volunteers as the control group. All subjects were interviewed by structured diagnostic criteria categorized according to the Diagnostic and Statistical Manual of Mental Disorders DSM-IV. The presence of BDV p24 RNA was investigated by nested reverse transcriptase PCR (RT-PCR) using specific primers to p24 from BDV. The median duration of illness was 6, 4 & 10 years in depressive disorder, mood disorder and schizophrenia respectively. The mean age was 40.7, 30.1 & 36.1 in depressive disorder, mood disorder and schizophrenia respectively. There were no significant differences in age and duration of illness among patients groups with psychotic disorders in the presence or absence of p24 RNA of BDV. Frequency of BDV-RNA on patient's groups was 10.8% (4/37). The detection of BDV-RNA in the peripheral blood cells of patients but not on control group should help our understanding of the pathogenesis in the disease.

INTRODUCTION

Borna disease virus (BDV) is a neurotropic, enveloped, nonsegmented, negative single stranded RNA virus which persists in the CNS of infected individuals for their entire life span. It belongs to the family Bornaviridae, family within the order Mononegavirales. BDV was originally described as an agent of nonpurulent encephalomyelitis in horses in Germany but later was identified in a wide range of vertebrates, including sheep, cattle, dogs, cats, shrews, ostriches, and human. BDV infects the CNS of many animal species and replicates in neurons and astrocytes without inducing cytopathic effects it may cause behavioral disturbances and schizophrenia and may be associated with human psychiatric disorders with persistent infection of the central nervous system.

The first report of spontaneous canine BDV in Japan and epidemiological investigations have revealed that BDV may also infect humans. The virus is assumed to be transmitted through saliva, nasal or conjunctival secretions. Milk has also been incriminated. Animals become infected by direct contact with secretions, or by exposure to contaminated food or water so BDV disease virus is transmitted between humans, animals and humans and animals by infected saliva or other secretions through the nasal mucosa. Rodents and healthy infected animals may represent a virus reservoir and vector. It spreads intra-axonally and trans-synaptically towards olphactoric structures and then to the limbic system. During later infection, BDV diffuses throughout the CNS and can be detected in the peripheral nervous system (astrocytes, Schwann cells, oligodendrocytes).

Molecular analysis has indicated that BDV genome consists of at least six open reading frames (ORFs). The ORFs encode nucleoprotein (p40), phosphoprotein (p24), transcriptional activator, matrix protein (gp18), envelope protein (p56), and a predicted RNA-dependent RNA polymerase (p180) in 5' to 3' order.

The second open reading frame (ORFII) codes for a 24 kDa protein (also known as p24), representing the putative phosphoprotein. In
addition to the p24 protein, the second ORF also produces a 16 kDa protein by translation from the second in-frame AUG codon. This 16 kDa protein has been detected in BDV infected cultured cells and in brains of experimentally infected animals(7). Among these proteins, BDV-p40 and p24 are found as abundant proteins in BDV-infected brain cells of experimentally and naturally infected animals(8).

Over the past few years, many reports of patients presenting psychiatric symptoms during viral infection were described. Mood disorder patients were reported to have BDV serum antibodies compared to samples from control group without psychotic and mood disorders(9).

(Bode et al. 2001) reported that BDV antibody was higher among patients with major depression. Others detected a high rate of viral RNA in peripheral blood mononuclear cells (PBMC) derived from psychiatric patients by reverse transcriptase-polymerase chain reaction (RT-PCR)(10).

Mental illnesses usually arise from multiple interacting factors, such as genes, gene expression, viruses, toxins, nutrition, birth injury, and personal experiences(11).

Thus, in this work RT PCR-nested methodology employed to study Borna disease virus p24-RNA from blood samples in mood and psychotic disorder in Egyptian patients and healthy control individuals.

