Serum zonulin levels are correlated with symptom severity independent from body mass index and gender in children with attention deficit hyperactivity disorder

Dikkat eksikliği hiperaktivite bozukluğu olan çocuklarda serum zonulin seviyelerinin belirti şiddeti, vücut kitle indeksi ve cinsiyet ile ilişkisi

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SUMMARY

Objective: It has been recently emphasized that neurodevelopmental disorders such as schizophrenia, autism spectrum disorder and attention deficit hyperactivity disorder (ADHD) may be related to the gut-brain axis. Zonulin is a protein that changes the integrity of tight junctions between gastrointestinal mucosa cells. We aimed to investigate serum zonulin levels and its relationship with symptom severity in children with ADHD. **Method:** 21 ADHD patients and 19 controls were included. Zonulin levels were obtained from blood specimens. Clinical severity of the ADHD symptoms was evaluated by Conner's Parents Rating Scale-Revised/Long Form (CPRS-R/L) and Conner's Teacher Rating Scale-Revised/Long Form (CTRS-R/L) in ADHD group. **Results:** There was no significant difference between the groups in terms of age, gender and body mass index. Mean serum zonulin level of the ADHD group was 13.45±9.08 and 21.32± 19.96 in the control group. There was no significant difference between groups (t=1.99, p=0.51). Significant correlation was found (R=0.82, p<0.01) between serum zonulin levels and CTRS-R/L scores in the ADHD group. This correlation persisted when BMI and sex variables were controlled (R=0.85, p<0.01). **Discussion:** We have found significant correlation between ADHD symptom severity and serum zonulin levels, whereas there was no significant difference between children with ADHD and controls.

Key Words: Attention deficit, zonulin, children, intestinal, gut

ÖZET

Amaç: Şizofreni, otizm spektrum bozukluğu ve dikkat eksikliği hiperaktivite bozukluğu (DEHB) gibi nörogelişimsel bozuklukların bağırsak-beyin ekseniyle ilişkili olabileceği son zamanlarda vurgulanmıştır. Zonulin, gastrointestinal mukoza hücreleri arasındaki sıkı bağlantıların bütünlüğünü değiştiren bir proteindir. DEHB'li çocuklarda serum zonulin düzeylerini ve semptom şiddeti ile ilişkisini araştırmayı amaçladık. **Yöntem:** 21 DEHB hastası ve 19 kontrol dahil edildi. Zonulin seviyeleri kan örneklerinden elde edildi. DEHB belirtilerinin klinik şiddeti, Conners' Ebeveyn Derecelendirme Ölçeği-Gözden Geçirilmiş/Uzun Form (CPRS-R/L) ve Conners' Öğretmen Derecelendirme Ölçeği-Gözden Geçirilmiş/Uzun Form (CTRS-R/L) ile değerlendirildi. **Bulgular:** Gruplar arasında yaş, cinsiyet ve vücut kitle indeksi (VKİ) açısından anlamlı fark yoktu. DEHB grubunun ortalama serum zonulin düzeyi 13.45 ± 9.08 ve kontrol grubunda 21.32 ± 19.96 idi. Gruplar arasında anlamlı fark yoktu (t = 1.99, p = 0.51). DEHB grubunda serum zonulin düzeyleri ile CTRS-R / L skorları arasında anlamlı korelasyon (R = 0.82, p <0.01) bulundu. Bu korelasyon, VKİ ve cinsiyet değişkenleri kontrol edildiğinde de devam etti (R = 0.85, p <0.01). **Sonuç:** DEHB semptom şiddeti ile serum zonulin düzeyleri arasında anlamlı bir ilişki bulunmakla birlikte, DEHB olan çocuklar ve kontroller arasında anlamlı bir fark bulunamamıştır.

Anahtar Sözcükler: Dikkat eksikliği, zonulin, çocuk, bağırsak

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INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a condition that starts from early childhood and causes loss of function in various areas such as school, social environment, work and family life due to symptoms in the areas of inattention, impulsivity and hyperactivity. According to data obtained from different communities around the world, the prevalence of ADHD is approximately 5%(1). Twin studies show that ADHD has 70-80% heritability (2). In a study which was conducted in Turkey, the prevalence of ADHD was reported to be 12.4% (3). The contribution of many environmental factors such as alcohol and smoking during pregnancy, low birth weight, preterm birth, pesticides containing organophosphate, zinc and lead exposure on the etiology of ADHD has been examined until today. Despite this, studies have reported that those other than preterm birth may be affected by some unmeasurable familial confounding factors and their role in etiology has not been elucidated (4).

