



Anti-Ganglioside, Anti-Glutamate, and Anti-Gad Antibody Levels in Attention-Deficit Hyperactivity Disorder

ORIGINAL
INVESTIGATION

Sevgi Özmen¹, Esra Demirci¹, Didem Behice Öztıp¹, Fatih Kardaş², Selma Gökahmetoğlu³, Hüseyin Per⁴

ABSTRACT

Objective: The etiology of Attention-Deficit Hyperactivity Disorder (ADHD) is still unclear. In the present study, we aimed to demonstrate the relationship among anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-glutamic acid decarboxylase (anti-GAD) antibodies, which are believed to be involved in the etiology of ADHD.

Materials and Methods: The study included 36 children who were diagnosed with ADHD according to DSM IV diagnostic criteria and 21 healthy children as the control group. In all subjects, anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-GAD antibodies were studied in the Microbiology Laboratory of Erciyes University, Medical School.

Results: The mean age was 9.34 years in the ADHD group, which consisted of 5 girls and 31 boys. The mean age was 7.8 years in the control group, which consisted of 8 girls and 13 boys. No significant differences were observed in the levels of anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-GAD antibodies between the ADHD and control groups.

Conclusion: Although the etiology is unknown in ADHD, it is believed that autoimmune factors may be involved in the etiology according to currently available information. However, there is a need for further studies with larger sample size to clarify the linkage between ADHD and anti-neuronal antibody levels.

Keywords: ADHD, autoantibodies, etiology

INTRODUCTION

ADHD is one of the most common neuropsychiatric disorders of childhood, which manifests with symptoms of hyperactivity and impulsivity (1). Its prevalence ranges from 8% to 12% worldwide (1-3). ADHD prevalence has been reported as 8.1–8.6% in community samples and as 8.6–29.4% in clinical samples from Turkey (4-6). ADHD incidence is 3–5 times higher in boys than in girls (7). The symptoms of ADHD diagnosed in childhood persist into adolescence in 50–80% and in adulthood in 30–50% (8).

Although ADHD is a heterogeneous disorder involving multiple causes, its etiology has not been fully elucidated yet (3). In general the majority of hypotheses about ADHD etiology focus on neurological, genetic, psychosocial, and environmental factors and brain dysfunction caused by several natal and postnatal causes (9). In recent years, a significant increase in the incidence of ADHD has emphasized the need to investigate factors that may be involved in ADHD etiology.

It has long been known that autoimmunity and resultant target antibodies (auto-antibodies) lead to various diseases in several tissues, including the central nervous system. The relationship between neurodevelopment disorders and autoimmune etiological factors has been frequently emphasized in recent studies. In particular, it was shown that the auto-antibodies against glutamate N-Methyl-D-aspartate (NMDA) receptors, glutamic acid decarboxylase (GAD), and gliadin are closely related with central nerve system disorders (10-12). In addition, there is an increasing body of evidence indicating that the autoimmune mechanism may play a role in the etiology of ADHD similar to studies on the etiology of other psychiatric disorders, such as schizophrenia, obsessive compulsive disorder, and anorexia nervosa (13-15). In one study, it was proposed that anti-GAD65 auto-antibodies may be associated with ADHD (16). It was shown that dopamine and glutamatergic pathways are involved in ADHD (17). In animal studies, it was found that there is a relationship between glutamate receptors related to motor activity and ADHD. It was also suggested that Glutamate Receptor, Ionotropic, N-methyl D-aspartate 2A (GRIN2A) gene polymorphisms may be a risk factor for ADHD (18). In literature, there is no study investigating the relationship between the anti-ganglioside antibodies and ADHD. Therefore, in this study we aimed to demonstrate the relationship among anti-ganglioside antibodies, anti glutamate receptor antibodies, and anti-GAD antibodies, which are believed to be involved in the etiology of ADHD.

