Prevalence and Titre of Antibodies to Cytomegalovirus and Epstein-Barr Virus in Patients with Autism Spectrum Disorder

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Abstract. Background/Aim: The etiology of autism spectrum disorders (ASD) is currently unknown. Few studies have explored the role of Cytomegalovirus (CMV) and Epstein Barr Virus (EBV) as potential etiological factors of ASD. The aim of the present study was to evaluate the seropositivity rate and antibody titre to CMV and EBV in children with ASD compared to same-aged healthy controls.

Patients and Methods: We compared the seropositivity rate and titre of antibodies to CMV and EBV in 54 children with ASD compared to 54 controls. Results: Seropositivity rate and titre of the two antibodies were not dissimilar between cases and controls. However, considering only patients with ASD, those seropositive for CMV tended to test worse to the major severity scales than the seronegative ones. Conclusion: Titre and seropositivity rate of antibodies to CMV and EBV are similar between children with ASD and healthy controls.

The Autism Spectrum Disorders (ASD) are multiple cognitive and developmental disorders and include Autistic Disorder (AD), Asperger Syndrome, Rett Syndrome, Childhood Disintegrative Disorder and PDD-NOS (Pervasive Developmental Disorder Not Otherwise Specified). According to the definition of the American Psychiatric Association, significantly abnormal or deficient social interaction, impaired communication and language abilities and considerable narrow pattern of activities and interests are the mainstays of ASD diagnosis. The prevalence of ASD is dramatically increasing. In fact, in the United States, it was estimated that ASD affected 1:2,000 children before the 80s and now is diagnosed in 1:88 newborns (1, 2), with a specific male dominance (5:1 ratio between males and females).

Nevertheless the prevalence trend and the enormous interest aroused by such complex and fascinating disorders, most cases of ASD lack a definitive etiological agent (1, 3). In fact, genetic predisposition and punctual gene abnormalities are thought to be able to explain only a small percentage of the cases of ASD, approximately less than 20% (4). In the other cases, it is generically stated that ASD develop from an interaction between genetic and environmental factors (5). Several authors have proposed different etiological hypotheses of ASD that include infections (6-11), vaccine employment (12, 13), vitamin deficiency (14-16), pesticide exposure (17), paracetamol use (18-20).

In this sense, a novel unifying hypothesis of the etiopathogenesis of ASD has been proposed by our group (3). We suggested that ASD are disorders of the immune system whose onset, in individuals with a background of genetic and environmental susceptibility (the latter likely due to vitamin D deficiency), can ultimately be triggered by an infection (notably a viral infection). The effects of this deranged immune response could, in turn, result in focal damages to specific central nervous system areas.

Therefore, viral infections have repeatedly been invoked as risk factors in the development of ASD (11, 13, 21-25), mainly the ones caused by herpesviridae (26-34). Several case reports have associated cytomegalovirus (CMV) infections with the onset of ASD (35-43). A study conducted in Egypt assessed the prevalence of anti-CMV IgG in patients with autism and age-matched healthy controls and a significant higher seropositivity rate was registered in the autistic group (43.3% vs. 7%) (44).

As regards to Epstein Barr Virus (EBV), it represents an actual obscure and challenging matter of study, since this virus has, above all others and more recurrently, been implicated as both an environmental trigger factor and as a
direct causative agent of central nervous system immunopathology of disorders such as multiple sclerosis (45-48) or autoimmune encephalomyelitis (49).

In this sense, according to the above-quoted unifying hypothesis, a study on possible virus-induced autoimmune phenomena that could affect the developing brain, thus generating anatomic abnormalities of neural connections, could be helpful in the understanding of such multi-faceted disorders.

The aim of the present study was to evaluate some specific viral exposure markers, notably the prevalence and the titre of anti-CMV and anti-EBV IgG antibodies, in a cohort of patients with ASD and in healthy controls.

Patients and Methods

Patients. Patients were recruited at the Child and Adolescent Neuropsychiatry Unit at the Second University of Naples and at the Department of Pediatrics of the University of Naples “Federico II”, Italy, between January 2010 and January 2013. Informed consent was obtained from all children’s parents or legally-authorized representatives and identifying information was removed from each sample. The Ethics Committee of the University of Naples “Federico II” approved the study (protocol number: 85/09). Inclusion criteria for cases were diagnosis of ASD (according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition, Text Revision (DSM-IV-TR)(50)) and informed consent signed by parents/guardians; the only exclusion criterion was the inability to sign an informed consent form.

Controls were recruited at the Division of Pediatric Surgery of the University of Naples “Federico II”, Italy, where they entered for minor surgical treatments. They went through an interview to exclude the presence of a possible ASD, which consequently disallowed their participation at the study.

In order to validate the diagnosis of autism, cases underwent the Autism Diagnostic Interview, Revised version (51) and were examined by means of the Childhood Autism Rating Scales (CARS) (52), the Autism Diagnostic Observation Schedule (ADOS)-Generic (53), the Griffith Mental Developmental Scales (GMDS) (54) and the Vineland Adaptive Behavior Scales (VABS) (55).

