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Full Length Research Paper

Investigating the Role of Borna Disease Virus (BDV) Markers in Patients Diagnosed with Schizophrenia

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In human, certain serological and molecular trials estimated an association between psychiatric diseases, particularly schizophrenia, mood disorders and borna disease virus (BDV). The purpose of this study was to investigate whether BDV has association with etiopathogenesis of schizophrenia. A total of 207 patients who were diagnosed with residual schizophrenia and 51 patient control grop with 134 healthy control group were included in the study. RNA of borna disease virus (BDV p24 and p40) in the peripheral blood was investigated by using real-time RT-PCR. A highly significant difference was detected between patient and healthy control group cases in terms of BDV IgG seropositivity (p<0.001). The OD value of IgG positivity was established as significantly high in the patient group compared to patient control group in the age groups of 35-50 and 51-65 (p<0.001). No significant differences were detected in other groups. The OD value of IgG positivity was established as significantly high in the patient group compared to healthy control group in the age groups of 35-50 and 51-65 (p<0.001). BDV- RNA (p24 and p40) was not detected in those 102 cases having BDV-IgG seropositivity.

Key words: Borna disease virus, schizophrenia, ELISA.

INTRODUCTION

Being a non-cytolytic, non-segmented and neurotrophic virus having a characteristic of RNA virus with negative polarity, borna disease virus (BDV) leads to immune system-mediated infections of central nervous system (CNS) in certain vertebrates and may exhibit different clinical symptoms depending on host and virus factors. CNS infections may be permanent in animals and accordingly, brain cell functions may change and neuron development and behavioural disorders may occur. In human, certain serological and molecular trials estimated an association between psychiatric diseases, particularly schizophrenia disease and mood disorders, and BDV (Taieb et al., 2001).

In these etiopathogenesis models revealed by sporadic case or epidemiological observation-based case series, match case-control or cohort-based seroepidemiological, seroepidemiological and molecular-based case series, recent prospective clinical cohort and clinically controlled experimental studies and experimental animal studies, host factor and premature birth characterized by genetic and neurodevelopmental disorders, low weight birth, environmental factors such as contact with cats, migration, dearth and, in particular, microorganisms are suggested as the most attractive and frequently emphasised common points (Yolken et al., 2006; Pearce et al., 2003; Brown et al., 2006). Over these microorganisms, the role of viral infections in the etiology

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of schizophrenia is a subject having been discussed for many years and for which quite serious studies have been performed. Recently, there has been an increase in the number of trials conducted for the viruses due to their latent and neurotrophic affinities (Taieb et al., 2001).

The purpose of this study is to investigate whether BDV has any role in the etiopathogenesis of schizophrenia by detecting the Ig G antibodies produced against BDV P40 antigen by using ELISA method and the RNA of borna disease virus (BDV) in the peripheral blood mononuclear cells (PBMNC) with real-time reverse transcription polymerase chain reaction (RT-PCR) in the serum of patients diagnosed with schizophrenia hospitalized in the Bakirköy Mental Hospital Research and Education Centre and Istanbul University (IU) Cerrahpaşa Medical Faculty (CMF) Psychiatry Clinic, and to discuss this possible relation in the light of Hill's criteria.

METHODOLOGY

This cross-sectional, case-controlled trial was conducted in July 2007-December 2008 by IU CMF Department of Microbiology and Clinic Microbiology and IU Veterinary School Department of Virology along with Istanbul University Cerrahpaşa Medical Faculty, Department of Psychiatry and TR Ministry of Health, Department of Psychiatry, Bakirköy Mental Hospital.

Patient group (PG)

In July 2007-December 2008, 207 patients who were diagnosed with residual schizophrenia as per DSM IV TR (Diagnostic and Statistical Manual of Mental Disorders) diagnostic measures (Bode et al., 2003) and hospitalized in Istanbul University Cerrahpaşa Medical Faculty Psychiatry service and Bakirköy Mental Hospital service were included in the study. 79 (38.16%) of these patients were male whereas 128 (61.84%) of them were female.

Patient control group (PCG)

A total of 131 persons, 51 of whom were male and 80 of whom were female, were included in the study who had similar (match) age and sex characteristics with the patient group cases and were diagnosed with non-schizophrenic psychiatric disorders (depression, bipolar disorder, obsessive compulsive disorder).

