Antinuclear, Cytoskeletal, Antineuronal Antibodies in the Serum Samples of Children with Tic Disorders and Obsessive Compulsive Disorders

Işık Görker1, Gülşen Akman Demir2, Nuray Gürel Polat3, Rukiye Eker Ömeroğlu4, Sema İçöz2, Piraye Serdaroğlu2, Ümran Tüzün5

1Department of Child and Adolescent Psychiatry, Faculty of Medicine, Trakya University, Edirne, Turkey
2Department of Neurology, Faculty of Medicine, Istanbul University, Istanbul, Turkey
3Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey
4Department of Pediatrics, Faculty of Medicine, Istanbul University, Istanbul, Turkey
5Freelance Medical Doctor, Istanbul, Turkey

ABSTRACT

Objective: As environmental factors, the role of Group A beta hemolytic streptococcus infections in the development of tic and obsessive compulsive disorders (OCD) is controversial. The autoimmune hypothesis states that during infection, formation of autoantibodies leads to an autoimmune disorder, which in turn results in movement disorders, tic disorders and/or OCD. In order to test this hypothesis, we assayed these antibodies in children and adolescents diagnosed with tic disorders and/or OCD.

Material and Methods: Children and adolescents who were diagnosed with either tic disorders or OCD according to DSM-IV criteria (n=28), were compared with healthy controls (n=15) having similar age and gender characteristics. Regardless of a streptococcus infection history, serum samples of all patients and controls underwent antinuclear, cytoskeletal, and antineuronal antibody assay using indirect immunofluorescence.

Results: The rates of antinuclear antibody positivity were 21% and 20% in the patient and control groups respectively (p>0.05). Antineuronal antibody was positive in 2 (7%) of 28 patients versus in 1 (6%) of 15 controls (p>0.05).

Conclusion: These results suggest that such antibodies may not be involved in the pathogenesis of tic disorders/OCD.

Key Words: Tic disorders, obsessive compulsive disorder, PANDAS, autoimmunity, child

Received: 19.11.2010 Accepted: 10.06.2011

Introduction

Childhood onset Obsessive Compulsive Disorder (OCD) and Tic Disorders are both neurobiological disorders which seem to arise from different etiological factors such as genetic predisposition, neurophysiological changes and regional brain dysfunction. Since there is a neuroanatomical dysfunction in the basal ganglia region in both of these pathologies and they have frequent comorbidity, they might be the result of different phenotypes of the same gene (1-4). Recently, environmental factors have also been regarded as responsible for the onset of OCD and tic disorders. The PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections) concept was developed when sudden onset obsessive-compulsive and/or tic symptoms were seen in children immediately after Group A beta-hemolytic streptococcus (GABHS) infection. Criteria for this group are: the presence of OCD and/or Tic Disorder, onset between 3 years of age to puberty, an episodic course characterized by a rapid onset of symptoms or worsening dramatically, a temporal relationship between GABHS infection and the onset or flaring of symptoms and abnormal neurologic findings during exacerbations (increased movements or choreiform movements or tics) (5-8). This hypothesis is similar to the mechanism of the Sydenham chorea, a movement disorder, which is seen after Rheumatic Fever induced by GABHS. This mechanism, called molecular mimicry, is a result of cross-reaction between neural tissue and antibodies formed against antigens. The damage seen in Sydenham chorea is in the form of inflammation in the basal ganglia. However, this damage might be seen as a set of neurological and psychiatric symptoms such as chorea, muscle weakness, motor and vocal tics, obsessions, compulsions, attention difficulties and emotional lability. Systematic evaluation of children with Sydenham Chorea shows that obsessive compulsive symptoms are seen frequently during the acute onset of the illness (9, 10). In a study (11) supporting this mechanism, antineuronal antibodies were assayed in the sera of children with Sydenham chorea. Almost half of these children had IgG antibodies which reacted with neuronal cytoplasms of caudate and subthalamic


