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Association of Borna disease virus with autism spectrum disorder in Turkish children

Türk çocuklarda Borna hastalığı virüsü ve otizm spektrum bozukluğu ilişkisi

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Abstract

Aim: Autism spectrum disorders are lifelong neurodevelopmental disorders whose pathogeneses are not fully understood. Borna disease virus is a neurotropic virus that affects the central nervous system. Considering the neuropsychiatric and behavioral effects of the virus, it can be suggested that it may play a role in autism spectrum disorder. However, there are insufficient evidence to support this. In this study, we aimed to investigate the presence of Borna disease virus in patients with autism spectrum disorders and healthy controls.

Methods: This case-control study, performed in children with autism spectrum disorders and a control group, included patients with autism who visited the Child and Adolescent Psychiatry outpatient clinic between December 2017 - December 2018. Borna virus positivity was assayed with the ELISA method in serum samples. Data was analyzed using SPSS version 22.

Results: The study included 63 children diagnosed with autism spectrum disorder and 31 healthy controls. The age range of autism patients was 3-14 years, their mean age was 7.83 (1.96) years, and The Childhood Autism Rating Scale score was 51.09 (5.71). The seropositivity rate for Borna disease virus in the autism and healthy control groups were 25.39% and 25.80%, respectively (P=0.966). For all patients, seropositivity rate was 25.53%.

Conclusion: No relationship was found between autism spectrum disorders and Borna disease virus. The clinical significance of Borna disease virus positivity in society is unknown. We conclude that Borna disease virus is not involved in the pathogenesis of autism spectrum disorders.

Keywords: Borna disease virus, Autism spectrum disorder, Seroprevalence, Etiology

Öz

Amaç: Otizm spektrum bozuklukları yaşam boyu nörogelişimsel bozukluklardır ve patogenezi günümüzde tam olarak anlaşılamamıştır. Borna hastalığı virüsü, merkezi sinir sistemini etkileyen nörotropik bir virüstür. Virüsün nöropsikiyatrik ve davranışsal etkileri göz önüne alındığında, otizm spektrum bozukluğunda rol oynayabileceği ileri sürülebilir. Ancak, bunu destekleyecek yeterli kanıt yoktur. Bu çalışmada otizm spektrum bozukluğuna sahip hastalarda ve sağlıklı kontrol grubunda Borna hastalığı virüsü varlığının araştırılması amaçlanmıştır.

Yöntemler: Bu çalışma otizm spektrum bozukluğu tanısı almış ve kontrol grubundaki çocuklarda yapılan bir vaka kontrol çalışmasıdır. Çalışmaya Aralık 2017 - Aralık 2018 tarihleri arasında Çocuk ve Ergen Psikiyatri polikliniğine başvuran otizmli çocuklar dahil edilmiştir. Serum örneklerinde ELISA yöntemi ile Borna virüsü pozitifliği değerlendirilmiştir. Veriler SPSS sürüm 22 kullanılarak analiz edilmiştir.

Bulgular: Çalışmaya otizm spektrum bozukluğu tanısı almış 63 çocuk ve 31 sağlıklı kontrol grubu dahil edildi. Otizm hastalarının yaş aralığı 3-14 yıl, ortalama yaş 7,83 (1,96) ve Çocukluk Otizmini Derecelendirme Ölçeği skoru ortalama 51,09 (5,71) olarak saptandı. Otizm grubunda ve sağlıklı kontrol grubunda Borna hastalığı virüsü seropozitiflik oranı sırasıyla %25,39 ve %25,80 idi (P=0,966). Tüm hastalar için seropozitiflik oranı %25,53 saptandı.

Sonuç: Otizm spektrum bozukluğu ile Borna hastalığı virüsü arasında ilişki saptanmadı. Borna hastalığı virüsünün toplumdaki pozitifliğinin klinik önemi bilinmemektedir. Sonuç olarak Borna hastalığı virüsünün, otizm spektrum bozukluğunun patogenezinde rol oynadığını düşünmüyoruz.

Anahtar kelimeler: Borna hastalığı virüsü, Otizm spektrum bozukluğu, Seroprevalans, Etyoloji

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Introduction

Autism spectrum disorders (ASD) are life-long neurodevelopmental disorders, characterized by insufficient social communication and limited repetitive behaviors or areas of interest [1,2]. The prevalence of ASD is increasing at alarming dimensions globally. Latest epidemiological studies have reported the prevalence of ASD as 1.5% [3]. Despite several studies explaining the etiopathogenesis of ASD, it is still not fully understood. Though there is a high genetic effect, it is a multifactorial disease resulting from mutual interactions with environmental factors [4]. Among these environmental factors, it is proposed that viral infections experienced in the early stages of development may be causative. The mechanisms causing neurodevelopmental disorders linked to viral infections are proposed to include direct central nervous system (CNS) infections, affecting other tissues in the body, triggering pathologies in the CNS or in the immune system or a combination of these factors. Borna virus is also among the suspected viruses [5].

