EREDETI KÖZLEMÉNY

ORIGINAL ARTICLE

Is autism spectrum disorder an inflammation?

Yüksel Sümeyra NARALAN[1], Abdulhakim Hasan GÜL[2], Konca ALTUNKAYNAK[3]

[1]Rize Recep Tayyip Erdogan University Faculty of Medicine, Department of Child and Adolescent Psychiatry, Rize, Turkey
[2]Ardahan State Hospital, Department of Medical Biochemistry, Ardahan, Turkey
[3]Erzurum Regional Training and Research Hospital, Department of Medical Biochemistry, Erzurum, Turkey

| English | https://doi.org/10.18071/isz.76.0212 | www.elitmed.hu

Correspondent:
Yüksel Sümeyra NARALAN, Rize Recep Tayyip Erdoğan Training and Research Hospital, Child and Adolescent Psychiatry, Rize, Turkey.
Tel: 05352932150,
e-mail: drsumeyrakaragoz@yahoo.com
https://www.orcid.org/0000-0002-7788-5711

Érkezett:
2022. augusztus 24.
Elfogadva:
2022. október 18.

Background and purpose – In our study, we aimed to evaluate inflammation by measuring serum Adenosine deaminase and dipeptidyl peptidase IV levels of individuals diagnosed with autism spectrum disorder and to determine its relationship with the Childhood Autism Rating Scale.
Methods – 37 children aged 2-12 years with a diagnosis of autism spectrum disorder and 27 children aged 2-12 years without any psychiatric disease were included in the study. Psychiatric examination and clinical evaluation according to DSM-5 diagnostic criteria for the diagnosis of autism spectrum disorder were performed on the children included in the study. The Childhood Autism Rating Scale was filled in by the researcher by interviewing the parents of the children diagnosed with autism spectrum disorder. 5 ml of venous blood samples were taken from the children in both groups in the morning on a full stomach.
Results – There was no statistically significant difference between the groups in terms of age, gender, and sociodemographic data. While serum adenosine deaminase levels were found to be statistically significantly higher in the group with autism spectrum disorder, serum dipeptidyl peptidase IV levels were found to be significantly lower. A positive correlation was found between dipeptidyl peptidase IV and Childhood Autism Rating Scale.
Conclusion – We think that inflammation may play a role in the etiology of autism spectrum disorder due to altered adenosine deaminase and dipeptidyl peptidase IV levels in children with autism spectrum disorder.

Keywords: autism spectrum disorder, adenosine deaminase, child, dipeptidyl peptidase IV

Az autizmus spektrumzavar gyulladás?
Naralan YS, MD; Gül AH, MD; Altunkaynak K, MD

Háttér és cél – Vizsgálatunkban a gyulladás értékelését tűztük ki célul autizmus spektrumzavarral diagnosztizált személyek szérumának adenozin-deamináz- és dipeptidil-peptidáz IV szintjének mérésével. Célunk volt továbbá a gyulladás és a Childhood Autism Rating Scale (Gyermekkori autizmust értékelő skála) közötti kapcsolat meghatározása.
Módszerek – A vizsgálatba 37, autizmus spektrumzavar diagnózissal rendelkező 2–12 éves gyermeket és 27, pszichiátriai betegséggel nem rendelkező 2–12 éves gyermeket vontunk be. Az autizmus spektrumzavar diagnózisának megállapítása érdekében a vizsgálatba bevont gyermekeken pszichiátriai vizsgálatot és klinikai értékelést végeztek, a DSM-5 diagnosztikai kritériumai szerint. A gyermekkori autizmust értékelő skálát a kutató az autizmus spektrumzavarral diagnosztizált gyermekek szüleivel készített interjúk segítségével töltötte ki. Mindkét csoportban 5 ml vénás vérmintát vettek a gyermekektől reggel, teli gyomorral.
Eredmények – A csoportok között nem volt statisztikailag szignifikáns különbség az életkor, a nem és a szociodemográfiai adatok tekintetében. Míg a szérum adenozin-deamináz szintje statisztikailag szignifikánsan magasabbnak bizonyult az autizmus spektrumzavarral küzdő csoportban, addig a szérum dipeptidil-peptidáz IV szintje szignifikánsan alacsonyabbnak bizonyult. Pozitív korrelációt találtunk a dipeptidil-peptidáz IV szintje és a gyermekkori autizmust értékelő skála pontszáma között.
Következtetés – Úgy gondoljuk, hogy a gyulladás szerepet játszhat az autizmus spektrumzavar etiológiájában, mivel az autizmus spektrumzavarban szenvedő gyermekeknél megváltozott az adenozin-deamináz- és dipeptidil-peptidáz IV szintje.