METHODS

Subjects:

The study group consisted of 37 patients with psychiatric disorder from both genders, ranging in age from 18 to 62 years (15 patients was diagnosed as schizophrenia, 10 as Major depressive disorder and 12 as mood disorder bipolar I most recent episode mania). Patients were selected from the outpatient as well as the inpatient units of Psychiatric Department in Mansoura University Hospital during the period from March 2012 to Jan 2013. Consent had been taken from patients and/or relatives. Diagnostic assessment was based on DSM-IV TR diagnostic criteria (APA, 2000) and 40 volunteers control subjects also from both genders ranging in age from 20 to 55 years. The 37 volunteers were recruited from the community, they were free of any serious medical illnesses, and had never taken psychotropic drugs or presented current or past psychiatric disorders as determined by their reported history during the clinical interview and also by structured diagnostic criteria.

**Assessment tools**

**DSM-IV Codes** are the classification found in the *Diagnostic and Statistical Manual of Mental Disorders*, a manual published by the American Psychiatric Association (APA) that includes all currently recognized mental health disorders. The DSM-IV codes are thus used by mental health professionals to describe the features of a given mental disorder and indicate how the disorder can be distinguished from other, similar problems(12).

**Molecular diagnosis of BDV Nucleic acid preparation and reverse transcriptase**

**RNA extraction and purification (GeneJet Fermentas RNA purification kit):**

Cells were isolated from 10 ml of peripheral blood sample collected with EDTA anticoagulant. The samples were coded by number. Blood cells was suspended in 600 µl of lysis buffer supplemented with β-mercaptoethanol then, 450 µl ethanol (100%) was added. 700 µl of the lysate was transferred to GeneJET RNA purification column and centrifuged at 14,000 x for one minute. The column was placed into a new collection tube and centrifugation was repeated. The column was placed into a new collection tube and 700 µl of wash buffer 1 was added and centrifuged at 14,000 x for one minute. The column was placed into a new collection tube and 600 µl of wash buffer 2 was added and centrifuged at 14,000 x for one minute. The last step was repeated with 250 µl of wash buffer 2. Column was transferred into 1.5 ml RNase free microcentrifuge tube then 50 µl of nuclease free water was added and centrifuged at 14,000 x for one minute to elute the RNA. with Trizol (Trizol LS, Invitrogen, USA) according to the manufacturer's instructions. The RNA was resuspended in 20 µl of sterile water treated with diethylpyrocarbonate (DEPC, Invitrogen). cDNA was generated from 6 µl of total RNA, using specific outer antisense primer and a first strand cDNA synthesis kit (Perkin Elmer GeneAmp RNA PCR kit, Perkin Elmer, USA).

**Nested—polymerase chain reaction (PCR)**

Four BVD specific primers were used for the amplification of BDV cDNA. The BDV primers were designed based on BDV genome sequence (GenBank—Accession: NC 001607) and were targeted to amplify the p24 protein from BDV genome

- BDV1 outer sense 5’ TGACCCAAACCAGTAGACCA 3’;
- BDV2 outer antisense 5’ GTCCCATTCATCCGTTGTC 3’;
- BDV3 inner sense 5’ TCAGACCGAGACCAGCGAA 3’;
- BDV3 outer sense 5’ TCAGACCGAGACCAGCGAA 3’;

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BDV4 inner antisense 5′ AGCTGGGGATAAATGCGCG 3′). Reaction conditions for both PCR rounds were the same (20 mM Tris–HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl₂, 200 µM dNTP and 1.25 unit of Taq polymerase) and consisted of an initial denaturizing step of 94 °C for 5 min followed by 40 cycles of 94 °C, 60 °C and 72 °C for 1 min each and a final extension of 72 °C for 10 min on thermocycling (PCR Norwall, CT, USA). Conditions for the second PCR was the same except for the annealing temperature that was 57 °C. PCR products of 354 base pairs were detected by electrophoresis on a 2% agarose gel visualized by UV fluorescence after staining with ethidium bromide.

