

Rev Biomed 2004; 15:141-147.

Serological survey of Borna Disease Virus in schizophrenic patients from Yucatan, Mexico.

Original Article

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SUMMARY.

Background. Borna Disease Virus (BDV) is an enveloped, nonsegmented negative-strand RNA. BDV causes central nervous system disease in many vertebrate species that is frequently manifested by behavioral abnormalities. BDV naturally occurring infections were initially thought to affect only horses and sheeps in certain geographic regions of central Europe. Serological data obtained from different laboratories over the last 15 years, and recent molecular studies have shown that BDV can infect humans and might be associated with certain neuropsychiatric disorders. We report the first seroprevalence study of BDV in Latin America.

Method. Serum samples were obtained from 70 patients with a diagnostic of schizophrenia, and from 70 healthy volunteers, matched by age, sex and social-economical level. These samples were analyzed by two Western blot using different recombinant BDV phosphoprotein (P) in our laboratory and The Scripps Research Institute in San Diego CA, USA.

Results. The groups of schizophrenic patients were 47 males and 23 females. The average age was 40 years (range 17 to 73 years). All them borned in the Yucatan Peninsula. Diagnostic classification was made by discharge, according to ICD-10. The study showed a prevalence of serum antibodies to BDV-P of 21.43% (15/70), whereas no antibodies were found in sera from healthy subjects. No relation was found between seroprevalence and gender, age and type of schizophrenia.

Conclusions. This is in our knowledge the first study aimed at examining the prevalence of BDV in Latin America. Our results are consistent with a other reports, and support the possible association between antibodies to BDV and neuropsychiatric disease. There is need to continue with studies on this area in order to know the magnitude of this problem.

(Rev Biomed 2004; 15:141-147)

Key words: Borna Disease Virus, schizophrenia, Yucatan, Mexico.

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Received April 27, 2004; Accepted September 7, 2004.

This paper is also available at <http://www.uady.mx/sitios/biomedic/revbiomed/pdf/rb041531.pdf>

RESUMEN.**Estudio serológico del virus de la Enfermedad de Borna en pacientes esquizofrénicos de Yucatán, México.**

Antecedentes. El Virus de la Enfermedad de Borna (VEB) es un virus envuelto, con una cadena de RNA simple de polaridad negativa. VEB causa enfermedad a nivel del Sistema Nervioso Central en muchos animales vertebrados, que frecuentemente se manifiesta como anormalidades en la conducta. La infección silvestre con el VEB ocurre de manera natural. De hecho fue detectada primero en caballos y en ovejas en algunas regiones de Europa Central. Los resultados serológicos obtenidos de diferentes laboratorios en los últimos 15 años y recientemente con la biología molecular, muestran que el VEB puede infectar a los seres humanos y que también puede estar asociado a ciertos trastornos psiquiátricos. Reportamos el primer estudio de seroprevalencia del VEB en humanos de Latinoamérica.

Metodología. Muestras de suero fueron obtenidas de 70 pacientes con diagnóstico de esquizofrenia y otras 70 muestras de suero se tomaron de personas sanas que participaron de manera voluntaria al estudio y que fueron pareados por edad, sexo y nivel socio-económico. Las muestras de ambos grupos fueron procesadas para analizar por Western-blot en nuestro laboratorio y en The Scripps Research Institute en San Diego, CA USA. Como antígeno se utilizó a la fosfoproteína (P) obtenida de la expresión con GST-P.

Resultados. El grupo de pacientes esquizofrénicos, fueron 47 hombres y 23 mujeres con un promedio de edad de 40 años (rango de 17 a 73 años). Todos ellos nacieron en la Península de Yucatán. Para su clasificación el diagnóstico se basó en el ICD-10. El estudio mostró una prevalencia de anticuerpos en los sueros contra la proteína GST-P de 21.43% (15/70), mientras que no se encontraron anticuerpos en el suero de las personas sanas. No se encontró relación entre el género, la edad y tipo de esquizofrenia y la presencia de anticuerpos contra VEB.

Conclusiones. Hasta donde sabemos, éste es el primer estudio en Latinoamérica que se lleva a cabo

para detectar anticuerpos contra el VEB en seres humanos. Nuestros resultados son semejantes a lo reportado en la literatura y apoyan la posible asociación entre la presencia de anticuerpos contra el VEB y los trastornos psiquiátricos. Sin embargo, se necesita continuar con más estudios para conocer si es realmente un problema de salud y cual podría ser su magnitud. (*Rev Biomed 2004; 15:141-147*)

Palabras clave: Virus de la Enfermedad de Borna, esquizofrenia, Yucatán, México.

INTRODUCTION.

Borna Disease Virus (BDV) is an enveloped, nonsegmented negative-strand (NNS) RNA virus with a genome of approximately 8.9 kb, with a characteristic gene organization of mononegaviruses. However, based on its unique genetical and biological features BDV is considered in the order of *Mononegavirales*, Family *Bornaviridae*, Genus *Borna Disease Virus* (1).