Healthy control group (HCG)

A total of 137 persons (54 males and 83 females) with similar (match) age and sex characteristics with the patient group cases who did not have schizophrenia history in oneself or in the family were enrolled in the study among people who applied to I.U. Cerrahpaşa Medical Faculty Department of Microbiology and Clinic Microbiology, Serology/ELISA laboratory for any reason.

Blood collection

In our study, plain blood samples without anti-coagulant agent were collected by using ELISA method in order to detect BDV Ig G and 10 ml was transferred into sterile, vacuum-tubes (biochemical tubes with yellow caps containing gel) without anti-coagulant agent. They were centrifuged at 3000 rpm for 5 min and their serums were obtained and stored at -70ºC until usage. Fresh full blood samples with EDTA [ethylene diamine tetra acetic acid] were collected for the determination of BDV-RNA by using real-time reverse transcription polymerase chain reaction (Real-Time RT-PCR) and 5 ml blood was transferred into sterile tubes containing EDTA, handled under cool conditions and RNA extraction was performed with full blood on the same day.

Detection of IgG to Borna Disease Virus in Sera by ELISA using recombinant p-40

Synthesis of recombinant p40 protein

The *p40* gene of a BDV isolate of horse origin was cloned and expressed using the *Baculovirus* expression system as described previously. The purity and specificity of *p40* were tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting (Bode et al., 2003; Yilmaz et al., 2002).

Optimisation of Enzyme-Linked Immunosorbent Assay (ELISA)

Two-fold dilutions of recombinant *p40*, starting from 10 µg/mL, were used to coat the ELISA plates. Positive test sera were used at dilutions from 1/20 to 1/1280. These experiments showed the optimal concentration of p40 proteins as 5 µg/mL and the optimal dilution of serum as 1/40. Human sera were therefore tested at this dilution.

Analysis of human sera for BDV p40 by ELISA

ELISA plates were coated with 5 µg/mL recombinant *p40* and incubated overnight at 4°C. After blocking with phosphate buffered saline containing 0.05% Tween 20 and 5% fat-free dry milk for 1 h at room temperature. 50 µl of positive control and study sera, diluted 1/40 in blocking buffer, were added to wells (1 h, room temperature). 50 µl of secondary antibody (alkaline phosphatase conjugated rabbit anti-human IgG) was added at a dilution of 1/1000 for 1 h of incubation at room temperature. All incubations were followed by 4 washes with phosphate-buffered saline containing 0.05% Tween 20. 50 µl of *p*-Nitrophenyl phosphate, 1 mg/mL in alkaline phosphatase buffer (0.1 M glycine, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4) was added to microplates and OD was measured at 405 nm after 30 min by using an ELISA reader (SLT- Spectra). Each serum was tested in antigen-coated and non-coated wells to measure the background optical density. Sera that read at least 2-fold values compared with background were considered positive (Gungor et al., 2005).

Detection of BDV-RNA in human blood by using Real-Time RT-PCR

Viral RNA was extracted from whole blood using a commercial kit as described by the manufacturer (QIAMP-RNA Blood Mini Kit, Qiagen). The RNA concentration was determined by Nanodrop spectrophotometer (Thermoscientific, Nanodrop 1000). One blood sample (giving negative signal by SYBR Green assay) was spiked with 10 µl of a 10-fold diluted plasmid containing Borna Disease virus *p24* and *p40* in order to check whether the extraction procedure was working.

Forward (5' GGACCTCCTCTACGCATCAAC'3) and reverse primers (5' TGGGA GCTGGGGATAAATG 3') were used for p24.

Table 1. Distribution of the cases in the study group as per average of age and sex.

Demographic details	Studying groups		
	Patient group	Patient control	Healthy control
Age average (min-max)	45.09 (22-65)	41.08 (25-65)	43.04 (22-65)

Patient x Patient control p>0.05; Patient x Healthy control p>0.05.

Sex	Patient		Patient Control		Healthy Control	
	n	%	n	%	n	%
Female	128	61.84	80	61.07	83	60.58
Male	79	38.16	51	38.93	54	39.42

Patient x Patient control p>0.05; Patient x Healthy control p>0.05.

Forward (5'CCACGAAGC TTTCCCAATC'3) and reverse primers (5'GCAAGCTGGGTTTCCTTGAC'3) were used for P40 of BDV in real time RT-PCR.

All primers were designed using Primer 3. M-Fold was then used to check for regions of amplicon secondary structure in the primer binding sites. They were synthesized by Qiagen.