Kulcsszavak: autizmus spektrum zavar, adenozin-deamináz, gyermek, dipeptidil-peptidáz IV

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by limitations in social communication, interaction, and restrictive repetitive diseases[1]. Although the etiopathogenesis of ASD is not known exactly, it is known to occur due to the interaction of genetic predisposition and environmental factors[2]. It is known that children with ASD often show immune system dysfunction[3]. It is thought that this immunological dysfunction is associated with changes in the complex and prenatal immune environment, which in turn causes an increased risk of autism[4]. Considering all these pieces of information, studies on the immune system were conducted in children with ASD. As a common finding in studies conducted in patients with ASD, it was observed that there was an increase in the Th2, a proportional increase in helper T2 (Th2) phenotype, and helper T1 cells; a decrease in Th1 cells and an atypical adaptive T cell response in which the ratio changed in favor of Th2[5]. It has been reported that there is a possible deterioration in Th1 and Th17 responses in children with ASD, making them more vulnerable to certain microbial infections[6, 7]. In addition, a decrease in the frequency of regulatory T (Treg) cells has been observed in children with ASD and it has been found that this decreased Treg level causes irregular Th1 and Th17 functions, and an increase in Th2[5]. These findings add to the theory that autism with altered T-cell activation may be a state of chronic inflammation in the central nervous system.

Adenosine deaminase (ADA) and dipeptidyl peptidase IV (DPP IV) are enzymes required for normal immune responses, especially involved in T lymphocyte activation and formation of cellular immunity. DPP IV is an exopeptidase that cleaves dipeptides. DPP IV is originated from T lymphocytes and shows its main activity in helper T cells. Cleavage of many peptides plays a key role in the T cell-mediated immune response and may result in an increase or decrease in the activity of peptides[8, 9]. When DPP is found in membrane-bound form in IV lymphocytes, it is called CD26, and its serum level reflects the amount of this membrane-bound form[9, 10]. CD26 plays a role in T cell activation and cytokine production. ADA is a purine metabolism enzyme, and it is found in higher amounts in T lymphocytes than in B lymphocytes[11]. ADA enzyme level is increased mainly during mitogenic and antigenic responses of lymphocytes. ADA inhibitors are known to inhibit lymphocyte blastogenesis. In addition, ADA ensures the destruction of adenosine, which accumulates in the body and has toxic effects on cells and DNA[12]. It can be found as an ectoenzyme in the form of membrane-bound DPP, which we call ADA CD26. The co-existence of ADA and CD26 leads to T cell activation. ADA and DPP IV have been investigated immunologically in some psychiatric disorders. In the studies, serum DPP IV and ADA activities were examined in adult patients with depression and panic disorder, and it was found to be higher than in healthy controls[11, 13, 14].

In the light of all these information, we aimed to evaluate inflammation by measuring serum ADA and DPP IV levels of individuals diagnosed with ASD and to determine its relationship with the Childhood Autism Rating Scale (CARS).

Material and method

Participants

A total of 64 children aged 2-12 years who applied to X Regional Training and Research Hospital Child and Adolescent Psychiatry Outpatient Clinic between May 2021 and September 2021 were included in the study. 37 children diagnosed with ASD according to DSM-5 diagnostic criteria were included in the study. The patient group consisted of children who were diagnosed with ASD for the first time and did not receive medication, while the control group consisted of 27 healthy children in the same age group who applied to the Erzurum Regional Training and Research Hospital Pediatrics Polyclinic and did not have any psychiatric disorders.

Inclusion criteria for both groups were being between the ages of 2-12, not having a chronic medical disease, especially immune system pathology, not having any infection signs and using drugs related to it in the last 1 week, and not entering puberty. To exclude possible diseases, clinical examinations of all children were performed by the pediatrician, and the results of routine laboratory tests were evaluated, including biochemical, hematology, and thyroid function measurements. None of the kids were taking nutritional supplements such as antioxidants etc.