BDV causes central nervous system (CNS) disease in many vertebrate species that is frequently manifested by behavioral abnormalities (2, 3). BDV naturally occurring infections were initially thought to affect only horses and sheeps in certain geographic regions of central Europe (3, 4), causing Borna Disease (BD) which is frequently a fatal immune-mediated neurologic disorder. However, naturally infected animals of different species have been now documented, suggesting that the natural host range of BDV, as well as its geographic distribution and prevalence may have been underestimated (5-8). Experimentally, BDV has a wide range of hosts from birds to rodents and non-human primates (3, 9-11). Genetic factors, immune status and age of the host, together with viral factors determine the incubation period, and are important for the heterogeneity of the symptoms and pathology associated with BDV infection (3, 5, 9, 12). Immune-mediated neuronal damage is responsible for the clinical symptoms of classic BD (4, 9, 13). In contrast, the mechanisms whereby BDV causes CNS disturbances in the

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absence of encephalitis remain largely unknown (3). A recent review summarized cross-sectional and longitudinal data which correlate mood disorders (14).

Serological data obtained from different laboratories over the last 15 years, and recent molecular studies have shown that BDV can infect humans and might be associated with certain neuropsychiatric disorders (6-8, 15-19). Therefore, the importance of a survey aimed at determining the prevalence of BDV in different geographic regions across the world. In this study we report the seroprevalence of BDV in schizophrenic patients and healthy control individuals from Yucatan, Mexico. This is, to our knowledge, the first study reporting the prevalence of BDV in Latin America.

MATERIALS AND METHODS.

Subjects.

Serum samples were obtained from 70 patients with a diagnosis of schizophrenia. These patients were admitted for treatment at the Psychiatric Hospital of Yucatan in Mexico. The groups of schizophrenic patients were 47 males and 23 females. The average age were 40 years old (range: 17 to 73 years). All them borned in the Yucatan Peninsula. Diagnostic classification was made by discharge, according to ICD-10 (20). Serum samples from 70 healthy volunteers matched by age, sex and social-economical level were used as controls.

The samples were obtained in accordance with the guidelines of the Declaration of Helsinki. The protocol of this study was approved by the Human Ethics Committee of Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", Universidad Autonoma de Yucatan, Mexico.

Western blot.

A total of 140 serum samples (70 patients and 70 healthy volunteers) were analyzed for the presence of antibodies to the BDV-P antigen by Western blot. Briefly, BDV full-length P from BDV strain He80 was received by the Scripps research Institute and the sequence was cited (21) and cloned in pGEX-5X-3, was expressed as a fusion protein with the glutathione S-transferase (GST) protein. GST alone was used as

a negative control. GST and GST-P recombinant proteins were produced in *E. coli* DH5, and purified by affinity chromatography using glutathione-sepharose 4B according to the manufacturer's instructions (Pharmacia Biotech AB, Uppsala, Sweden). Purified GST and GST-P proteins were separated by 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (22). After transfer, unspecific binding sites were blocked by incubation of nitrocellulose membranes in TBST-milk (10 mM Tris-HCl pH 8.0, 150 mM NaCl, and 2% Tween-20, and 1% nonfat milk) at 4°C overnight, and the membranes were cut in 0.5 cm strips. Each human serum sample was diluted 1:25 in blocking buffer and was incubated overnight at 4°C with one strip containing GST-P recombinant protein, and one strip as negative control containing GST alone. Rabbit polyclonal serum to BDV-P, and the corresponding pre-immune rabbit serum was used as external positive and negative controls, respectively. Membranes were washed five times in TBST and incubated for 1 hour at 25°C with the appropriate secondary antibody (horseradish peroxidase-conjugated goat anti-human or goat anti-rabbit IgG), diluted 1/500 in blocking buffer. After three washes in TBST, bound antibodies were revealed with diaminobenzidine in the presence of hydrogen peroxide.

Eight positive serum samples for BDV and ten serum samples from healthy volunteers were independently analyzed for BDV antibodies by Western blot at the Scripps Research Institute, La Jolla CA, USA, using recombinant BDV phosphoprotein (P) as a target.

Statistical Analysis.

Group comparisons were carried out using the Chi-square and Fisher tests. Comparison between sex groups and type of schizophrenia among the patient's population were done using the Mantel-Haenszel corrected Chi-square test, using the Epi-Info 6.04b software free from CDC. The adopted level of significance was 0.05

RESULTS.