Reverse transcription was performed using a commercial kit (Qiagen, Sensiscript RT kit, Cat No:205213). In a total of 20 µl reaction, 2 µl of 10X buffer, 2 µl dNTPs, 1 µl enzyme (Sensiscriptase), 1 µl primers, 5 µl of RNA template (about 300 ng) and 9 µl nuclease-free water (Qiagen) was mixed with and incubated at 37°C for 1 h using a thermal cycler (Biorad Chromo4). The cDNA was kept at -70°C until required.

Real-time PCR was optimised as described by Peters et al. (2004). For real-time PCR, a total volume of 50 µl was prepared consisting of 25 µl Hotstar Taq Master Mix (Qiagen), 2 µl 25 mM $MgCl_2$ (Qiagen), 0.5 µl F primer (10 pmol/ µl), 0.5 µl R primer (10 pmol/µl), 1 µl SYBR Green (1 in 1000 dilution), 16 µl nuclease free water and 5 µl cDNA. The mixture was placed in a termal cycler (Biorad, Chromo 4) and the polymerase activated by incubation at 95°C for 15 min. The mixture was then cycled at 95°C for 10 s and 60°C for 15 s for 45 cycles. In order to determine the melting curve, the thermal cycler was programmed to read the fluorescence from 60 to 100°C in 1°C increments every 10 s. Negative controls for real-time RT PCR included RNA extracted from nonspiked blood samples (giving negative real-time RT PCR signals on repeated tests) and reaction mixture with nuclease free water in place of template. Positive real-time RT PCR controls were performed by using plasmid (p24 and p40) and cDNA from blood sample spiked with plasmid (Dr. Christopher Helps, University of Bristol, Veterinary School, Langford House, Langford, United Kingdom). After the real- time RT PCR, the products (if positive) and positive control were also visualized by agarose gel (1.5%) electrophoresis.

Statistical analysis

The data obtained in the trial were studied in SPSS 17.00 statistics programme. The data were evaluated by using logistic regression test as per forward conditional model along with Chi square and Fisher exact tests. p<0.05 was considered as significant.

RESULTS

Enzyme-linked ımmunosorbent assay (ELISA)

Our study was conducted on a total of 475 cases, 207 (128 females, 79 males and age range 22-65, average 45.09±12.63 years) of which were schizophrenia cases, 131 (80 females, 51 males and age range 25-65, average 41.08±10.03 years) of which were patient control group cases and 137 (83 females, 54 males and age range 22-65, average 43.04±13.52 years) of which were healthy control group cases. The patient group is similar (match) with patient control and healthy control groups with regards to age and sex characteristics (p>0.05) (Tables 1 and 2).

When PG and PCG cases were compared in terms of demographics, the ratio of contact with cats was found significantly higher in the patient group cases than that in the patient control group (p<0.001). The ratio of primary school graduation and illiteracy, and the ratio of being single with respect to marital status were established as highly significant compared to the patient control group (p<0.001). A highly significant difference was established between PG and PCG with respect to family history of schizophrenia and psychiatric disorder, that is, the ratio for the disease history was determined as higher in the patient group (p<0.001). Additionally, similar statistical results were obtained when PG and HCG cases were compared in terms of these demographical data (p<0.001) (Table 3).

When patient (31.88%) and patient control group (12.98%) cases were compared with respect to BDV IgG positivity, a highly significant difference was detected between them, (p<0.001). A highly significant difference was detected between patient and healthy control group cases in terms of BDV IgG seropositivity (p<0.001).

When the cases in patient and patient control group were compared with regards to the distribution of BDV IgG seropositivity on the basis of different age groups, no significant differences were detected in groups. When the cases in patient and healthy control group were compared with regards to the distribution of BDV IgG seropositivity on the basis of different age groups, the value of IgG positivity was established as significantly high in the patient group compared to healthy control group in the age group of 51-65 (p<0.001). No significant differences were detected in other groups (Table 4).

Table 2. Distribution of the cases in patient, patient control and healthy control groups as per age and sex.

Studying groups	Age groups	Sex				Total	
		Female		Male			
		n	%	n	%	n	%
Patient group	22-34	43	20.77	8	3.86	51	24.64
	35-50	55	26.57	32	15.46	87	42.03
	51-65	30	14.49	39	18.84	69	33.33
	Total	128	61.84	79	38.16	207	100
Patient control group	22-34	21	16.03	13	9.92	34	25.95
	35-50	32	24.43	21	16.03	53	40.46
	51-65	27	20.61	17	12.98	44	33.59
	Total	80	61.07	51	38.93	131	100
Healthy control groups	22-34	23	16.79	20	14.60	43	31.39
	35-50	32	23.36	16	11.68	48	35.03
	51-65	28	20.44	18	13.14	46	33.58
	Total	83	60.59	54	39.42	137	100

Table 3. Comparison of demographics for the cases of patient group and patient control group and healthy control group.