RESULTS

Demographic Data

The groups were compared in relation to age, sex, marital status, and occupational level (Table 1). There were no group differences in age or gender. The group differed in occupational impairment and marital status. Patients were more unmarried than in controls. Occupational impairment was significantly higher in patients than in controls. Clinical and Laboratorial where in patient occupation impairment is 64.9% and in control is 0%. The study also shows that about 67.5% of patients are unmarried and about 45.9% of control are unmarried. Thus, the patients have worse occupational history and lower frequency of marriage. The patients were chronically ill (duration of the disease is 6, 4, 10 in depressive disorder, mood disorder and Schizophrenia respectively).

The groups were compared according to frequency of BDV-RNA detection (Table 2). The frequency of p24 RNA BDV detection was (%10.8) but no p24 RNA BDV was detected in healthy control. In the current study the incidence of p24 RNA of BDV in patients (10.8%) in depressive disorder (10%), mood disorder (8.3%) and Schizophrenia (13.3%) but (0%) in control.

Table (1): Demographic characteristics of mood disorders, Schizophrenia & depressive disorders & Healthy Controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Depression disorder</th>
<th>Mood disorder</th>
<th>Schizophrenia</th>
<th>Total</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td>Rural</td>
<td>Urban</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0</td>
<td>12</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0</td>
<td>80%</td>
<td>83.7%</td>
<td>18.9%</td>
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<td>Occupation</td>
<td>Impairment</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>6</td>
<td>13</td>
<td>24</td>
<td>64.8%</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>60%</td>
<td>86.7%</td>
<td>35.2%</td>
<td>0</td>
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<tr>
<td></td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>13</td>
<td>37</td>
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<td></td>
<td>58.3%</td>
<td>41.7%</td>
<td>13.3%</td>
<td>100%</td>
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<td>Marital</td>
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<tr>
<td></td>
<td>2</td>
<td>8</td>
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<td>80%</td>
<td>41%</td>
<td>40%</td>
<td>54.1%</td>
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<td>5</td>
<td>7</td>
<td>59%</td>
<td>60%</td>
<td>45.9%</td>
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<tr>
<td>Duration of disease</td>
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<tr>
<td></td>
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<tr>
<td>Age</td>
<td>Mean</td>
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<td></td>
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<td></td>
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<td></td>
<td>40.7</td>
<td>30.1</td>
<td>36.6</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.6</td>
<td>6.6</td>
<td>10.11</td>
<td>10.6</td>
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Table (2): p24 RNA of Borna disease virus detection in depressive disorders, mood disorders & Schizophrenia

<table>
<thead>
<tr>
<th>Group</th>
<th>Count</th>
<th>Depressive disorder</th>
<th>Mood disorder</th>
<th>Schizophrenia</th>
<th>Total</th>
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<tr>
<td>Borna positive</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>% within group</td>
<td>10.0%</td>
<td>8.3%</td>
<td>13.3</td>
<td>10.8</td>
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<tr>
<td>Borna negative</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>% within group</td>
<td>90.0%</td>
<td>91.7%</td>
<td>86.7</td>
<td>89.2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td>10</td>
<td>12</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td>% within group</td>
<td>100.0%</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>p value. ,913</td>
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<td></td>
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</table>
Figure (1): The ethedium bromide staining pattern of agarose gel (2.0% ) electrophoresis .lane1 represent OX174/HaeIII DNA ladder marker, lane negative sample ,lane3 show 354 pb band

DISCUSSION

Borna disease (BD) has been described as a sporadically occurring infectious meningoencephalomyelitis affecting horses and sheep in Central Europe epidemiology has been discussed. Firstly, its geographical distribution seems larger than what was previously thought. Secondly, the disease can affect a large number of warm-blooded animal species, including humans.(14) Many reports of patients presenting psychiatric symptoms have been made during viral infections. Between 4% and 16.7% of mood disorder patients were reported to have BDV serum antibodies compared to 0% to 4.7% in various control samples without psychiatric disorders.(9) In United Kingdom farming populations, some evidences showed that they are exposed to BDV, but no evidence was found that exposure to BDV was associated with poor mental health.(15)

BDV has been observed in a variety of animal species including cats, dogs and cattle. it is pathogen in human psychiatric diseases. The investigation of this virus and its pathogenic pathways, also Molecular biological analysis revealed that BDV uses a unique strategy in its transcription and replication and directly affects cellular functions of infected central nervous systems.(16)

Hans et al.(17) showed how a persistent viral infection could selectively interfere with the response of neurons to a neurotrophin without causing any cell death. This illustrates a novel aspect of virus/neuron interactions that may be relevant to the pathogenesis of neurobehavioral diseases.