Virological tests. In order to detect the specific IgG antibodies to Epstein-Barr viral capsid antigens (VCA) and to CMV in human serum a chemiluminescent immunoassay (CLIA) technology has been performed (LIAISON EBV IgG assay, LIAISON CMV IgG II assay, DiaSorin S.p.A. – Saluggia (VC), Italy).

In detail, for the determination of specific IgG to VCA or to CMV, the p18 synthetic peptide or a CMV antigen, respectively, were used for coating magnetic particles (solid-phase), then mouse monoclonal antibodies were linked to isoluminol derivatives (isoluminol-antibody conjugates). During the first incubation, VCA antibodies or CMV antibodies present in calibrator, samples or controls bound to the solid phase. During the second incubation, the antibody conjugates reacted with VCA- or CMV-IgGs already bound to the solid surface. After each incubation, the unbound material was removed with a wash cycle.

Subsequently, the starter reagents were added, hence inducing flash chemiluminescence reactions. The light signal, thus the amount of isoluminol-antibody conjugate, was measured by a photomultiplier as relative light units (RLU) and was indicative of antibody titre using Spearman’s rho test. For ADOS and CARS (Total Score) 36 (33-40)

<table>
<thead>
<tr>
<th>Scale</th>
<th>Score</th>
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<tbody>
<tr>
<td>GMDS (Developmental Quotient)</td>
<td>50.5 (43-59)</td>
</tr>
<tr>
<td>VABS (Adaptive Quotient)</td>
<td>50 (38-59)</td>
</tr>
<tr>
<td>ADOS (Language)</td>
<td>5 (4-6)</td>
</tr>
<tr>
<td>ADOS (Interaction)</td>
<td>10 (8-12)</td>
</tr>
<tr>
<td>ADOS (Total Score)</td>
<td>16 (13.5-18)</td>
</tr>
<tr>
<td>CARS (Total Score)</td>
<td>36 (33-40)</td>
</tr>
</tbody>
</table>

Data are given as median and interquartile range (IQR). GMDS: Griffith Mental Developmental Scales; VABS: Vineland Adaptive Behavior Scales; ADOS: Autism Diagnostic Observation Schedule; CARS: Childhood Autism Rating Scales.
Results

We enrolled 100 children in our study, 54 with ASD (19 with AD and 35 with non-AD ASD) and 46 controls. None of the controls was found to be affected by a neuropsychiatric disorder. The mean age was 6.1 years for cases (SD=2.5 years) and 5.9 years for controls (SD=2.8 years) (p=0.852).

Males outnumbered females, both among cases (41/54, 75.9%) and controls (39/46, 84.8%, p=0.270). The neuropsychiatric and clinical features of the children with ASD are shown in Table I.

We assessed the rate of seropositivity for the two herpes viruses in cases and controls. As shown in Table II, the rate of seropositivity was similar in cases and controls.

We measured and compared antibody titres for EBV (VCA-IgG) and CMV in cases and controls. As shown in Table III, the titres of antibodies against the two herpes viruses did not differ between cases and controls.

In a logistic regression analysis model, which included age and gender, it was confirmed that the presence or the titre of anti-CMV or anti-EBV antibody was not an independent predictor for health status (ASD condition or health).

We also evaluated the levels and seropositivity rate of the two antibodies in three specific categories (children with AD, children with non-AD ASD and controls). As shown in Table III, neither the seropositivity rate for the two viruses nor antibody titres differed significantly among the three groups.

Therefore, neither antibody level nor seropositivity rate was associated with ASD status.

We correlated severity of GMDS and VABS scales with CMV and EBV antibody levels using Spearman’s Rho test. None of them showed a significant correlation with the antibody levels. For CMV, p-values were -0.130 (p=0.353) and -0.091 (p=0.518) and for EBV were 0.14 (p=0.922) and 0.047 (p=0.737) for GMDS and VABS, respectively.

Finally, we evaluated the median values of severity scales in seropositive and seronegative children with ASD for the two antibodies. GMDS was 47 (IQR: 42.75-54.25) vs. 54 (IQR: 43-68, p=0.073) and VABS was 45 (IQR: 34.75-53.50) vs. 52 (IQR: 38-64, p=0.054) in anti-CMV seropositive and seronegative patients, respectively. GMDS was 50 (IQR: 44-60) vs. 50.5 (IQR: 42.25-58, p=0.823) and VABS was 52 (IQR: 36.5-59) vs. 47 (IQR:40.25-61.25, p=0.761) in anti-EBV seropositive and seronegative patients, respectively.

Discussion and Conclusion

Our study shows that patients with ASD have similar rates of CMV and EBV contact markers compared to healthy controls. This finding even corroborated by the results of a multivariate model that included potential confounding variables such as age and gender.

Herpes viruses are a family of DNA viruses characterized by the ability to engender acute infection, survive in latency status or recurrently reactivate in response to different kinds of triggers. They support a complex interaction with the immune system, that can explain their suggested role as factors or co-factors of autoimmune diseases (56, 57).
In conclusion, the rate of seroprevalence and titre of anti-CMV and EBV antibodies are similar in subjects with ASD and same-aged healthy controls.

Conflicts of Interest

Each Author certifies that he or she has no commercial associations that might pose a conflict of interest in connection with the submitted article.

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