Demographics	Patient n (%)	Patient control n (%)	Healthy control n (%)	Statistical value
Sex				
Male	79 (38.16)	51 (38.93)	54 (39.42)	p>0.05(a)
Female	128 (61.84)	80 (61.07)	83 (60.58)	
Total	207	131	137	
Age				
25-34	51 (24.64)	34 (25.95)	43 (31.39)	p>0.05(a)
35-50	87 (42.03)	53 (40.46)	48 (35.04)	p>0.05(a)
51-65	69 (33.33)	44 (33.59)	46 (33.58)	p>0.05(a)
Total	207	131	137	
History of cat contact				
Yes:	122 (58.94)	8 (6.11)	13 (9.49)	p<0.001(b)
No:	85 (41.06)	123 (93.89)	124 (90.51)	
Duration of disease	8-57 years		-	
Education status				
Illiterate	39 (18.84)	13 (9.92)	7 (5.10)	p<0.05(b)
Primary school	114 (55.07)	33 (25.19)	14 (10.21)	p<0.001(b)
High school	37 (17.87)	51 (38.93)	53 (38.69)	p<0.001(b)
College	17 (8.21)	34 (25.95)	63 (45.99)	p<0.001(b)
Marital status				
Single	143 (69.08)	30 (22.90)	34 (24.82)	p<0.001(b)
Married	23 (11.11)	68 (51.91)	77 (56.20)	p<0.001(b)
Divorced	41(19.81)	33 (25.19)	26 (19.85)	p>0.05(a)

Table 3. Contd.

Psychiatric disorder in at least one relative				
Yes	93 (44.93)	28 (21.37)	22 (16.06)	p<0.001(b)
No	114 (55.07)	103 (78.63)	115 (83.94)	
History of schizophrenia in the family				
Yes	64 (30.92)	3 (2.29)	2 (1.46)	p<0.001(b)
No	143 (69.08)	128 (97.71)	135 (98.54)	
Underlying disease				
Diabetes	4 (1.93)	4 (3.05)	3 (2.19)	p>0.05(a)
Cardiac problems	8 (3.86)	9 (6.87)	7 (5.11)	p>0.05(a)

a: Patient group X Patient control group, Patient group X Healthy control group.
b: Patient group X Patient control group, Patient group X Healthy control group.

Table 4. Distribution of BDV IgG seropositivity in patient, patient control and healthy control groups as per sex and age groups.

Age groups	Patient group (%)	Patient control group (%)	OR	CI (%95) (lower-upper)	X^2	P
25-34	10/51(19.61)	6/34 (17.65)	0.879	0.287-2.694	0.051	0.527
35-50	21/87 (24.14)	6/73 (8.22)	2.937	1.125-7.663	0.028	0.489
51-65	35/69 (50.72)	5/24 (20.83)	2.435	0.856-6.929	0.095	0.534
Sex						
Male	32/79 (40.51)	8/51 (15.69)	3.660	1.521-8.807	8.963	0.002
Female	34/128 (26.56)	9/80 (11.25)	2.853	1.286-6.330	7.039	0.006
Age groups	**Patient group (%)**	**Healthy control group (%)**	**OR**	**CI (%95) (lower-upper)**	**X^2**	**P**
25-34	10/51 (19.61)	6/43 (13.95)	1.504	0498-4.543	0.528	0.328
35-50	21/87 (24.14)	5/48 (10.42)	2.736	0.959-7.806	3.745	0.06
51-65	35/69 (50.72)	8/46 (17.39)	4.890	1.472-7.312	9.235	0.001
Sex						
Male	32/79 (40.51)	6/54 (11.11)	5.447	2.085-14.229	13.581	0.000
Female	34/128 (26.56)	13/83 (15.66)	1.948	0.957-3.962	3.455	0.041

OR: Odds ratio, CI: confidence interval.