Although time management and information processing problems are frequently mentioned in the pathophysiology of ADHD, some changes in inflammatory mechanisms are thought to play a role in the formation of neuropsychiatric diseases such as schizophrenia, bipolar disorder, and post-traumatic stress disorder through pathways such as glial activation, neuronal damage, increase in oxidative stress, alteration of the neurotransmitter metabolism and the blood-brain barrier (5). There are some data indicating that the risk of ADHD increases in the presence of atopic immune disorders such as eczema, asthma, rheumatoid arthritis, type 1 diabetes and hypothyroidism, and evidence has been obtained from recent studies indicating that serum cytokine levels of individuals with ADHD are higher than the normal population (6).

It has become a frequently emphasized issue in recent research that the gut microbiota is important for the physiology and development of the host, and the deterioration of the microbiota has an effect on brain functions and behavior. It is known that the alteration of the intestinal flora for various reasons activates some pathways and causes the tight connections between cells to loosen, and that the loosened epithelium - that is, the "leaky gut", plays a role in the development of autoimmune diseases by increasing the antigen exposure of the organs (7). In recent years, it has been also reported in some cases that neurodevelopmental disorders may also occur through this mechanism (8). In addition to immune-mediated mechanisms, it is also suggested that some bacteria that grow excessively in microbiota facilitate the emergence of ADHD symptoms by increasing the production of neurotransmitters such as dopamine, serotonin and GABA. Although it is not known exactly whether these molecules can directly cross the blood brain barrier, it has been reported that the modified microbiota can contribute to neurotransmitter synthesis by causing an increase in peripheral tryptophan (9).

Zonulin, the precursor to haptoglobin (HP2), is a protein that causes changes in the integrity of tight junctions between gastrointestinal mucosa cells. Enteric bacteria and intestinal tissue exposed to gliadin increase zonulin secretion through chemokines (10,11). It has been reported that zonulin, which is higher in serum samples of celiac patients compared to healthy controls, is also associated with the etiology of neuropsychiatric disorders such as chronic inflammatory demyelinating polyneuropathy, multiple sclerosis (MS), and schizophrenia (12,13). There is a study in which increased serum zonulin levels were found in individuals with ADHD diagnosis compared to the control group (8). The number of studies examining the relationship between parameters related to intestinal permeability and ADHD in this age group is very limited.

In this study, we examined the intestinal permeability of individuals with ADHD who did not receive any medical treatment by determining their serum zonulin levels and we aimed to contribute to the literature by investigating the relationship between these levels and symptom severity.

METHOD

Study Sample

This study was conducted in Aksaray University Medical Faculty. Participants were recruited from the outpatient clinic of child and adolescent psychiatry. Children and adolescents within 8-12 years old who have diagnosed as ADHD and admitted to the clinic for the first time have been included in the patient group (n=21). Nine ADHD patients have ODD (oppositional defiant disorder) comorbidty. Newly diagnosed and psychotropic medication-naive patients were selected. Patient group have been compared with healthy controls (n=19) similar to the patient group in terms of age and sex. Exclusion criteria for the ADHD group were determined as major physical, allergic, endocrine or neurologic (such as epilepsy) diseases; those with body mass index (BMI) percentile ≥ 95%; those who use corticosteroids or any other drugs that affect the immune system in any time; and having an active infection within the past month. Those with comorbid depression, anxiety, mental retardation, autism spectrum disorder (ASD) and psychotic disorders were excluded from the study.

Control group was recruited from outpatient clinic for pediatric at the same hospital. The Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime Version-DSM-5 (K-SADS-PL-DSM-5) was used to screen healthy controls for psychiatric disorders by psychiatrist after physical examination by a pediatrician. The following exclusion criteria were implemented for control group: the existence of psychiatric disorders such as mental retardation, ASD, ADHD, schizophrenia, bipolar disorder, major depression, obsessive-compulsive disorder and anxiety disorders; major physical, allergic, endocrine or neurological diseases; those with body mass index (BMI) percentile ≥ 95%; those who use corticosteroids or any other drugs that affect the immunological system in any time and having an active infection within the past month. The control group consisted of healthy children and adolescents matched by age and sex who applied to the hospital for a routine checkup. The study was reviewed and approved by the Ethics Committee at Ankara City Training and Research Hospital Ethical Committee. The study's subjects, and parents of the subjects were briefed about the purpose of the study, and written consent was obtained from each of them.