Procedure

A clinical interview based on DSM-5 diagnostic criteria was conducted by the first author of the study to diagnose all children and their parents who met the inclusion criteria and volunteered for the study. The interviewer filled out the Childhood Autism Rating Scale (CARS) by asking the families of the children diagnosed with an autism spectrum disorder. The study was approved by the Erzurum Regional Training and Research Hospital Clinical Research Ethics Committee (19.04.2021 decision no: 2021/08-154). All families were informed verbally and in writing about the study and signed the informed consent form prepared in accordance with the rules set out in the Declaration of Helsinki.

Venous blood samples in the amount of 5 ml were taken from the participants of both groups, between 9:00 and 11:00 in the morning on a full stomach. Collected blood samples were centrifuged at 4000 *x*g relative centrifugal force for 10 minutes to separate the plasma from the cells. After separating the obtained sera, they were stored in eppendorf tubes at -80°C until biochemical

analysis. Serum ADA and DPP IV levels were measured by ELISA method and results were given in ng/mL. The budget of the study was covered by the researchers, and no financial support was received.

Statistical analysis

After the data were obtained, statistical evaluations were made in the "SPSS (Statistical Package for Social Sciences) 22.0" package program. Kolmogorov-Smirnov test was used to test whether the variables obtained from the data were suitable for normal distribution. Since the age and gender parameters of the sociodemographic data were not normally distributed, they were analyzed using the Mann-Whitney U test. Independent sample T test was used to compare serum ADA and DPP IV levels of ASD and control groups. Spearman Correlation test was applied to the data to examine the relationship between serum ADA and DPP IV levels of the ASD group and CARS. Statistical significance level (p) was accepted as 0.05.

Results

No significant difference was found between the ASD (n=37) and Control (n=27) groups in terms of age (9.19±2; 9.7±1.9, respectively) (p>0.05). Gender distribution was similar between groups (p>0.05). Patient and control groups did not differ significantly in parents' education and income levels, or any medical condition or adverse life events. Likewise, no significant difference was found between the groups in terms of delivery type or trauma, surgical operation, and autoimmune disease in the postpartum period. In addition, there was no difference in the use of drug supplements by the children and in the history of psychiatric disease in the parents.

Serum ADA levels of the patients were significantly higher than those of the control group **(Table 1)**. Serum DPP IV levels of the patients were found to be significantly lower than those of the control group **(Table 1)**. A significant positive correlation was found between DPP IV levels and CARS scores **(Table 2)**.

Table 1. *Comparison of ASD and control group data*

	ASD (n=37)	Control (n=27)	p Value
Demographic Data			
Age (mean±sd)	5 ± 2,6	6.4± 3.8	p = 0.260*
Gender (M/F)	27/10	18/9	p = 0.588*
Biochemical data			
ADA (mean±sd)	110±23.9	86±30.6	p=0.001**
DPP IV (mean±sd)	354±201.8	524±215	p=0.002**

**Mann-Whitney U Test, **Independent sample T-Test*

Table 2. *Correlation analysis of CARS and DPP IV levels in the ASD group*

		CARS
Serum DPP IV	*r*	0,831
	p	0,000

Spearman correlation test, r = correlation coefficient

Discussion

In our study, we aimed to evaluate the place of inflammation in the etiology of autism spectrum disorder by measuring the serum ADA and DPP IV levels of children diagnosed with ASD and to determine its relationship with the Childhood Autism Rating Scale (CARS).

In our study, we found that the serum level of the ADA enzyme, which plays an important role in the T cell-mediated immune response, is higher in children with ASD than in healthy children. In their study investigating purine metabolism in children with ASD, *Stubb* et al. found that serum ADA levels were significantly lower than in healthy controls[15]. *Bottini* et al. investigated the ADA gene polymorphism in children with ASD and claimed that the decrease in ADA activity due to ADA gene polymorphism may be a risk factor for the development of autism[16]. In the study of *Hassan* et al. in which they evaluated possible metabolic changes in boys with ASD in 2019, serum ADA levels were found to be lower than in healthy controls[17]. In our study, the high serum ADA level, which has an important role in the immune system, in children with ASD is a finding that is inconsistent with the previous literature. The increased level of adenosine deaminase may have occurred due to an increase in the mitogenic and antigenic responses of lymphocytes in children with ASD. The inconsistency of our study with the decrease in serum ADA levels in the literature suggests that the immune response may be increased in individuals with newly diagnosed ASD and then decreased with negative feedback. In addition, increased serum ADA levels can be interpreted as increased levels of the adenosine deaminase enzyme as a reaction to the accumulation of adenosine and causing deviations in DNA synthesis.