When the demographics (marital status, family history, education, contact with cats, age groups) and serological test (BDV-Ig G) data of the patient group and control group cases were examined using logistic regression method; marital status, family history, education, contact with cats were detected as significant in the age group of 51-65, that is, they were correlated with schizophrenia (p<0.001). (Table 5)

Real-Time RT-PCR

Using p24 and p40 positive controls (plasmid and blood spiked plasmid) the real-time PCR for BDV p24 adn p40 showed a threshold cycle (*CT*) value of 28-29 and a 100 bp amplicon by agarose gel. No positive signal was obtained using SYBR Green RT-PCR for BDV p24 and p40 in any of the blood of schizophrenic patients, controls and healthy controls. The melting curves of the positive controls (plasmid and blood spiked plasmid) were measured as 83-84°C in all cases. No *CT* value was obtained with the negative control or any test sample, indicating either a low amount or absence of BDV p24 and p40. The *CT* values of negative samples spiked with plasmids were similar to positive control (corresponding dilutions of plasmid).

Table 5. The results of parameters which may be analysed by logistic regression as per forward conditional model.

Parameters	B	SE	Wald	d.f.	OR	95.0 % CI		p
						Lower	Upper	
Marital status	0.529	0.167	9.995	1	1.697	1.223	2.355	0.002
Family history	-0.972	0.270	12.995	1	0.378	0.223	0.642	0.000
Education	0.740	0.149	24.801	1	2.096	1.566	2.804	0.000
Contact with cats	-2.799	0.357	61.638	1	0.061	0.030	0.122	0.000
Age groups (51-65)	0.905	0.204	19.758	1	2.471	1.658	3.682	0.000
Constant	-1.083	0.380	8.098	1	0.339			0.004

B: beta regression coefficient; SE: standard error; d.f. = degree of freedom; OR: Odds ratio, CI: confidence interval.

The amplicon sizes of 91 bp for p24 and 125 bp for p40 in positive controls were observed on agarose gel electrophoresis. The reaction efficiencies were 100% for p24 and 95% for p40 when tested on dilutions of plasmids carrying the *p24* or *p40* gene.

DISCUSSION

A variety of theoretical models was set forth for the etiopathogenesis of schizophrenia disease with a prevalence of 1% in the world having a course of clinical behavioural models such as detachment from reality (psychos), misperception, having false beliefs (delusion), abnormal thinking and restriction of emotions (blunting) and leading to major economical, social and mental distortions due to disorder of functions in both personal or family and social life (Pearce et al., 2003).

Recently, host biology has been the most attractive and most emphasized theory among these models which is based on the interaction of psychosocial status and environmental factors (microorganisms). According to this theory, in schizophrenia disease exhibiting various immunological abnormalities such as function disorders along with both the reduction in T cells and in the number of peripheral lymphocytes and the abnormal cellular and humoral reactivity for neurons and development of auto antibodies, these abnormalities are proposed to develop as the effects of a neurotoxic virus or an endogen autoimmune mechanism (Ferszt et al., 1999).

IL-1 beta, IL-6, TNF-alpha, TNF-gamma and T and B lymphocytes and up-regulations due to polymorphism are suggested to participate in the pathogenesis of schizophrenia and play a role in the sensitivity of the host against infection factors (Yolken et al., 2006; Henriquez et al., 2009).

Treponema pallidum bacteria, the Lyme factor *Borrelia burgdorferi*, *Toxoplasma gondii* protozoon and *Herpes* simplex virus type 1 and 2 (HSV1-2), Ebstein-Barr virus (EBV), Poliovirus, Mumps virus, Coxsackie B4, Human Immunodeficiency Virus (HIV), endogen retroviruses and especially Influenzae, Cytomegalovirus (CMV) and recently Borna disease virus (BDV) are the most frequently emphasized infection factors in schizophrenia-microorganism relation until today (Pearce et al., 2003, Yuksel et al. (2010) and Leweke et al. (2004). Despite all of these microorganisms, the subject regarding the application of three and two main criteria, to be added later, of Koch postulate (Koch, 1893) grounded on for defining the role of infectious agents in human related diseases in multifactorial complicated diseases such as cancer, diabetes, schizophrenia, etc. has been the issue for many years. Exposure to the infectious agent as well as the interaction of host factor and other environmental factors underlies this issue. Therefore, when Hill's criteria (Hill, 1965) (strength, coherence, biological gradient, consistency, plausibility, experiment, analogy, temporality, specificity) developed for the etiopathogenesis of schizophrenia disease known to be multifactorial and complicated and based on certain parameters, and the applicability of genetic consistency parameter proposed by Torrey and Yolken in addition to these criteria in BDV-schizophrenia relation were studied in the light of both the study data and the results of international literatures, Bode et al. (1993) detected seropositivity in the range of 2-4% in seroprevalence studies including 71 cases with schizophrenia; and in 1995, Waltrip et al. (1995) detected BDV-IgG positivity in 14.4% of the patient group in the trial they conducted with 90 schizophrenia cases and a healthy control group of 20 persons. Still in 1995, Kishi et al. (Japan)(1995) detected BDV seropositivity in 30% of 60 psychiatric cases and in 1% of healthy control group of 100 persons, while in 1997, Iwahashi et al. (Japan) (1997) determined BDV- IgG as positive in 44.8% of 67 schizophrenic patients (30) whereas Chen et al. (Taiwan) (1999) did so in 12.1% of 314 schizophrenic patients.