¹Department of
Child Psychiatry, Erciyes
University Faculty of
Medicine, Kayseri, Turkey

²Department of Pediatric
Endocrinology, Erciyes
University Faculty of
Medicine, Kayseri, Turkey

³Department of Microbiology,
Erciyes University Faculty of
Medicine, Kayseri, Turkey

⁴Department of
Child Neurology, Erciyes
University Faculty of
Medicine, Kayseri, Turkey

Submitted
26.05.2014

Accepted
28.06.2014

Correspondance
Sevgi Özmen MD,
Department of
Child Psychiatry, Erciyes
University Faculty of
Medicine, Konya, Turkey.
Phone: +90 352 207 66 66
e.mail:
drsevgiozmen@gmail.com

This study was presented at the
24th International Child and
Adolescent Psychiatry Congress,
9-12 April 2014, Konya, Turkey

©Copyright 2015
by Erciyes University School of
Medicine - Available online at
www.erciyesmedj.com

MATERIALS and METHODS

This study included 35 children with no known psychiatric, neurological, or metabolic disease who presented to the Child Psychiatry Department of Erciyes University Medical School in Kayseri, between May 2012 and May 2013 and were diagnosed as having ADHD according to DSM IV diagnostic criteria. For the assessment of severity of ADHD, we used Turgay ADHD scale. Twenty-one healthy, volunteer children with no neurological, metabolic, or psychiatric disorders were employed as the control group. Written informed consent was obtained from the parents of all children. In all subjects, the blood samples drawn were centrifuged at 1000 rpm for 15 minutes. In the sera obtained, anti-ganglioside antibodies (Immunoblot technique), anti-glutamate receptor antibodies [indirect immunofluorescent antibody (IFA) technique], and anti-GAD antibodies (ELISA technique) were studied at the Microbiology Laboratory of Erciyes University, Medical School. Erciyes University Ethical Committee approved of the study with the number 2011/447 on August 02, 2011.

Statistical analysis

All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) (IBM SPSS Inc, Chicago, IL, USA) version 17.0. The Mann Whitney test was used to compare groups, whereas the chi-square test was used to compare quantitative variables. We performed correlation analysis between the quantitative variables using Pearson's correlation test. $P < 0.05$ was considered as statistically significant.

RESULTS

The mean age was 9.34 years in the ADHD group, which consisted of 5 girls and 31 boys. The mean age was 7.80 years in the control group which consisted, of 9 girls and 13 boys. There was a history of thyroid dysfunction in the mothers of 4 patients and in the second degree relatives of 5 patients ($p < 0.05$). It was found that there was a history of type II diabetes mellitus in the second degree relatives of 2 patients in the ADHD group and more common history of type II DM in the ADHD group ($p < 0.05$). No significant difference was observed in the levels of anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-GAD antibodies between groups ($p > 0.05$). We failed to demonstrate a significant correlation between antibody levels and age of onset or disease severity. When gender was assessed, no significant correlation was detected in antibody levels between male and female genders.

DISCUSSION

In this study, no significant difference was observed in the levels of anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-GAD antibodies between groups, and we found more common history of thyroid dysfunction and type II diabetes mellitus in the ADHD group relatives.

ADHD is a heterogeneous disorder of unknown etiology. The symptoms of ADHD may occur in conditions such as Fragile X, fetal alcohol syndrome, low birth weight, and, less commonly, thyroid disorders with genetic basis. However, such cases account for only minority of ADHDs. Some potential causes have been proposed in previous studies, including genetic factors, brain in-