In our study, we found that DPP IV levels, which play an important role in T cell-mediated immune response and are closely related to ADA, are statistically significantly lower in children with ASD compared to healthy controls. In a study conducted by *Frenssen* et al. in 2015, they compared 18 cases diagnosed with ASD between the ages of 13-20 with healthy individuals and

found no significant difference between DPP IV levels[18]. However, the relationship between the Child Behavior Rating Scale (CBCL) and DPP IV was examined, and a significant positive relationship was found especially in the attention, aggression, and expressive behaviors subscales. In their study, *Hunter* et al. investigated opioid peptides and DPP IV in individuals with autism spectrum disorder, 10 children aged 2-10 years with ASD were compared with adults aged 25-55 years who developed normally, and serum DPP IV levels were evaluated by measuring ELISA[19]. In that study, no statistically significant difference was found between the two groups, and CD26-labeled cells were measured as a percentage, and it was found that these cells were lower in children with ASD than in healthy adults. In our study, the finding of low serum DPP IV in children with ASD does not seem to be compatible with the literature. However, the fact that Hunter et al.[19] found CD26-labeled cells to be low may be consistent with our finding. The comparison of our study with a healthy group, similar in age and gender, with newly diagnosed ASD, and the selection of the age group as the pre-adolescent age group may cause different results from previous studies in the literature. Decreased levels of DPP IV may be found as negative feedback against inflammation in children with ASD. In addition, as far as we know, it is seen that ADA levels were not determined and the relationship between them could not be determined in other studies conducted with DPP IV, which is the first study in the literature to evaluate serum ADA and DPP IV levels together in children with autism. The increase in serum ADA levels in our study may have shown that the increase in T cell mediated immune response may have decreased the amount of membrane DPP IV, that is, CD26, by binding with ADA.

The limitations of our study are that it had a small number of samples, it included data from a single center, and inflammation indicator cytokines were not included in the study. The strengths of the study are that the participants did not use drugs, the study had an adolescent age group, individuals with medical disease comorbidities were not included, and similar groups were compared in terms of age and gender.

In summary, to the best of our knowledge, this is the first study in which serum ADA and DPP IV were investigated together in children with ASD. Our study shows that there may be a relationship between inflammation and ASD in children. In addition, this study is a precursor to future studies in which the effect of immune system modulation on symptoms of children with ASD is evaluated.

Conclusion

We found that the serum ADA and DPP IV levels, which play a role in the T cell-mediated immune response of children with ASD, were significantly different those of healthy children. As far as it is known, our study is the first in the literature in which serum ADA and DPP IV levels were evaluated together in children with ASD. Although many studies have been conducted on neuroinflammation and immune systems in children with ASD, we think that more studies should be conducted in various age groups by associating them with symptoms, since there are not enough studies on this subject for ADA and DPP IV. Our research is important in that it draws attention to further evaluation of ASD in terms of inflammation. There is a need for studies with large samples, comparing children, adolescents, and adult age groups, and evaluating cytokines and inflammatory cells, which are indicators of inflammation.

———

AUTHORSHIP CONTRIBUTION STATEMENT – Abdulhakim Hasan Gül conducted the analyses. Konca Altunkaynak conducted the analyses, revised the manuscript critically, and wrote the final version of the article. Yüksel Sümeyra Naralan designed the study, collected the data, wrote a first draft, and revised the manuscript critically. All authors approved the final version of the manuscript.