Address for Correspondence: Dr. Işık Görker, Department of Child and Adolescent Psychiatry, Faculty of Medicine, Trakya University, Edirne, Turkey
Phone: +90 532 355 92 77 Fax: +90 284 235 27 30 E-mail: isikgorker@trakya.edu.tr
nuclei (11). More recently, Kiessling et al. (12) assayed the sera of children diagnosed with movement disorders (Attention Deficit Hyperactivity Disorder, tic disorders, choreiform movements) to assess the relationship of streptococcus infection and movement disorders. Anti-neuronal antibody levels and ASO levels of children with movement disorders were found to be significantly higher in this study. In recent studies, antineuronal and antinuclear (ANA) antibodies of children and adults with Tourette's Disorder were also significantly higher than those of the control group (13, 14). A study in which children diagnosed with Tourette's Disorder were compared to healthy controls, antistreptolysin O (ASO) titers of children with Tourette's Disorder who had comorbidity with Attention Deficit Hyperactivity Disorder (ADHD) were significantly higher than those who did not have this comorbidity (15).

Group A beta-hemolytic streptococcus antigens show cross reactions with tissues other than the brain and give rise to inflammation in the organs. Formation of antibodies against heart muscle and striated muscles in Rheumatic Fever results in damage to these tissues. ANA is usually positive in autoimmune illnesses. ANA can be found as either positive or negative in the sera of children with Tourette’s syndrome and Sydenham chorea (11, 13, 16).

In our study, the immune-mediated hypothesis in the etiology of OCD and/or Tic Disorder was taken into consideration. Thus, development of serum antibodies against neural, nuclear and cytoskeletal antigens was investigated in patients with onset of obsessive compulsive and/or tic symptoms at the time of referral to our clinic.

Patients and Methods

Children and adolescents between age 6 to 14 years (mean age 10.03±2.05 years), who were referred to the Department of Child and Adolescent Psychiatry and diagnosed with either Obsessive Compulsive Disorders and/or Tic Disorders according to DSM-IV criteria (17), formed the study group (n=28) and were compared with a healthy control group (n=15) of similar age (mean age 9.97±2.12 years) and gender characteristics. During the interview, parents stated that these children did not have any infections before referral and were not on any antibiotic or antiinflammatory treatment. Regardless of streptococcal infection history, subjects were diagnosed with OCD, Tic Disorder and OCD + Tic Disorder comorbidity according to DSM-IV. None of the cases had any comorbid diagnoses. Diagnoses were established through a best estimate consensus procedure performed by two child psychiatrists. Severity of the tic symptoms was assessed by the Yale General Tic Severity Assessment Scale (18, 19). For the assessment of obsessive compulsive symptoms, the Yale Brown Obsessive Compulsive Scale for children was used (20, 21). Venous blood samples were obtained from both patient and control groups. The serum samples were kept at -20°C until the antibody assays.

This study was reviewed and approved, by the local Ethics Committee of the Istanbul University Faculty of Medicine (Registration number: 388) The study was also supported by the Research Fund of Istanbul University with the project number: 632312003

Evaluation of antineuronal antibodies

IgG class antineuronal antibodies were analyzed by indirect immunofluorescence. The serum samples were kept at -20°C prior to the study and, after being brought to room temperature, they were diluted in a 1/10 ratio with phosphate buffer solution (PBS). Diluted samples were incubated for 30 minutes at room temperature with cerebrum/cerebellum tissue samples which were prepared using primate brain (EUROIMMUN AG Lübeck, Germany). After being washed with PBS+Tween they were incubated for 30 minutes with FITC-tagged anti-human IgG. After another washing with PBS and Tween, they were covered with PBS-glycerol. Prepared samples were stored at +4°C. Each of these samples was evaluated by two investigators blind to the diagnoses within 24 hours. Sera known to contain antineuronal antibodies (Anti Hu+, Anti Ri+, AntiYo+) were used as positive controls. The studied sample was considered as positive if nuclear or cytoplasmic fluorescence was shown in the cerebrum and/or cerebellum neurons similar to that of known positive controls.