jury, neurotransmitters, nutrients/supplements, toxic substances and psychosocial factors (3). There is emerging evidence indicating that autoimmune mechanisms may also play a role in ADHD besides other causes. There is evidence suggesting that anti-neuronal antibodies may be involved in the etiopathogenesis of autism, neuropsychiatric systemic lupus erythematosus, and multiple sclerosis (19, 20). In addition, there has been an increase in the number of studies evaluating autoimmunity in the etiology of psychiatric diseases such as obsessive compulsive disorder, Tourette syndrome, and anorexia nervosa (13-15). Antibodies against glutamate NMDA receptors, GAD, and gliadin are closely related to CNS disorders (16, 18). Brain areas, in which auto-antibodies have a direct effect include the hypothalamus, hippocampus, amygdala, and limbic system components and these areas account for emotional response and memory. In studies on ADHD, HPA dysfunction, low plasma cortisol levels, and volume changes in the hypothalamus, hippocampus, and amygdala have been identified (21, 22). Therefore, it is believed that there may be a relationship among ADHD etiology and levels of anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-GAD antibodies. In a study by Rout et al. (16), it was shown that there is a relationship between GAD65 and disease in autism and ADHD. In another study, it was suggested that the GRIN2A gene polymorphism may be a risk factor for ADHD (18). In our study, no significant relationship was found among ADHD and levels of anti-ganglioside antibodies, anti-glutamate receptor antibodies, and anti-GAD antibodies. This could be due to the limited number of subjects and lack of age- and sex-matched control group. No significant correlation was detected between antibody levels and age of onset or disease severity. In literature, it was shown that the female gender is a risk factor for auto immune diseases (23). However, in our study, no significant correlation was detected between antibody levels and gender in the ADHD group. This could be due to the limited number of girls in our study population. However, higher numbers of boys in this study group concordant with the findings of that higher prevalence of ADHD was observed among boys than girls (7). In our study, the finding that there was no correlation between ADHD and auto-antibodies may be due to the higher prevalence of ADHD among boys. Moreover, familial auto-immune diseases were observed to be significantly more common in the ADHD group. The relationship among ADHD, a neurodevelopmental disease, and familial HLA-DR4 positivity was shown in previous studies (24, 25). This association may explain the burden of familial auto immune diseases in the patient group. However, further studies with larger sample size are needed on this topic.

CONCLUSION

Further studies using different antibodies are needed to determine the relationship between ADHD and autoimmunity, since the etiology of ADHD, the most common neuropsychiatric disorder of childhood, is still unclear.

Our study was crosssectional and our sample size was small. There were gender differences between the control and ADHD group. These were the limitations of our study.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authors' Contributions: Conceived and designed the experiments or case: SÖ, ED, DBÖ, FK, SG, HP. Performed the experiments or case: FK, SG, HP. Analyzed the data: SÖ, ED. Wrote the paper: SÖ, ED. All authors have read and approved the final manuscript.

