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Evaluation of suPAR Levels in Attention Deficit Hyperactivity Disorder Etiopathogenesis

ORIGINAL ARTICLE

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ABSTRACT

Objective: Although a strong inflammatory basis has been demonstrated, the pathophysiology of attention deficit hyperactivity disorder (ADHD) has not been defined clearly. The aim of the present study was to investigate whether soluble urokinase plasminogen activator receptor (suPAR), one of the inflammatory disruptors, plays a role in the etiology of ADHD.

Materials and Methods: The study population comprised 50 patients aged 7–13 years, diagnosed with ADHD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, without any chronic or other psychiatric disease, and 25 healthy controls. Parents of the children in the study group completed the Conners' Parent Rating Scale—Revised Short, and teachers completed the Conners' Teacher Rating Scale—Revised Short. Enzyme-linked immunosorbent assay kits were used to measure suPAR levels in plasma samples.

Results: The mean plasma suPAR level of patients with ADHD was 2.92±1.74 ng/ml, the suPAR level of the controls was 2.54±1.05 ng/ml, and there was no significant difference in suPAR levels between ADHD and controls (Z=0.084, p=0.933). No correlation was found between plasma suPAR levels and ADHD severity as assessed by Conners' parent and teacher scales.

Conclusion: The role of inflammatory systems and mediators in ADHD was emphasized in many studies, and many important data on ADHD etiopathology were obtained. However, we found no significant relationship between ADHD and suPAR levels. Further research is needed with large samples.

Keywords: suPAR, ADHD, inflammation, child

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INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder with complex etiology; genetic, biological, and environmental factors (pre–postnatal risks and environmental toxins) have all been considered as potential risk factors (1). Increasing evidence shows that inflammation plays a role in the etiology of ADHD (2). Studies have shown that infections during pregnancy, at birth, or in early childhood and chronic inflammatory diseases in childhood increase the risk of ADHD (3).

In recent studies investigating the etiology of ADHD, the relationship between ADHD and polymorphism in proinflammatory genes has been emphasized (4). It has been reported that inflammatory mediator levels are higher in patients with ADHD than in healthy control groups (5). Inflammatory mediators (cytokines) have also been reported to play an important role in tryptophan metabolism and dopaminergic pathways in the brain, which are also implicated in ADHD. Alterations in proinflammatory and anti-inflammatory cytokines may be influential in the pathogenesis of ADHD (5). In addition, it has been shown that the administration of cytokines, such as interleukin-1β, interleukin-2 (IL-2), and interleukin-6 (IL-6), can cause neurotransmission changes similar to those seen in ADHD, such as increased norepinephrine and reduced dopamine levels in studies with rodents (6).

It is known that protein kinase activity (PKA), which controls inflammatory responses in the brain, controls the expression of some important cytokines, such as IL-6, and also induces the production of the urokinase plasminogen activator receptor (uPAR), which is known to play a critical role in brain development (5, 7).

When brain damage occurs, PKA is weakened, and IL-6 production, an anti-inflammatory cytokine, is increased (8). PKA inhibition and elevated levels of IL-6 have been shown to cause ADHD, leading to hippocampal neuronal death and neuronal differentiation (9).

Soluble urokinase plasminogen activator receptor (suPAR), the soluble form of uPAR, has emerged as a valuable indicator of the activation state of the immune system. suPAR molecule is involved in various immuno-

logical functions, such as cell adhesion, migration, differentiation, proliferation, and angiogenesis (10). suPAR, a proinflammatory molecule, and its relationship with psychiatric illnesses have been the subject of many studies, and the role of suPAR in psychiatric diseases has not yet been fully elucidated (11–13). suPAR levels in patients with schizophrenia and depression and who attempted suicide were found to be higher than those in the healthy control groups (12, 13).

Although there are studies in the literature regarding the relationship between PKA, IL-6, and ADHD development, to our knowledge, there is no study investigating the relationship between suPAR and ADHD. Based on this information, the aim of the present study was to investigate the relationship between ADHD and the suPAR molecule, which is a stabilizing inflammatory marker, to contribute to the etiopathogenesis of ADHD, and to gain a new perspective on treatment methods.

MATERIALS and METHODS

Participants

Subjects were recruited from the Outpatient Clinic for Child and Adolescent Psychiatry Department of Erciyes University in Kayseri. Treatment-naive, 50 children (13 girls and 37 boys) aged 7–13 years, with a diagnosis of ADHD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5), were enrolled in the study. Exclusion criteria included the presence of neurological, metabolic, and endocrine diseases, acute or chronic infections, smoking, psychiatric diagnosis except conduct disorder, oppositional defiant disorder, enuresis, and encopresis. The control group consisted of 25 (11 girls and 14 boys) unrelated healthy volunteers aged 7–13 years who were not affected by a major physical/neurological illness or a psychiatric disorder, neurological, metabolic, and endocrine diseases, and acute or chronic infections.