Evaluation of antinuclear and cytoskeletal antibodies

In this analysis, the indirect immunofluorescence technique was used. The serum samples were diluted with PBS in a 1/10 ratio and were studied along with positive and negative controls, using Hep 20-10 (human) and monkey liver samples (EUROIMMUN AG, Lübeck, Germany). After incubation for 30 minutes in a moist and dark environment, all serum samples were washed with PBS for 5 minutes. Anti human IgG was incubated with FITC tagged monoclonal antibody for 30 min. in a moist and dark environment. After being washed it was covered by PBS-glycerol. Each of the samples was evaluated by fluorescence microscopy by two investigators both blind to the diagnoses of the cases. During the evaluation, the sera of the cases were compared with that of positive controls for ANA (homogeneous, speckled, nucleolar, SS.A). For the evaluation of cytoskeletal antibodies, the same task was carried out in the sera of the cases and controls using serum positive for cytoskeletal antibodies.

Statistical analysis

Statistical analysis of the study data were carried out with SPSS for the Windows 10.0 program. When study results were evaluated, the Fisher Exact chi-square test was used for comparison of the qualitative data. The results were expressed as 95% confidence intervals and the significance level was adjusted to p<0.05.

Results

Antibody positive case numbers in study groups are shown in Table 1. Nuclear type antineuronal antibodies were positive in two cases (7%) in the patient group and one case (6%) in the control group. Antineuronal antibody findings were not statistically significant between the two groups (p>0.05). When ANAs were evaluated, the difference was not statistically significant between the groups: six cases in the patient group (21%) had positive results, whereas there were three cases in the control group (20%) (p>0.05). Cytoskeletal antibodies were found to
be negative. This case was diagnosed with OCD+Tourette Disorder. Positive and negative results of antineuronal antibodies were shown in Figure 1 and Figure 2, whereas positive and negative ANA results were shown in Figure 3 and Figure 4, respectively.

Table 1. The number of antibody-positive individuals in patient and control groups

<table>
<thead>
<tr>
<th>Antibodies characteristics (n)</th>
<th>Patient Group (n=28)</th>
<th>Control Group (n=15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antineuronal/ Nuclear</td>
<td>2</td>
<td>1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ANA/ Homogenous</td>
<td>5</td>
<td>1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ANA/ SS.A</td>
<td>1</td>
<td>1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ANA/ Nucleolar</td>
<td>-</td>
<td>1</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

ANA: antinuclear antibody, SS.A: Sjögren Syndrome Anti-Ro

Discussion

We found a similar antibody profile in patients with tic disorders and OCD and healthy controls. In our study, there was no statistically significant difference between patients diagnosed with OCD and/or tic disorder and healthy controls as far as antineuronal antibody levels were concerned. Two children with OCD in the patient group, which was formed regardless of streptococcus infection history, had positive antineuronal antibodies (7%). In the healthy control group, one child had positive antineuronal antibodies (6%). The majority of cases with OCD and tic disorder had negative results for antineuronal antibodies, which might be considered as a lack of correlation between two entities. However, in our study, primate cerebral cortex neurons and cerebellum were used for the assessment of these antibodies. The Biochip Mosaic kit does not include specific basal ganglia sections. Hence,
an antibody specific to the basal ganglia region could not be demonstrated. In studies assessing antineuronal antibodies using human caudate putamen and globus pallidus sections, antineuronal antibody levels were significantly elevated in children with Tourette's Disorder, tic disorders and choreiform movements, all of which are classified under the heading of movement disorders (12, 14). In the study by Singer and co-workers, anti-basal ganglia antibodies were investigated, and children with PANDAS diagnosis and healthy controls showed similar antibody characteristics. However, antibodies were found against epitopes in the caudate fraction of the cases with tic and PANDAS (22). In a very recent study of children, antibrain antibody profiles were compared in children with OCD, OCD-PANDAS and OCD-Cronic Tic Disorder to evaluate antineuronal antibodies, ASO and putative antigens. The conclusion was controversial due to finding differences between the groups (23).