Borna disease virus (BDV) is an enveloped, unsegmented, single-stranded RNA virus with negative polarity belonging to the *Mononegavirales* order. It is a neurotropic virus affecting the central nervous system, especially the limbic structures [6,7]. Borna disease (BD) was first described in the 18th century as an epidemic disease in horses. Initially considered to be a chronic progressive meningoencephalitis causing neurological symptoms in horses and sheep, it was later identified to affect all warm-blooded mammals. BDV may cause behavioral abnormalities like anxiety, aggressiveness, cognitive deficiencies, and hyperactivity without encephalitic changes in animals [8-10]. Behavioral disease studies in animals have led to the thought that this disease may be associated with some neuropsychiatric diseases in humans. It is thought that BDV may infect humans and animals by direct contact with infected secretions, contaminated food, and vertical routes [8,11]. Serologic evidence in humans was first reported in 1985 [12]. Though the links with a variety of diseases have been topics of study through the years, the outcomes caused in humans are not clear [13]. In humans, BDV was associated with four cases of encephalitis in Germany and resulted in the death of 3 [14]. The properties of the virus and the virus genome being shown in psychiatric diseases like schizophrenia and bipolar disease, which affect a high proportion of the general population but have unknown etiology, have strengthened the possibility of this relationship [15-18]. Whether BDV contributes to neurodevelopmental disorders in children or its effects are unknown. Young animals have been shown to be more susceptible to persistent Bornavirus infection [19]. There is a need for advanced studies about the effect of this neurotropic virus in children, especially in early childhood.

Considering the neuropsychiatric and behavioral effects of BDV, it is proposed to possibly play a role in ASD pathogenesis. However, there are insufficient evidence to support this. This study aimed to investigate the presence of BDV among ASD patients and healthy controls.

Materials and methods

Ethics

The details of the study were explained to the families of the patients and their consents were obtained. The study was approved by Ordu University Clinical Research Ethics Committee (2017/126) and conducted according to the principles of Helsinki Declaration.

Selection and description of participants

This case control study involved ASD patients admitted to the Child and Adolescent Psychiatry outpatient clinics between December 2017 and December 2018. The age range of ASD patients was 3-14 years and their mean age was 7.83 (1.96) years. The healthy control group was selected from those attending the same department and pediatric department due to minor complaints in the similar age range. The study included 63 ASD patients (19 females and 44 males) and 31 healthy children (14 females and 17 males) in the control group. Patients were diagnosed by a pediatric psychiatrist according to Diagnostic and Statistical Manual of Mental Disorders (DSM) 5 criteria [1]. Childhood Autism Rating Scale (CARS) was applied to assess the severity of autism in patients. Children with comorbid diseases in the ASD group or those with unclear diagnoses were excluded from the study.

Childhood Autism Rating Scale

Childhood Autism Rating Scale comprises 15 items and is used to measure the severity of autism. CARS is assessed after detailed interviews with the family and observation of the child. The items assess relationships with people, imitation, listening response, emotional response, object use, body use, adaptation to change, visual response, smell, taste and touch responses, fear or nervousness, verbal communication, non-verbal communication, activity level, level and consistency of intellectual response and general impression. They are scored from 1 to 4 and the total score is calculated. Scores below 30 are normal, those between 30-36.5 indicate mild-moderate autism, while a score of 37 or higher denote severe autism [20].

Detection of Borna Disease Virus

Ten milliliter blood samples from patients were separated while drawing blood for routine tests. Blood samples were centrifuged at 3000 rpm for 15 minutes. Separated serum samples were stored under appropriate conditions (-80°C) in the microbiology laboratory and tested for Bornavirus protein with the human Borna disease virus (BDV) ELISA kit (Abbexa Ltd., Cambridge Science Park, Cambridge, CB4 0EY, UK). This kit is pre-coated with a polyclonal IgG antibody specific for BDV and detects BDV protein in sera. There is no known cross-reactivity with other proteins/viruses. The results are assessed as positive or negative according to the manufacturer's recommendations.

Statistical analysis

The statistical package program Statistical Package for Social Sciences (IBM SPSS for Windows, Ver.22) was used in the calculations. Means and standard deviations were obtained for continuous variables while categorical variables were summarized using frequency and percentage. The student's t-test was applied to assess differences between numerical variables. Chi-square test was used to compare categorical variables. Kruskal-Wallis test was performed to compare the means of more than two groups. *P*-value <0.05 was considered statistically

significant. Considering Cohen's chi square analysis criteria, the average power was calculated as 0.30, alpha=0.05 and n (sample width) =94, and the power was 0.837 [21]. G-Power 3.1.9.2 statistics program was used to power and sample size.

Results

The study included 63 ASD patients and 31 healthy controls. The age range of the children participating in the study was 3-12 years. The mean CARS score of ASD patients was 51.09 (5.71). Detailed information about the children is summarized in Table 1. The seropositivity rate for BDV in the ASD and control groups were 25.39% (n=16) and 25.80% (n=8), respectively, which were similar (*P*=0.096). Borna disease virus seroprevalences are presented in Table 2. Its incidence was insignificantly different among females and males in the ASD group (42.85% vs. 20.83%, respectively) (*P*=0.098). There was no difference when compared to categorized age groups. The seropositivity rate in all individuals included in the study was 25.53% (n=24). The mean titrations of the healthy individuals, mild-heavy and severe autism patients were similar (χ2=2.042, *P*=0.360). Distribution of titer median values by severity of disease is shown in Figure 1.

