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SHORT COMMUNICATION

Investigation of schizophrenic patients from Istanbul, Turkey for the presence of West Nile virus

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Abstract Association of some neurotropic viruses like Borna Disease virus and Herpes virus with schizophrenia is better explained. However, the role of West Nile virus (WNV) infection in schizophrenia is not well documented. Therefore, this study was performed to investigate possible association between schizophrenia and presence of antibodies and WNV RNA in schizophrenic patients. For this, 200 blood samples from patients with schizophrenia and 200 from control groups were collected in Istanbul, Turkey. WNV RNA was not detected in any of the 200 patients and 200 controls analyzed by real-time RT–PCR. One hundred and twelve sera of schizophrenic patients and 162 of controls were analyzed for the presence of IgG antibodies to WNV by a commercial IgG-ELISA (Euroimmun, Germany). Antibodies to WNV were detected in 6 schizophrenic patients and 5 controls. ELISA positive patients had antipsychotic therapy. The difference between groups in terms of seropositivity to WNV was not statistically significant ($p = 0.887$, $p = 0.148$). Known symptoms of schizophrenia were observed in these patients, and interestingly majority had close contact to cats in the past and come from agricultural area of Turkey where potential area of mosquitoes and bird habitat. In conclusion, the results of this study show that antibodies to WNV in people do not seem to be associated with schizophrenia. However, detecting antibodies to WNV in schizophrenic patients suggests that WNV infection should be considered in endemic areas as it may play role in psychiatric diseases.

Keywords Schizophrenia · ELISA · Real-time RT–PCR · West Nile virus

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Introduction

According to WHO reports, the global burden of mental illness is estimated to be 450 million in the world [1]. Neurotropic viruses like Borna Disease virus (BDV) and West Nile virus (WNV) have recently been payed attention because of the predilection for latency, which may be associated with the occurrence of psychiatric disorders like schizophrenia [2]. WNV is an enveloped single stranded RNA virus in the family of *Flaviviridae*. Transmission occurs mainly by mosquitoes among birds, horses, and people causing acute and chronic illness associated with neurologic disorders [3, 4].

WNV infection in people is generally subclinical. In clinical cases, most people develop West Nile febrile accompanied by other symptoms like myalgia, malaise,

headache, and gastrointestinal distress [4, 5]. However, neuroinvasive disease (West Nile meningitis, West Nile encephalitis, and West Nile poliomyelitis) seen only in small number of cases [6, 7]. Individual somatic complaints, like fatigue, weakness, memory problems, and lack of concentration, may be described by persons recovering from WNV as well as by those recovering from neuroinvasive disease [8, 9]. However, persistent neurologic impairments (movement disorders, functional disability, and parkinsonism), fatigue, epilepsy, and cognitive complaints such as depression and behavioral disorders may occur after West Nile neuroinvasive disease [5, 10].

As it is known, association of BDV and Herpes virus with schizophrenia is better explained [2]. However, the role of WNV infection in schizophrenia is not well documented. Therefore, this study was performed to investigate possible association between schizophrenia and presence of antibodies and WNV RNA in the blood of schizophrenic patients.

Materials and methods

Study population and collection of samples

This study consisted of 3 groups of people. Group I consisted 200 patients with schizophrenia from Bakırkoy Mental Health Hospital located in Istanbul, Turkey; Group II consisted 100 blood donors with no history of progressive neurologic disorders from Cerrahpasa Medical School as healthy control and were screened for the presence of antibodies to HIV, HCV, Syphilis, and HBsAg, and Group III consisted 100 sera from the patients with other anxiety and depressive disorders from the Psychiatry Department of the Cerrahpasa Medical Faculty. Sera and blood were collected from all groups, and they were transferred and kept in a cold storage.

ELISA

One hundred and twelve (112) sera from schizophrenic patients (Group I) and 82 sera from control (Group II) and 80 sera from healthy control (Group III) were analyzed by ELISA. A commercial ELISA kit was used to analyze human sera for the presence of antibodies (IgG) to WNV. ELISA was performed as described by the manufacturer (Euroimmun, Germany, Cat. No: E-100126AA).