Furthermore, in another study carried out in cases varying in age distribution from childhood to adulthood, frozen sections of rat brain striatum were used and antineuronal antibody levels of patients with Tourette Disorder were significantly higher than that of healthy controls (13). Although specific basal ganglia sections were not used in our study, two cases in the patient group (7%) and 1 case in the control group (6%) had positive findings for antineuronal antibodies. How can one explain antineuronal antibody findings without using a specific region? In a very recent study in adults with Tourette's Disorder, a quantity of M proteins, which is the major destructive part of Group A streptococci, were thought to have a cross reaction with the epitopes in brain tissue of human beings to start the immune process. A group of adults with Tourette's Disorder had higher serum titers for antibodies against M12 and M19 proteins compared to that of the controls (24). In fact, a group of children with PANDAS had D8/17 monoclonal antibody reactivity, a specific marker for Rheumatic fever, which was higher than the control group. D8/17 marker frequency is a genetic trait. These findings might show that there is a subgroup of children having OCD and tic symptoms with a genetic predisposition to streptococcal immunity similar to the mechanism of rheumatic fever (8). Antineuronal antibodies can be shown in 20-40% of healthy controls (14, 25). This, in fact, stresses the importance of detailed studies to clarify the reasons for the formation of post-streptococcal neuropsychiatric symptoms.

ANA, which is usually positive in autoimmune disorders, was also assessed in the sera of the cases. Six cases in the patient group (21.4%) had a positive ANA, and five of these showed homogeneous staining properties. Two of these cases were diagnosed with OCD+Tourette Disorder, and three cases had an OCD diagnosis. SS-A staining was seen in a case with Tourette Disorder. 20% of the control group were ANA positive. These findings are not statistically significant. In a study by Singer et al. (1998), 41 children and adolescents with Tourette Disorder were investigated and 2% were found to be positive for ANA (14). Morshed et al. (2001) compared three groups of patients with Tourette Disorder, Sydenham Chorea and Autoimmune Disorders to healthy controls, finding a higher level of positive ANA in patients with Tourette Disorder (26).

In our study, the results did not show a statistically significant difference. Although having a positive ANA might be considered a sign of an autoimmune disorder, none of the patients had any symptoms related to an autoimmune disorder in their histories. Homogeneous staining seen in these cases was considered nonspecific.

Antistreptolysine O (ASO) levels of children and adults diagnosed with Tourette Disorder, OCD and ADHD were investigated, and cases diagnosed with OCD and ADHD which had an increase in the volume of basal ganglia also had higher ASO titers (27). In other studies, ASO levels were found to be high in cases with Tourette Disorder. The same studies showed high ASO titers in the pediatric control group (13, 14, 25). A recent study attempted to investigate the temporal coexistence of streptococcus infections and exacerbations of obsessive-compulsive/tic symptoms in patients with OCD and/or tic disorder diagnosis and extensive symptom exacerbations. There was a positive correlation between obsession compulsion symptom severity and Group A streptococcus antibody titers. In the same study, tic symptoms showed seasonal fluctuations, being the worst in Fall and Winter (28).

There are several limitations to this study. First of all, all patients and controls were assessed regardless of a streptococcus infection history. Therefore, ASO levels of all cases could not be evaluated. Secondly, the results obtained here by the use of a kit which consisted of both cerebral and cerebellar cortex tissue samples and which was not prepared specifically to include basal ganglia may reflect the method's sensitivity. Hence, no antibody specific to the basal ganglia region could be demonstrated. Thirdly, the lack of a sufficient number of patients diagnosed with Tic Disorder and OCD in the limited time of our study has prevented us from reaching more meaningful findings. However, this study was a preliminary study for further studies which we have planned. In follow-up studies, where concomitant clinical assessment and laboratory investigations can be made, if antibodies against these antigens are related to a specific receptor or a single neuron type and this can be shown by the epitopic studies mentioned above, the etiology of childhood onset OCD and Tic Disorder can be determined. Further studies are needed in larger groups of patients and controls in order to evaluate our aforementioned hypothesis.

Conflict of Interest
No conflict of interest was declared by the authors.

References