References

1. *American Psychiatric Association.* Diagnostic and statistical manual of mental disorders. Fifth Edition. Köroğlu E, Trans. Ed. Ankara: Physicians Publication Association 2013. p. 41-51. https://doi.org/10.1176/appi.books.9780890425596
2. *Herbert MR.* Contributions of the environment and environmentally vulnerable physiology to autism spectrum disorders. Curr Opin Neurol 2010;23:103-10. https://doi.org/10.1097/WCO.0b013e328336a01f
3. *Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J*. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 2011;25:40-45. https://doi.org/10.1016/j.bbi.2010.08.003
4. *Goines PE, Croen LA, Braunschweig D, Yoshida CK, Grether J, Hansen R, et al.* Increased midgestational IFN-γ, IL-4, and IL-5 in women giving birth to a child with autism: a case- control study. Mol Autism 2011;2:13. https://doi.org/10.1186/2040-2392-2-13
5. *Noriega, DB, Savelkoul HF*. Immune dysregulation in autism spectrum disorder. European journal of pediatrics 2014;173(1):33-43. https://doi.org/10.1007/s00431-013-2183-4
6. *Geschwind DH*. Advances in autism. Annu Rev Med 2009;60:367-80. https://doi.org/10.1146/annurev.med.60.053107.121225
7. *Jyonouchi H, Geng L, Streck DL, Totuner A*. Immunological characterization and transcription profiling of peripheral blood (PB) monocytes in children with autism spectrum disorders (ASD) and specific polysaccharide antibody deficiency (SPAD): a case study. J Neuroinflammation 2012;9(4):1-12. https://doi.org/10.1186/1742-2094-9-4

8. *Maes M, Meester I, Vanhoof G, Scharpe S, Bosmans E, Vandervorst C, et al*. Decreased serum dipeptidyl peptidase IV activity in major depression. Biol Psychiatry 1991;30:577-86. https://doi.org/10.1016/0006-3223(91)90027-J
9. *Elgün S, Keskinege A, Akan H, Karaca L*. Serum dipeptidyl peptidase IV activity correlates with the T cell CD26 antigen Clin Chem Lab Med 1999;37(8):839-40. https://doi.org/10.1515/CCLM.1999.126
10. *Van West D, Monteleone P, Di Lieto A, Meester I, Durinx C, Scharpe S.* Lowered serum dipeptidyl peptidase IV activity in patients with anorexia and blumia nervosa. Eur Arch Psychiatry Clin Neurosci 2000;250:86-92. https://doi.org/10.1007/s004060070040
11. *Herken H, Akyol O, Yılmaz R, Tutkun H, Savas H, Ozen ME, et al*. Nitric oxide, adenosine deaminase, xanthine oxidase and superoxide dismutase: alterations by antidepressant treatment. Hum Psychopharmacol Clin Exp 2006;21:53-9. https://doi.org/10.1002/hup.742
12. *Lizuka H, Koizumi H, Kamigaki K, Aoyagi T, Miura Y*. Two forms of adenosine deaminase in pig epidermis. J Dermatol 1981;8:91-5. https://doi.org/10.1111/j.1346-8138.1981.tb02573.x
13. *Rapaport MH*. Circulating lymphocyte phenotypic surface markers in anxiety disorder patientsand normal volunteers. Biol Psychiatry 1998;43:458-63. https://doi.org/10.1016/S0006-3223(97)00263-1
14. *Yarpuz AY, Yilmaz A, Soykan A, Elgün S, Kumbasar H*. Levels of Adenosine Deaminase and Dipeptidyl Peptidase IV in Patients with Panic Disorder. Turkish Journal of Psychiatry 2008;19(2).
15. *Stubbs G, et al.* Adenosine deaminase activity decreased in autism. Journal of the American Academy of Child Psychiatry 1982;21(1):71-4. https://doi.org/10.1097/00004583-198201000-00012
16. *Bottini N, et al*. Autism: evidence of association with adenosine deaminase genetic polymorphism. Neurogenetics 2001;3(2):111-3. https://doi.org/10.1007/s100480000104
17. *Hassan MH, et al*. Possible metabolic alterations among autistic male children: clinical and biochemical approaches. Journal of Molecular Neuroscience 2019;67(2):204-16. https://doi.org/10.1007/s12031-018-1225-9
18. *Frenssen F, et al*. Prolyl endopeptidase and dipeptidyl peptidase IV are associated with externalizing and aggressive behaviors in normal and autistic adolescents. Life sciences 2015;136:157-62. https://doi.org/10.1016/j.lfs.2015.07.003
19. *Hunter LC, et al*. Opioid peptides and dipeptidyl peptidase in autism. Developmental Medicine and Child Neurology 2003;45(2):121-8. https://doi.org/10.1111/j.1469-8749.2003.tb00915.x