The sociodemographic characteristics of the children and adolescents included in the study were assessed using the semi-structured sociodemographic information form prepared by the researcher. All children were interviewed using the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS-PL) by a child and adolescent psychiatrist (14, 15).

The diagnoses of ADHD were made according to the DSM-5 criteria. Parents completed the Conners' Parent Rating Scale (CPRS), and teachers completed the Conners' Teacher Rating Scale (16–19). The treatment of the patients was arranged after the completion of the research protocol.

The study was approved by the local ethics committee of Erciyes University Medical Faculty (no. 2017/129).

Materials

Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version

The K-SADS-PL scale was developed by Kaufman et al. (15) after the publication of DSM-IV in 1994. Turkish validity and reliability studies were performed by Gökler et al. in 2004 (14). K-SADS-PL allows the screening of >20 different psychiatric disorders.

Conners' Parent Rating Scale

The CPRS consists of a total of 48 items and of subtests questioning psychopathology. These subtests question attention deficiency and hyperactivity, behavioral difficulties, and anxiety symptoms. The Turkish version of the CPRS validity and reliability studies was done by Dereboy et al. (20).

Evaluation of suPAR Levels

Blood samples

Venous blood samples of patients and controls were drawn from an antecubital vein between 8:00 and 09:00 a.m. after an overnight fast. Blood samples collected in anticoagulated tubes were centrifuged for 10 min at 4000 rpm, and plasma was stored at –80 °C until assayed. Serum suPAR levels were measured using commercial enzyme-linked immunosorbent assay kits following the manufacturer's protocols (Biovendor Research and Diagnostic Products, Czech Republic) (sensitivity: 5.1, assay range: 7.8–500 pg/ml).

Statistical Analysis

Data were analyzed using the SPSS 20 (Statistical Package for the Social Sciences for Windows; SPSS Inc., Chicago, IL, USA) package program for statistical analysis. The normal distribution of continuous variables with two groups was evaluated by the Shapiro–Wilk test. The Independent Sample t-Test was used to compare variables with normal distribution, and the Mann–Whitney U test was used to compare variables with non-normal distribution between the groups. The Pearson chi- square test was used to compare categorical variables. Data are presented as mean±standard deviation for variables with normal distributions and as median (interquartile range) for variables with non-normal distributions. A p value <0.05 was considered as statistically significant for all analyses.

RESULTS

Our study group consisted of 50 (13 girls and 37 boys) children and adolescents with ADHD and 25 (11 girls and 14 boys) healthy children and adolescents. The mean age of the ADHD group was 9.16±1.74 years, and the mean age of the control group was 9.88±1.53 years. There was no significant difference between the groups when both groups were compared with respect to mean age (Z=1.831, p=0.067) and gender (x^2=2.482, p=0.115).

Comorbid conditions for ADHD included conduct disorder (18%), oppositional defiant disorder (30%), enuresis (8%), and encopresis (2%).

It was determined that 70% (n=35) was combined presentation of ADHD, and 30% (n=15) was attention deficit presentation of ADHD.

ADHD subgroups were evaluated with respect to Conners' parent scores. The mean score of patients with ADHD attention deficit presentation was 48.20±21.17, and the mean score of patients with ADHD combined presentation was 49.86±19.31 (Table 1).

The ADHD group was assessed with respect to Conners' teacher scores, ADHD was 41.49±13.45 points in combined presentation, and ADHD was 29.67±6.09 in presentation of attention deficit (Table 1).

Table 1. Conners' scores of ADHD presentations

	ADHD presentation	Mean±SD
Conners' parent form	ADHD-attention deficit (n=15)	48.20±21.17
	ADHD-combined (n=35)	49.86±19.31
Conners' teacher form	ADHD-attention deficit (n=15)	29.67±6.09
	ADHD-combined (n=35)	41.49±13.45

ADHD: Attention deficit hyperactivity disorder; SD: Standard deviation

The mean plasma suPAR level of patients with ADHD was 2.92±1.74 ng/ml, the controls suPAR level was 2.54±1.05 ng/ml, and there was no significant difference between ADHD and the controls (Z=0.084, p=0.933) suPAR levels (Table 2). No correlation was found between plasma suPAR levels and ADHD severity as assessed by Conners' parent and teacher scales (p>0.05).

suPAR levels evaluated with respect to ADHD presentations were 2.75±1.58 for combined presentation and 3.34±2.06 for attention deficit presentation. There was no statistically significant difference between the two presentations of ADHD with respect to suPAR levels (Z=0.922, p=0.357) (Table 3).