Tools

Demographic and clinical information have been reported in a form prepared by researchers. BMI were calculated for each patient. Each patient underwent a detailed diagnostic evaluation by child and adolescent psychiatrist by using The Schedule for Affective Disorders and Schizophrenia for School-Aged Children, Present and Lifetime Version-DSM-5 (K-SADS-PL-DSM-5) (15). Conners' Parents Rating Scale and Conners' Teacher Rating Scale were used during the diagnostic procedure.

Conners' Parents Rating Scale- Revised/Long Form (CPRS-R/L): CPRS-R/L consists subscales of cognitive problems/inattention, oppositionality, hyperactivity, anxiety-shyness, perfectionism, social problems, and psychosomatic symptoms. DSM-IV index, ADHD index, and Global Index according to DSM-IV diagnostic criteria are used for contribution. Parents are requested to answer items taking the last month into consideration. Each item is answered as one the following four choices: Not true at all (rarely), somewhat true (sometimes), quite true (mostly), and completely true (almost always) (16).

Conners' Teachers Rating Scale-Revised/Long Form (CTRS-R/L): CTRS-R/L includes 38 items, 6 subscales, and additionally 3 assistant scales based on the ADHD symptoms in DSM-IV: ADHD index, Conner's Global Index and DSM-IV Symptoms Index. Teachers are requested to evaluate children/adolescent's behaviors while taking the last one month into consideration. For each item, four answer choices: Not true at all (rarely), somewhat true (sometimes), quite true (mostly), and completely true (almost always) (17).

Biochemical Analysis

Venous blood specimens were collected between

8.00 and 10.30 a.m. after overnight fasting. The blood samples were centrifuged at 4000 rpm for 5 min at 4Co, and the separated serum was stored at -80Co until the time of the assay. Serum zonulin levels were measured using commercial enzyme-linked immunosorbent assay kits following the protocols of the manufacturers (Serum Zonulin Sunredbio, China; Cat No:201-12-5578; Lot: 202003). The results of the analysis were presented in ng/dl. Intra- and inter-assay coefficients of variation of zonulin kit was intra-assay CV <10%, inter-assay CV <10%.

Statistical Analysis

The statistical analysis was conducted using SPSS 23. Shapiro–Wilkinson test was used to determine the normality of the variable distribution. Relationships between dichotomous variables were assessed with the x^2 test. The scale scores and biochemical parameters of the patient and control group were compared using the Mann–Whitney U test according to the distribution properties. The correlation between serum zonulin levels and the psychological test scores was evaluated by the Spearman correlation coefficient. A value of p less than 0.05 (two-tailed) was considered statistically significant.

RESULTS

Twenty-one ADHD patients and nineteen healthy controls were included in this study. There was no significant difference in terms of sex (x^2=2.50, p=0.11). In the ADHD group, the mean age was 9.85 (SD=2.79) years and the control group's mean age was 8.72 (SD=3.27) years. There was no significant difference in terms of age between ADHD and control groups. (u=160,5, z=-1.05, p=0.29). The mean BMI was 21.79 in the patient group (SD=1.88) and 21.99 (SD=2.47) in the controls. There was no significant difference regarding mean BMI (u=190, z=-0.25, p=0.81). Demographic variables were presented in Table 1.

When we examined the relation of Conners' scores between the groups, CTRS total score (u=50.5, z=-4.03, p <0.001), CTRS ADHD index (u=63.5, z=-3.70, p <0.001), CPRS total score (u=0, z=-5,40, p <0.001), and CPRS ADHD index (u=9.0, z=-5.17, p <0.001) sub-scores were found to be statistically significant.