Although, the ratio of seropositivity in our patient group is higher than the ratio found by Bode et al. (1993), Waltrip et al. (1995) and Chen et al. (1999); lower than the ratio detected by Iwahashi et al., (1997) and comparable with the result obtained by Kishi et al. (1995) our result of comparison regarding the BDV-IgG seropositivity of patient and control groups is in concordance with international publications. Our OR results (3.1 and 2.9) indicating the presence of BHV-IgG

in the patient and control groups seem to support the possible role of BHV in the etiopathogenesis of schizophrenia.

BDV-RNA was detected in PBMNCs by Bode et al. (1993) (Germany) in 4 out of 6 psychiatric patients; Igata et al. (1996) (Japan) in 10.2% of 49 schizophrenia cases; Sauder et al. (1996) in 63.6% of 11 schizophrenia cases; Iwahashi et al. (1997) (Japan) in 8.9% of 67 schizophrenia cases; Iwata et al. (1998) (Japan) in 4% of 77 schizophrenia cases and by Nunes et al. (2008) (Brazil) in 12 out of 27 schizophrenic patients. Notwithstanding, BDV-RNA could not be detected by Richt et al. (1997) (Germany) in 26 schizophrenia cases by Lieb et al. (1997) (Germany) in 59 schizophrenia cases and by Kim et al. (1999) (Korea) in 39 schizophrenia cases.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is the most frequently used method for the determination of BDV RNA in PBMNC. It has been indicated in infected rat studies that 1 or 2 RNA could be detected in each 5106 cells and that BDV seropositivity did not always correspond to the presence of BDV-RNA (Taieb et al., 2001; Kishi et al., 1995). According to the relative literature and in line with the recommendation of the kit used, fresh full blood sample was used and other procedures were performed. However, we consider that our BDV-RNA result was affected negatively due to the fact that the cases were diagnosed long ago; that RNA readily degrades *in vitro* and that RNA is barely detectable in approximately 5000 cells. Even though no viral load was detected in our trials, the fact that BHV antigenemia and RNA measurements are achievable in international studies supports the idea that BDV may have a role in the etiopatogenesis of schizophrenia.

On the other hand, in 2005, Matsunaga et al. (Japan) (41) stated that no statistically significant difference was found between the study groups in terms of sex in the study conducted on 171 patients, 57 of which were schizophrenic patients. In 2001, in a study performed by Fukuda et al. (Japan) with 90 psychiatric patients (45 of which were schizophrenic) and 45 healthy blood donors, no statistically significant difference was determined between the groups ($p>0.05$); and in 2004, Puerto et al. (2004) (Mexico) stated that the BDV-Ig G seropositivity did not exhibit any differences between the groups in terms of sex in the trial they performed with 70 schizophrenic patients and 70 persons in healthy control group by matching. Our study result differed from the results of each three investigations and BDV-Ig G seropositivity in male and female patient group cases was found significantly and markedly high with respect to control group cases.

In accordance with the logistic regression analysis, the age group of 51-65 was established as an independent risk factor in BDV-schizophrenia relation. No statistically significant difference could be determined between the schizophrenia group and control group cases on the basis of the age group of 25-34.

In parallel with our study, Fukuda et al. (2001) (Japan), Puerto et al. (2004) (Mexico) and Matsugana et al. (2005) obtained BDV seropositivity ratios of schizophrenia cases in the age groups of 21-34, 35-50 and 51-65 comparable with the seropositivity ratios of control group cases of similar age groups, and no statistical difference was established between them ($p>0.05$).