There was no statistically significant difference in suPAR levels between the control group and combined presentation of ADHD and attention deficit presentation of ADHD (Z=0.316, p=0.752 and Z=0.798, p=0.425, respectively).

DISCUSSION

In our study, although the plasma suPAR level, which is a marker of inflammation and immunological activation, tended to be higher in the ADHD group than in the controls, the difference was not statistically significant. There was no significant difference between the ADHD subgroups and controls. No correlation was found between plasma suPAR levels and ADHD severity as assessed by Conners' parent and teacher scales.

There is a limited study on the neuroinflammatory bases of ADHD (21), whereas there is no study evaluating suPAR level with ADHD. Recent studies have demonstrated that there is a possible relationship between immune processes and inflammatory mediators (e.g.,., cytokines) in ADHD. The dysregulation of proinflammatory cytokines is suggested to play an important role in the etiopathogenesis of ADHD (22). Oades et al. reported that IL-2, IL-6, IL-10, and IL-16 levels tend to increase, and IL-13 and interferon gamma levels are higher in children with ADHD group than in other groups (2). Another study showed that IL-6 and IL-10 levels are higher in subjects with ADHD than in the control group (23). It has been emphasized that PKA, which controls the inflammatory responses in the brain, controls the expression of some important cytokines, such as IL-6, induces the production of the uPAR (5, 7), and plays a critical role in brain development. In one study, the authors suggested that plasminogen activators, inhibitors, and uPAR are integral to the pathogenesis of depression (24). In addition, it was reported that the cerebral cortical neurons secrete uPAR during the course of post-hypoxic recovery, and uPAR plays a role in the regeneration of the central nervous system (25). Although hypoxia is known to have been implicated in the etiology of ADHD (26), to the best of our knowledge, there is no study of the association of ADHD and uPAR.

Inflammatory stimulant-releasing proteases cause circulating uPAR release from the cell surface and formation of the soluble form of suPAR. Higher suPAR levels are considered as an indicator of low-grade inflammation and have a prognostic value in various diseases (27). Inflammatory parameters, such as C-reactive protein (CRP), IL-6, and procalcitonin, correlate with suPAR levels (28). Among the psychiatric disorders, suPAR has only been studied in schizophrenia and bipolar disorder (BD). In assessing the studies evaluating the relationship between psychiatric disorders and suPAR, suPAR levels were significantly higher than healthy controls in a study investigating the role of inflammation in the etiology of schizophrenia (12). In another study, the plasma suPAR

Table 2. Comparison of groups with respect to suPAR levels

	ADHD (n=50) Median (1st–3rd quartiles)	Control (n=25) Median (1st–3rd quartiles)	Comparison
suPAR levels (ng/ml)	2.30 (1.98)	2.20 (0.75)	Z=0.084 p=0.933

ADHD: Attention deficit hyperactivity disorder

Table 3. Comparison of presentations of ADHD with the control group with respect to suPAR levels

	ADHD combined (n=35) Median (1st–3rd quartiles)	ADHD attention deficit (n=15) Median (1st–3rd quartiles)	Comparison
suPAR levels (ng/ml)	2.20 (1.70)	2.70 (2.60)	Z=0.92 p=0.357

ADHD: Attention deficit hyperactivity disorder

levels of male patients with schizophrenia who were in acute state were evaluated and compared with healthy controls, but there was no significant difference (11). Serum suPAR levels are found to be lower in patients with BD with acute periods than in patients with euthymic episodes and healthy controls (29). In addition, it was found that individuals with high suPAR levels were more likely to attempt to commit suicide (13).

Although a possible role of inflammation in the etiology of ADHD is shown in studies performed, we found no significant relationship between ADHD and suPAR levels. More studies are needed with suPAR in the pathophysiology of psychiatric disorders and should be evaluated in more detail in relation to ADHD.

Limitations

Further longitudinal studies with larger sample size might be more explanatory in understanding the role of suPAR in the pathophysiology of ADHD. In addition, white blood cell count, CRP, and suPAR levels were not correlated in our study. Moreover, unevaluated factors, such as history of alcohol abuse and liver disease, high body mass index, unhealthy diet, and low- to high-density lipoprotein levels, should be evaluated with suPAR levels.

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Ethics Committee Approval: The study was approved by the local ethics committee of Erciyes University Medical Faculty (no. 2017/129).

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

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