Table 1: Characteristics of the groups

Characteristics	Total ASD patients (n=63)			
	Mild-moderate ASD n=26 (%)	Severe ASD n=37 (%)	Healthy control n=31 (%)	*P*-value
Gender, Female/Male	4/22 (15.4/84.6)	10/27 (27/73)	14/17 (45/55)	0.173
Age mean (SD)	5.67 (1.99)	6.44 (2.60)	8.75 (2.71)	0.097
CARS mean (SD)	32.82 (3.68)	54.85 (2.7)	-	-

ASD: Autism spectrum disorders, CARS: Childhood Autism Rating Scale

Table 2: Borna disease virus seroprevalence of groups

	Total ASD (n=63)			
	Mild-moderate ASD	Severe ASD	Control group	χ2/*P*-value
BDV positive (n/%)	5 / 19.2	11 / 29.7	8 / 25.8	0.87/0.64
BDV negative (n/%)	21 / 80.8	26 / 70.3	23 / 74.2	0.87/0.35
Titers mean (SD)	0.160 (0.17)	0.237 (0.25)	0.270 (0.41)	2.04/0.36

BDV: Borna disease virus, ASD: Autism spectrum disorders

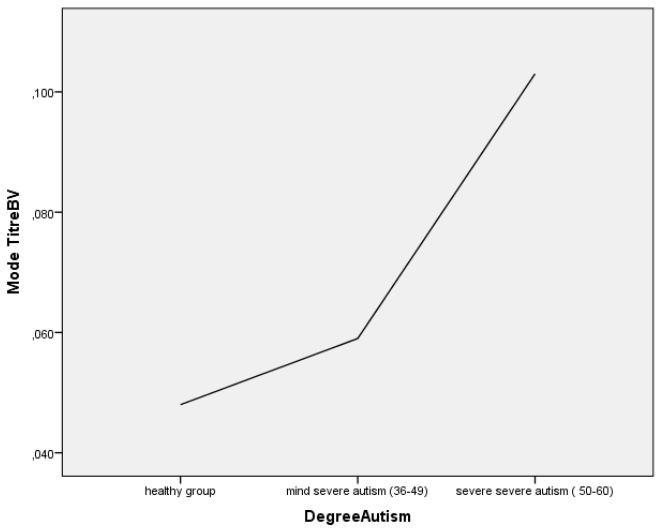

Figure 1: Distribution of titer median values according to the severity of the disease

Discussion

Neuropathology associated with BDV has been demonstrated in animal models. Studies show that infected neonatal rats developed autism-like behavior changes [22-24]. In a study of rats, Hans et al. [25] showed that the virus caused significant changes in synaptic functions, especially damaging neuronal functions, and noted that this may cause neurobehavioral disease in humans. BDV's neurotropic characteristics and ability to create persistent noncytolytic infections and proven outcomes in animal studies strengthen the possibility that viral contact may cause a pathology in humans. It is unknown whether BDV contributes to neurodevelopmental disorders in children. There is a need for advanced studies about the effect of this neurotropic virus in children, especially in the early childhood period. Though BDV has been researched in the etiology of many neuropsychiatric diseases, the number of studies regarding autistic children are limited. No study was observed in the literature, except for the work of Honda et al. [26]. A study of autistic children by Honda et al. reported the antibody prevalence against Bornavirus in children with ASD as 7.4%. In this study, positivity was higher in the 2-5 age group, and there was no significant difference according to gender. In our study, BDV positivity was 25.39% in the ASD group and there was no significant difference compared to the control group. There was no difference between age groups in our study. Similarly, no significant difference was found between genders. Both the number of BDV positivity and titer values were insignificantly higher in the severe autism group compared to those with mild-moderate autism. In another study in children, BDV seroprevalence was investigated. Patti et al. [27] reported 59% BDV seroprevalence in healthy children aged 0-18 years. This rate is quite high and showed the need for risk assessment in children. Unlike our study, male gender, and positivity at the age of 1-3 were higher. None of these studies had a control group.

Previously, Güngör et al. [28] researched the presence of BDV among patients with subacute sclerosing panencephalitis in Turkey and did not identify a significant correlation but reported similar seropositivity rates of 22% in the general population. Determination of positive values in both the patient and control groups are an indicator of presence of BDV contact in our country. However, our results do not support the theory that BDV may contribute to the pathogenesis of ASD.

Limitations

The small number of patients is a limiting aspect of our study. Furthermore, the effects of BDV should be investigated especially at early ages, which is a critical period in the neurodevelopmental process.

Conclusion

In our study, BDV positivity was 25.39% in the ASD group and there was no significant difference compared to the control group. The clinical significance of Borna disease virus positivity in society is unknown. We conclude that Borna disease virus is not involved in the pathogenesis of autism spectrum disorders.

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