RNA extraction and reverse transcription

Viral RNA was extracted from whole blood using QIAamp RNA Blood Mini Kit (Cat. No 52304, Qiagen) as described by the manufacturer. The amount of RNA in the extracted material was measured using Nanodrop spectrophotometer (Nano Drop 2000c, Thermo scientific, Waltham-USA).

Reverse transcription was optimized and performed in two steps as suggested by others [11, 12]. For the first step, 9 µl of RNA template was mixed with 1 µl Random Hexamers (Promega) and incubated at 70°C for 5 min followed by cooling to 4°C using a thermal cycler (Bio-Rad, Chromo-4). For the second step, a total volume of 20 µl reaction mixture was prepared consisting of 10 µl RNA/primer mixture from the first step, 4 µl 5X RT buffer, 2.4 µl 25 mM $MgCl_2$, 1 µl dNTPs (Qiagen), 1.6 µl nuclease-free water (Qiagen), 1 µl reverse transcriptase (Improm II, Promega). The mixture was returned to the thermal cycler and incubated at 20°C for 5 min, 42°C for 30 min, and 70°C for 15 min before being cooled to 4°C. To each cDNA sample, 30 µl of nuclease-free water was added and kept at −70 until required.

Real-time PCR

For the real-time PCR, the method was used as explained by others [11–13]. A total volume of 25 µl reaction mixture was prepared consisting of 12.5 µl Hotstar Taq Master Mix (Qiagen), 1 µl 50 mM $MgCl_2$ (Qiagen), 1 µl (10 pmol/µl) F primer: ProC-F1 5′CCT GTG TGA GCT GAC AAA CTT AGT, 1 µl (10 pmol/µl) R primer, ProC-R 5′GCG TTT TAG CAT ATT GAC AGC C [13], 0.5 µl SYBR Green (1 in 1,000 dilution), 4 µl nuclease-free water and 5 µl cDNA. The mixture was placed in a thermal cycler (Bio-Rad, Chromo-4) and the polymerase activated, by incubation at 95°C for 15 min. The mixture was then cycled at 95°C for 15 s and 60°C for 60 s for 45 cycles. In order to determine the melting curve, the thermal cycler was programed 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 seronegative human blood and reaction mixture with nuclease-free water in place of template. Positive real-time RT–PCR control was performed by using RNA extracted from WNV (Dr. Robert S. Lanciotti, Diagnostic & Reference Laboratory Arbovirus Diseases Branch Centers for Disease Control & Prevention, Fort Collins, Colorado, USA). Human G3PDH was used as PCR control. After the real-time RT–PCR, the products were visualized by agarose gel (2%) electrophoresis.

Statistical analysis

All statistical analyzes were carried out using SPSS 16.0 (Statistical Packages for Social Sciences; SPSS Inc., Chicago).

Results

ELISA and real-time PCR

ELISA

Antibodies to WNV were detected in 6 of schizophrenic patients, 4 in Group II (healthy control), and 1 in Group III (people with anxiety and depressive disorders but no history of progressive neurologic disorder). Remaining sera were found to be negative for the presence of IgG antibodies to WNW.

PCR

WNV RNA was not detected in any of the patient analyzed by real-time RT–PCR. However, positive signal was obtained with SYBR Green in positive control but not in negative control (Fig. 1).