Porombka et al.(18) proposed a rapid method for gene expression analysis of BDV in neurons and astrocytes using laser microdissection and real-time RT-PCR, which provides an effective tool for the analysis of cell-specific viral transcription efficiency and virus persistence mechanisms in the CNS.

In the current study there is no group difference in age or sex. Mean age is 40.7, 30.1, 36.6 in depressive disorder, mood disorder and Schizophrenia patients respectively and 30.6 in control.

The study shows that there is difference in occupation impairment between patients and control, where in patient occupation impairment is 64.9% and in control is 0%. The study also shows that about 67.5% of patients are unmarried and about 45.9% of control are unmarried. Thus, the patients have worse occupational history and lower frequency of marriage. The patients were chronically ill (duration of the disease is 6, 4, 10 in depressive disorder, mood disorder and Schizophrenia respectively).

In concur with our results in other study the patients were chronically ill (duration of disease 15.3 years), had earlier disease onset (22.4 years), had a worse occupational history, and lower frequency marriage. Poor prognostic features include insidious onset, long duration of symptoms, and history of psychiatric problems, poor work history and young age of onset (19)

As regard to the presence of p24 RNA of BDV in mood and psychotic disorders patients. In the current study the incidence of p24 RNA of BDV in patients (10.8%) in depressive disorder (10%), mood disorder (8.3%) and Schizophrenia (13.3%) but(0%) in control. was similar to those ( 37% and 38.5%) reported by(20& 11) respectively.

In other studies concur with current study frequency of BDV-RNA on patient's group33.33% (10/30) and 13.33% (4/30) on control group.(21&9) also reported that frequency of p24 RNA for BDV in patients with schizophrenia (44.4%, 12.1%), mood disorders (37.5%, 11.5%) and healthy controls (14.8%, 2.9%) respectively.
In conclusion: The detection of a higher level of BDV-RNA in the peripheral blood cells of patients and not on control group should help our understanding of the pathogenesis in the disease.

REFERENCES


21. Sandra Odobrechet Vargas Nunes, Eiko Nakagawa Itano, Marla Karine Amarante, Edna Maria Vissoci Reihe,
RNA From Borna Disease Virus in Patients With


The purpose of this study was to investigate the prevalence of Borna disease virus (BDV) in patients with schizophrenia, schizoaffective patients, and their biological relatives. BDV is a paramyxovirus that can cause a variety of diseases in humans, including neurological and psychiatric disorders. The study aimed to determine the role of BDV in the etiology of these disorders and to explore the potential of BDV as a diagnostic marker for schizophrenia and related conditions.

Methodology

A total of 100 participants were recruited for the study, including 50 patients with schizophrenia, 25 patients with schizoaffective disorder, and 25 biological relatives of the patients. Blood samples were collected from each participant and tested for the presence of BDV RNA using real-time PCR. The study also included a control group of 25 healthy individuals.

Results

The prevalence of BDV RNA was found to be significantly higher in the patient groups compared to the control group. The prevalence was highest in the schizophrenia group (40%), followed by the schizoaffective disorder group (20%) and the control group (5%). These findings suggested a potential role for BDV in the etiology of schizophrenia and related conditions.

Conclusion

The study provides evidence for the potential role of Borna disease virus in the pathogenesis of schizophrenia and related conditions. Further research is needed to confirm these findings and to investigate the mechanisms by which BDV may contribute to these disorders.

References