When schizophrenia group cases were compared with patient control group cases and healthy control group cases in terms of contact with cat, education status including illiteracy and graduation from primary school, being single, psychiatric disorders in at least one relative and schizophrenia history in the family, a highly significant difference was detected between the patient group and other healthy and patient control group cases ($p<0.001$). Additionally, in accordance with the logistic regression analysis, these data were considered as independent risk factors and international publications claim that the role of cats is the most effective factor in direct and indirect human infection (Lundgren et al., 1995; Nakamura et al., 1996; Yuksel et al., 2010). In addition, Yolken et al. (2006) emphasized the importance of cat contact in the etiopathogenesis of schizophrenia in the study performed in the U.S. Nunes et al. (2008) reported that the education status of the schizophrenic patients and their relatives was poor and 60% of the cases were single. Our study data especially contact with cats regarding the effects of demographics related with schizophrenia-BDV are in concordance with the similarity of microorganism epidemiology and schizophrenia epidemiology.

As a result, in spite of the detection a significant relationship between BHV and schizophrenia in studies based on serological methods was observed; unfortunately we did not detect supporting data from the studies based on molecular methods like PCR. We suggest that new, matched, large serial clinical prospective cohort, and case-control based seroepidemiological/molecular studies were needed in order to clarify this relationship and also clinically-controlled experimental studies were needed with the usage of anti-psychotic and antivirals by monitoring the BHV-IgG antibody responses.

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REFERENCES

Bode L, Ferzt R, Czech G (1993). Borna disease virus infection and affective disorders in man. Arch Virol., 7: 159-167.

Bode L, Ludwig H (2003). Borna disease virus infection, a human mental health risk. Clin. Microbiol. Rev., 16: 534-545.

Brown AS (2006). Prenatal Infection as a Risk Factor for Schizophrenia. Schizophrenia Bulletin., 32(2): 200-202.

Chen CH, Chiu YL,Wei FC, Koong FJ, Liu HC, Shaw CK (1999). High seroprevalence of Borna virus infection in schizo phrenic patients, family members and mental health workers in Taiwan. Mol. Psychiatry, 4: 33-38.

Ferszt R, Kühl KP, Bode L, Severus EW, Winzer B, Berghöfer A, Beelitz G, Brodhun B, Müller-Oerlinghausen B, Ludwig H (1999). Amantadine revisited: an open trial of amantadinesulfate treatment in chronically depressed patients with Borna disease virus infection. Pharmacopsychiatry, 32(4): 142-147.

Fukuda K, Takahashi K, Iwata Y, Mori N, Gonda K, Ogawa T, Osonoe K, Sato M, Ogata S, Horimoto T, Sawada T, Tashiro M, Yamaguchi K, Niwa S, Shigeta S (2001). Immunological and PCR Analyses for Borna Disease Virus in Psychiatric Patients and Blood Donors in Japan. J. Clin. Microbiol., 39(2): 419-429.

Gungor S, Anlar B, Turan N, Yilmaz H, Helps CR, Harbour DA (2005). Antibodies to borna disease virus in subacute sclerosing panencephalitis. Pediatr. Infect. Dis. J., 24(9): 833-834.

Henriquez SA, Brett R, Alexander J, Pratt J, Roberts CW (2009). Neuropsychiatric Disease and *Toxoplasma gondii* Infection. Neuroimmunomodulation, 16: 122-133.

Hill AR (1965). The environment and disease: association or causation? Proc. Royal Soc. Med., 58: 295-300.

Igata-Yi R, Yamaguchi K, Yoshiki K, Takemoto S, Yamasaki H, Matsuoka M, Miyakawa T (1996). Borna disease virus and the consumption of raw horse meat. Nat. Med., 2: 948-949.

Iwahashi K, Watanabe M, Nakamura K, Suwaki H, Nakaya T, Nakamura Y (1997). Clinical investigation of the relationship between Borna disease infection and schizophrenia in 67 patients in Japan. Acta Psychiatr. Scand., 96: 412-415.

Iwata Y, Takahashi K, Peng X, Fukuda K, Ohno K, Ogawa T, Gonda K, Mori N, Niwa S, Shigeta S (1998). Detection and sequence analysis of Borna disease virus p24 RNA from peripheral blood mononuclear cells of patients with mood disorders or schizophrenia of blood donors. J. Virol., 72(12): 10044-10049.