Description of the seropositive schizophrenic patients

All patients had antipsychotic therapy. One of the patients is 82 years old, born in a city of the south east of Mediterranean region in Turkey. This region is relatively hotter than to the North and West part of Turkey and therefore potential area of mosquitoes and bird habitat. She comes from an agricultural area and her family used to keep cat, dog, sheep, goat, cattle, and horses. After time passed, she had psychiatric problems which indicated schizophrenia in 1967. She was then hospitalized in Bakirkoy Mental Health Hospital located in Istanbul. During this period, social maladaptation, aggressiveness, visual and auditory hallucination, paranoid and persecutive delusion, obtuse affective association disorder and psychomotor activity retardation were observed. Second patient is 50 years old and female. She was borne in the North part of Turkey (Black Sea region) and was diagnosed 27 years ago. She has a history of cat contact, and she is still in the Bakırkoy Mental Health Hospital. Third patient is 46 years old and male. He comes from South East Anatolia and was diagnosed 16 years ago. He has a history of cat contact and still in the Bakırkoy Mental Health Hospital from time to time. Fourth patient is 55 years old and female. She was borne in South East Anatolia and was diagnosed 21 years ago. She has a history of cat contact and still in the Bakırkoy Mental Health Hospital. Fifth patient is 50 years old and male. He comes from South East Anatolia and was diagnosed 22 years ago. He has a history of cat contact and still in the Bakırkoy Mental Health Hospital. Sixth patient is 65 years old and male. He was borne in Inner Anatolia and was diagnosed 32 years ago. No history of close contact to animals and he is still in the Bakırkoy Mental Health Hospital from time to time.

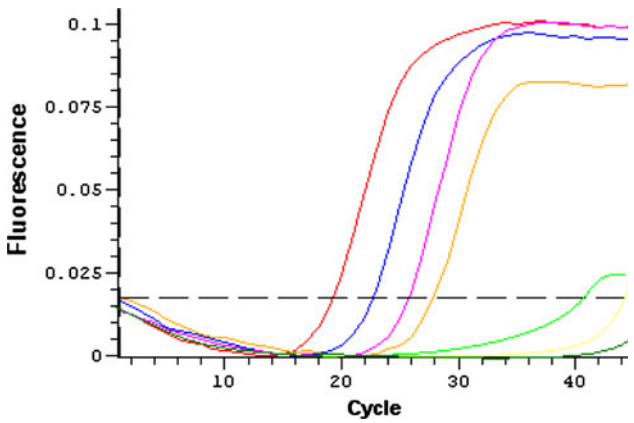

Fig. 1 Real-time PCR of blood samples and controls using West Nile virus primers. From *left* to *right*: *first*, *second*, *third,* and *fourth* line: dilution of positive control from 1:10 to 1:10,000; *sixth* and *seventh line*: blood samples tested; *last line* below threshold: negative control

Statistical analysis

The difference between groups for being seropositive to West Nile virus was not statistically significant. The $p = 0.148$, $X2 = 2.098$, OR = 0.233, and 95%CI = 0.028–1.976 were obtained when Group I (schizophrenic patients) and Group III (people with anxiety and depressive disorders but no history of progressive neurologic disorder) compared. While $p = 0.887$, $X2 = 0.020$, OR = 0.911, and 95%CI = 0.249–3.331 were obtained when Group I (schizophrenic patients) and Group II (healthy control) compared.

Discussion

According to CDC report, by November 2006, more than 23,500 cases of human WNV infection (including 9,700 cases of neuroinvasive disease resulted in 904 fatalities) occurred in the United States [14]. Although many reports described the acute and short-term features of human WNV infection, long-term or chronic squeal is not well documented [5, 7–9, 15–17].

Persistent neuropsychological impairment [16] and persistent parkinsonism have been observed in some patients following WNV infection [8, 10]. Interestingly, impairment was not related to subjective complaints of physical or emotional distress, or premorbid intellectual abilities. Persistent cognitive impairment in West Nile virus infection may be due to prolonged or permanent damage to the central nervous system [8]. Studies related to neuroimaging and histopathology have indicated that WNV and other flaviviruses show distinct neurotropism to

the basal ganglia, including the substantia nigra resulting in neuronophagia and cell death [7, 17, 18]. It is possible that involvement of substantia nigra in WNND may initiate parkinsonism by the natural senescent loss of dopaminergic neurons in older age [19]. The patient in this study was 82 years old, and similar damage to brain cells might have been occurred due to aging.