In this study we described for the first time the prevalence of serum antibodies to BDV in schizophrenic patients, and healthy control individuals, in Yucatan, Mexico. The patient population examined included mostly seriously ill individuals. Examination of clinical records indicated that all patients analyzed here had an initial clinical onset of schizophrenia at least three years before their blood samples were collected. So, for this study range of illness was 3 to 22 years, with an average of 8 years. Two patients had been diagnosed with schizophrenia more than 20 years before prior sample collection for this study. The majority of the patients, 63(90%) were classified in the low social-economical status; all the patients were homeless and had been hospitalized multiple times (from 3 to 20 times, with an average of 10 times) due to their lack of compliance with the drug treatment prescribed. The most frequent diagnosis (90% of cases) was paranoid schizophrenia (ICD-10, F20.0). All these cases presented mixed signs of positive and negative manifestations. Six of the 70 cases (8%) were diagnosed with hebephrenic schizophrenia (ICD-10, F20.1), and the remaining patients corresponded to catatonic schizophrenia (ICD-10, F20.2).

Fifteen of seventy (21.43%), sera from

schizophrenic patients showed specific antibodies to BDV-P. None of the healthy controls showed seroreactivity. This result indicated a significant difference in BDV seroprevalence between these two groups ($p < 0.0001$, Mantel-Haenszel corrected Chi-square). BDV seropositive cases were similarly distributed among males and females (9/52 males against 6/18 females, $p=0.18$) (table 1) Our results showed that paranoid schizophrenia was diagnosed in 89.99% of the patients, and we only found antibodies against BDV-P in them.

Eight positive and five negative sera originally characterized in Merida laboratory were also independently analyzed by Western blot at The Scripps Research Institute in La Jolla California at Dr. Juan Carlos de la Torre's laboratory. Results obtained in La Jolla's laboratory were identical to those obtained in Merida, Mexico.

DISCUSSION.

Different numbers of BDV seroprevalence in humans with neuropsychiatric disorders had been previously reported (15, 23-25). These discrepancies are likely related to the absence of methodological standardisation on viral genomes, standardized serologic tests and the technical difficulties by the

Table 1
Comparison of results obtained using the ICD-10 classification with the positive and negative schizophrenic patients, to BDV-P.

Diagnosis (ICD-10) (n=70)	Sex	Cases (%)	BDV-P + (%)
Paranoid schizophrenia (F20.0)	M	47 (67.14)	9 (19.1)
	F	16 (22.85)	6 (37.5)
Hebephrenic schizophrenia (F20.1)	M	4 (5.71)	0
	F	2 (2.85)	0
Catatonic schizophrenia (F20.2)	M	1 (1.45)	0
	F	0	0

ICD= International Classification of Diseases 1992.

M= Male

F= Female

BDV-P = Phosprotein of Borna Disease Virus

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frequency of low antibody titers to BDV in human sera, and possible controversy in *Bornavirus* research (14) with the necessary non-reproducibility of published data on BDV (26).

The finding of BDV seropositives among healthy control individuals is consistent with the results of other seroepidemiological studies, where is reported BDV seroprevalences from 0% to 4% in the healthy control individuals examined (16, 27-29).

This is to our knowledge, the first study aimed to examining the prevalence of BDV in Latin America. Our results are consistent with other reports on the distribution of BDV, and support its possible association with certain neuropsychiatric diseases. However, this antibody-based prevalence may be far too low when compared to recent studies detecting up to 30% infections in the healthy population by use of other markers (14).

The natural reservoir of BDV is unknown and there is only very limited knowledge about the epidemiology and circulation of BDV in nature (3, 4, 7, 9, 14). The majority of the schizophrenic patients studied belong to a low socio-economic level status. This condition and the behavioral abnormalities of patients with schizophrenia might contribute to a significant higher risk of exposure to infectious agents present in the environment. This, in turn, might have influenced the prevalence of BDV among psychiatric patients studied here. Nevertheless, the seroprevalence (21.43%) of BDV found in schizophrenic patients from Yucatan is similar to that described in other studies involving patients from other world geographic regions, but with mood disorder among others (7, 16, 24, 30). In the case of schizophrenia, two studies report a lower seroprevalence than our results (4% and 8%) (31, 32), and an other study made in Japan reported 21.9% of seroprevalence against the protein p10 of BDV (33).

The differences between these results could be explained because the reactive human sera appear to exhibit frequently low avidity for BDV antigens (14). The validity of a diagnostic test can be determined by measuring the rate of sensitivity and specificity, and, in general, the more sensitive the test the lower the

specificity and viceversa. On the other hand, the detection of antibodies by ELISA must be influenced by the presence of antigen and immune complex. In this way the ELISA used by Bode would be useful for future studies (14, 34).

According to our results, it's important to extend this type of epidemiological study to patients with other psychiatric diseases, and to look for reservoirs or infected animals (horses, bovines and sheep) in the Yucatan, to determine the magnitude of BDV infections.

ACKNOWLEDGEMENTS.

This work was supported by a grant from CONACYT No. 27882M. We are also grateful to Zaggy Gabriel Puc Cetz and Alicia Zapata-Peraza MD, for their technical assistance. We thank María García Miss PhD for English correction.

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