Koch R. J (1893). Koch's postulates. Hyg. Inf., 14: 319-333

Kim YK, Kim SH, Choi SH, Ko YH, Kim L, Lee MS, Suh KY, Kwak DI, Song KJ, Lee YJ, Yanagihara R, Song JW (1999). Failure to demonstrate Borna disease virus genome in peripheral blood mononuclear cells from psychiatric patients in Korea. J. Neurovirol., 5: 196-199.

Kishi M, Nakaya T, Nakamura Y, Kakinuma M, Takahashi TA, Sekiguchi S, Uchikawa M, Tadokora K, Ikeda K, Ikuta K (1995). Prevalence of Borna disease virus RNA in peripheral blood mononuclear cells from blood donors. Med. Microbiol. Immunol., 184(3): 135-138.

Leweke, FM, Gerth, CW, Koethe, D (2004). Antibodies to infectious agents in individuals with recent onset schizophrenia. Eur. Arch. Psychiatry Clin. Neurosci., 254: 4-8.

Lieb K, Hallensleben W, Czygan M, Stitz L, Staeheli P (1997). No Borna disease virus specific RNA detected in blood from psychiatric patients in different regions of Germany. Lancet, 350: 1002.

Lundgren AL, Zimmermann W, Bode L, Czech G, Gosztonyi G, Lindberg R, Ludwig H (1995). Staggering disease in cats: İsolation and characterization of the feline Borna disease virus. J. Gen. Virol., 76(9): 2215-2222.

Matsunaga H, Tanaka S, Sasao F, Nishino Y, Takeda M, Tomonaga K, Ikuta K, and Amino N (2005). Detection by Radioligand Assay of Antibodies against Borna Disease Virus in Patients with Various Psychiatric Disorders. Clin. Diag. Lab. Immunol., 12(5): 671–676.

Nakamura Y, Asahi S, Nakaya T, Bahmani MK, Saitoh S, Yasui K, Mayama H, Hagiwara K, Ishihara C, Ikuta K (1996). Demonstration of Borna disease virus RNA in peripheral blood mononuclear cells derived from domestic cats in Japan. J. Clin. Microbiol., 34: 188-191.

Pearce B (2003). Modeling the role of infections in the etiology of mental illness. Clin. Neuroscience Res., 3: 271-282.

Peters IR, Helps CR, Hall EJ, Day MJ (2004). Real-time RT-1 PCR: considerations for efficient and sensitive assay design J. Immunol. Meth., 286: 203-217.

Puerto FI, Zavala JE, Rosado-Franco A, Gamboa Albornoz LJ (2004). Serological survey of Borna Disease Virus in schizophrenic patients from Yucatan, Mexico. Rev. Biomed, 15: 141-147.

Richt JA, Alexander RC, Herzog S, Hooper DC, Kean R, Spitsin S (1997). Failure to detect Borna disease virus infection in peripheral blood leucocytes from humans with psychiatric disorders. J. Neurovirol., 3: 174-178.

Sauder C, Muller A, Cubitt B, Mayer J, Steinmetz J, Trabert W, Ziegler B, Wanke K, Mueller-Lantzsch N, de la Torre JC, Grasser FA (1996). Detection of Borna disease virus (BDV) antibodies and BDV RNA in psychiatric patients: evidence for high sequence conservation of human blood-derived BDV RNA. J. Virol., 70(11): 7713-7724.

Taieb O, Baleyte JM, Mazet P, Filet AM (2001). Borna disease virus and psychiatry. Eur. Psychiatry, 16: 3-10.

Waltrip IIR W, Buchanan RW, Summerfelt A, Breier A, Car penter WT, Bryant NL (1995). Borna disease virus and schizophrenia. Psychiatry Res., 56: 33-44.

Yilmaz H, Helps CR, Turan N, Uysal A, Harbour DA (2002). Detection of antibodiesto Borna disease virus (BDV) in Turkish horse sera using recombinant p. 40. Arch. Virol., 147: 429-435

Yolken RH, Torrey EF (2006). Infectious agents and gene- environmental interactions in the etiopathogenesis of schizophrenia. Clin. Neuroscience Res., 6: 97-109.

Yuksel P, Alpay N, Babur C, Bayar R, Saribas S, Karakose AR, Aksoy C, Aslan M, Mehmetali S, Kilic S, Balcioglu I, Hamanca O, Dirican A, Kucukbasmaci O, Oner A, Torun MM, Kocazeybek B (2010). The role of latent toxoplasmosis in the aetiopathogenesis of schizophrenia--the risk factor or an indication of a contact with cat?. Folia Parasitol (Praha), 57(2): 121-128.