Depression and behavioral changes in WNV infection have also been reported by many researchers [15, 20]. A year after the outbreak of WNV in New York City, 38% of patients subjectively reported depression [15]. Limited but growing data are available on the long-term cognitive and functional outcomes of WNV infection. After the 2002 Louisiana epidemic of WNV infection, six (38%) of 16 patients developed tremors and parkinsonism at 8 months after acute illness [7]. In West Nile virus infection, persistent symptoms like muscle pain, muscle weakness, fatigue, and headache were self-reported after few months post illness [3, 8, 9, 15, 20]. However, persistent neuropsychological impairment reported by others was not related to subjective complaints [16].

In this study, social maladaptation, aggressiveness, visual and auditory hallucination, paranoid and persecutive delusion, obtuse affective association disorder, and psychomotor activity retardation were observed in a schizophrenic patients. Some of these symptoms at least to some extend may be attributed to West Nile virus infection although no association was found between seropositivity and being schizophrenic. However, number of seropositive patients was higher than controls.

ELISA and plaque reduction neutralization test (PRNT) are used to detect antibodies in West Nile virus infection. Molecular techniques like RT–PCR are also used to detect viral RNA in the blood, cerebrospinal fluid (CSF), and brain tissue. However, in chronic cases, blood samples are not preferred since virus disappears from the blood [3, 21]. In this study, antibodies to WNV were found in 6 schizophrenic patients. No positivity was detected in any other patients and controls. Failure to demonstrate WNV RNA in the blood of schizophrenic patients can be explained since WNV can only be detected in the blood during acute period of infection. Similar results were obtained by other investigators meaning that chance of detecting WNV RNA in the blood decreases after the acute illnes [3, 21]. Therefore, presence of WNV RNA was not always associated with seropositivity and vice versa [3, 21]. In this context, seropositive CSF samples and sera were also analyzed by RT–PCR and WNV RNA was found in 57 and 14%, respectively [3]**.** To correlate the results of seropositivity with WNV RNA, CNS sample is needed since WNV RNA can be detected in the brain [3, 21]. However, in this study, it was difficult to obtain a brain tissue from the patient to be analyzed by PCR.

Schizophrenic patients described in this study, originated from different regions of Turkey. One of those patients come from the south eastern Mediterranean region in which mosquito and bird population is high and antibody to West Nile virus was detected in 29 of 181 human sera previously [22]. A correlation between the global distribution of schizophrenia with tick-borne flaviviruses was found especially tick-borne encephalitis carried by the *Ixodes* tick species in North America [1, 23]. Although it is not yet reported, similar correlation might exist between schizophrenia, mosquito population, and WNV infection. Most patients described in this study originally come from an agricultural area, and most had contact to animals. It has been reported that WNV infection in horse and bird population used to predict future West Nile virus infection in human [4, 24]. Life style (living in the densely populated mosquito area and presence of birds and horses) of these patients (described in this study) in the past might support the idea they might have had the West Nile virus in the early ages and virus might have caused persistent damage to the brain. Alternatively they might have had the virus after the occurrence of schizophrenia.

Findings on human WNV infection from endemic areas in Africa and the Middle East indicates that WNV infection in these areas has mild feature with few symptomatic illness in children [6]. However, in recent epidemics of WNV infection in Israel and Eastern Europe, development of neurologic illness particularly in older persons is more frequent like in the North America [25, 26]. This indicates that West Nile virus infection might be causing long-term clinical outcome in the Middle East. Patients described in this study might be result of exposure to West Nile virus that initiated the changes in the brain. Like in all diseases, occurrence of schizophrenia is multifactorial and therefore social life, West Nile virus and unknown factors might have affected to develop schizophrenia in cases described here.

Conclusions

The results of this study show that antibodies to WNV in people do not seem to be frequently associated with schizophrenia. However, detecting antibodies to WNV in 6 schizophrenic patients suggests that one should consider the possible association between WNV infections and psychiatric diseases in endemic areas although the difference was not significant in this study. However, future studies are needed to show the role of West Nile virus in the pathogenesis of neurologic disorders.

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Conflicts of interest None.

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