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

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed, Text Revision. APA, Washington DC, 2000.
- Barkley RA. Attention Deficit Hyperactivity Disorder: A Handbook for Diagnosis and Treatment. Guilford Press, New York, 2006.
- Biederman J, Faraone SV. Attention deficit hyperactivity disorder. *Lancet* 2005; 366(9481): 237-48. [\[CrossRef\]](#)
- Erşan EE, Doğan O, Doğan S, Sümner H. The distribution of symptoms of AttentionDeficit/Hyperactivity Disorder and Oppositional Defiant Disorder in school age children in Turkey. *Eur Child Adolesc Psychiatry* 2004; 13(6): 354-61. [\[CrossRef\]](#)
- Ercan ES. Epidemiological Aspect of Attention Deficit Hyperactivity Disorder. *J Pediatr Sci* 2010; 6(2):1.
- Polanczyk G, Rohde LA. Attention Deficit Hyperactivity Disorder: The epidemiology of lifetime. *Current Opinion in Psychiatry* 2007; 20(4): 386-92. [\[CrossRef\]](#)
- Kaplan HI, Saddock BJ, Greeb JA. Kaplan and Saddock's Synopsis of Psychiatry: Behavioural Sciences Clinical Psychiatry. Baltimore, Williams and Wilkins. Seventh Ed. 1994. p. 1063-9.
- Weiss M, Weiss G. Attention deficit hyperactivity disorder. In *Child and Adolescent Psychiatry, A Comprehensive Textbook*. Ed M Lewis. Lippincott Williams Wilkins, 3rd ed. Philadelphia, 2002. p. 645-51.
- Levent F. The effects of electromagnetic field on attention deficit hyperactivity disorder in the course of the disease and the etiology. *New Symposium Journal* 2011; 49(3): 165-72.
- Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immun* 1993; 7(1): 97-103. [\[CrossRef\]](#)
- Mostafa GA, Al-Ayadhi LY. A lack of association between hyperserotonemia and the increased frequency of serum anti-myelin basic protein auto-antibodies in autistic children. *J Neuroinflammation* 2011; 8: 71. [\[CrossRef\]](#)
- Vianello M, Tavolato B, Giometto B. Glutamic acid decarboxylase autoantibodies and neurological disorders. *Neurol Sci* 2002; 23(4): 145-51. [\[CrossRef\]](#)
- Luo F, Leckman J.F, Katsovich L, Findley D, Grantz H, Tucker D.M, Lombroso P.J, King R.A, Bessen D.E. Prospective longitudinal study of children with tic disorders and/or obsessive-compulsive disorder: relationship of symptom exacerbations to newly acquired streptococcal infections. *Pediatrics* 2004; 113(6): 578-85. [\[CrossRef\]](#)
- Smitka K, Papezova H, Vondra K, Hill M, Hainer V, Nedvidkova J. The Role of "Mixed" Orexigenic and Anorexigenic Signals and Autoantibodies Reacting with Appetite-Regulating Neuropeptides and Peptides of the Adipose Tissue-Gut-Brain Axis: Relevance to Food Intake and Nutritional Status in Patients with Anorexia Nervosa and Bulimia Nervosa, *International Journal of Endocrinology* 2013; 483145. [\[CrossRef\]](#)
- Titulaer MJ, Dalmau J. Antibodies to NMDA receptor, blood-brain barrier disruption and schizophrenia: a theory with unproven links. *Mol Psychiatry* 2014; 19: 1054. [\[CrossRef\]](#)
- Rout UK, Mungan NK, Dhossche DM Presence of GAD65 autoantibodies in the serum of children with autism or ADHD. *Eur Child Adolesc Psychiatry* 2012; 21(3): 141-7. [\[CrossRef\]](#)
- Carlsson ML. On the role of cortical glutamate in obsessive compulsive disorder and attention-deficit hyperactivity disorder, two phenomenologically antithetical conditions. *Acta Psych Scand* 2000; 102(6): 401-13. [\[CrossRef\]](#)
- Turic D, Langley K, Mills S, Stephens M, Lawson D, Govan C et al. Follow-up of genetic linkage findings on chromosome 16p13: evidence of association of N-methyl-Daspartate glutamate receptor 2A gene polymorphism with ADHD. *Mol Psychiatry* 2004; 9(2): 169-73. [\[CrossRef\]](#)
- Mostafa GA, Ibrahim DH, Shehab AA, Mohammed AK. The role of measurement of serum autoantibodies in prediction of pediatric neuropsychiatric systemic lupus erythematosus. *J Neuroimmunol* 2010; 227(1-2): 195-201. [\[CrossRef\]](#)
- Levin LI, Munger KL, Rubertone MV, Peck CA, Lennette ET, Spiegelman D, et al. Temporal relationship between elevation of Epstein-Barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. *JAMA* 2005; 293(20): 2496-500. [\[CrossRef\]](#)
- Hulvershorn LA, Mennes M, Castellanos FX, Di Martino A, Milham MP, Hummer TA, Roy AK. Abnormal amygdala functional connectivity associated with emotional lability in children with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 2014; 53(3): 351-61. [\[CrossRef\]](#)
- Chen YH, Chen H, Liu YY, Lin GX, Wei LX, Chen DL. Function of the hypothalamus-pituitary-adrenal axis in children with attention deficit hyperactivity disorder. *Zhongguo Dang Dai Er Ke Za Zhi* 2009; 11(12): 992-5.
- Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol*. 2014 May 2. (in press) [\[CrossRef\]](#)
- Aureli A, Sebastiani P, Del Beato T, Marimpietri A, Melillo V, Sechi E, Di Loreto S. Investigation on the possible relationship existing between the HLA-DR gene and attention deficit hyperactivity disorder and/or mental retardation. *Immunopathol Pharmacol* 2008; 21(4): 985-91.
- Lee LC1, Zachary AA, Leffell MS, Newschaffer CJ, Matteson KJ, Tyler JD, Zimmerman AW. HLA-DR4 in families with autism. *Int Pediatr Neurol* 2006; 35(5): 303-7. [\[CrossRef\]](#)