Table 1: Demographic and clinical characteristics of patients with ADHD and controls

	ADHD (n:21)	Controls (n:19)	*z/?2*	p
Age (years)	9.85 – 2.79	8.72 – 3.27	-1.05[a]	0.29
Sex				
Boy/girl	16/5	10/9	2.50[b]	0.11
BMI	21.79–1.88	21.99–2.47	0.25[a]	0.81

BMI: Body Mass Index; a: Mann-Whitney U test; [b]: Chi-square test

When compared, mean serum zonulin level in the ADHD group was 16.88 (SD=9.67) and 13,34 (SD=7.15) in the control group. There was no significant difference in terms of zonulin levels between groups (u=148, z=-1.39, p=0.16) (see Table 2 and Figure 1).

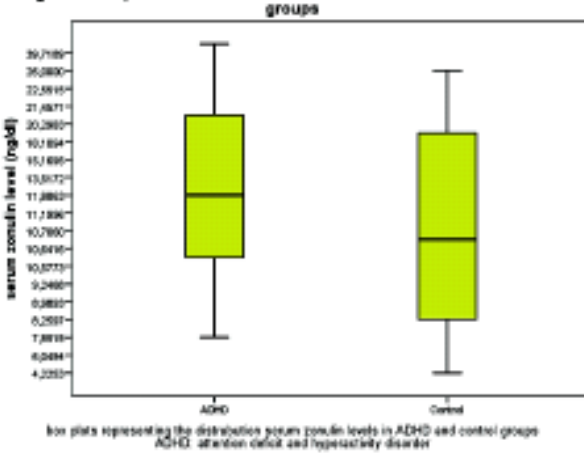
Table 2: Zonulin levels in ADHD and control groups

	ADHD	Control	u/z	p	Min/max value in ADHD group
Zonulin (ng/dl)	16.88 – 9.67	13.34 – 7.15	148/-1.39[a]	0,16	7.85/41.80

[a]: Mann-Whitney U test

When we examined the relationship between serum zonulin levels and CTRS total scores in the ADHD group, significant correlation was found (rs=0.905, p<0.01). Similar correlation existed with CPRS total score (rs=0.888, p<0.01) (Figure 2). There was no correlation between zonulin levels and total CTRS scores (rs= 0.214, p= 0.38) as well total CPRS scores (rs = -0.258, p = 0.28) in the control group.

Figure 1: comparision of serum zonulin levels between ADHD and control groups



box plots representing the distribution serum zonulin levels in ADHD and control groups
ADHD: attention deficit and hyperactivity disorder

Table 3. Correlation values of variables in ADHD group (n: 21)

		1	2	3	4	5	6	7	8	9	10	11	12	13
1.	Serum zonulin levels (ng/dl)	1												
1.	CTRS total scores	.905ᵃ, <.01ᵇ	1											
1.	CPRS total scores	.888ᵃ, <.01ᵇ	.973ᵃ, <.01ᵇ	1										
1.	CTRS. ADHD index subscale	.786ᵃ, <.01ᵇ	.900ᵃ, <.01ᵇ	.876ᵃ, <.01ᵇ	1									
1.	CTRS. opposition subscale	.694ᵃ, <.01ᵇ	.611ᵃ, <.01ᵇ	.590ᵃ, <.01ᵇ	.523ᵃ, <.01ᵇ	1								
1.	CTRS. social problems subscale	.838ᵃ, <.01ᵇ	.842ᵃ, <.01ᵇ	.781ᵃ, <.01ᵇ	.699ᵃ, <.01ᵇ	.620ᵃ, <.01ᵇ	1							
1.	CTRS. restlessness-impulsivity subscale	.794ᵃ, <.01ᵇ	.912ᵃ, <.01ᵇ	.898ᵃ, <.01ᵇ	.923ᵃ, <.01ᵇ	.512ᵃ, =.01ᵇ	.651ᵃ, <.01ᵇ	1						
1.	CTRS. emotional lability subscale	.599ᵃ, <.01ᵇ	.606ᵃ, <.01ᵇ	.584ᵃ, <.01ᵇ	.923ᵃ, <.01ᵇ	.533ᵃ, =.01ᵇ	.753ᵃ, <.01ᵇ	.451ᵃ, <.05ᵇ	1					
1.	CPRS. ADHD index subscale	.630ᵃ, <.01ᵇ	.726ᵃ, <.01ᵇ	.792ᵃ, <.01ᵇ	.717ᵃ, <.01ᵇ	.277ᵃ, =.22ᵇ	.615ᵃ, <.01ᵇ	.608ᵃ, <.01ᵇ	.370ᵃ, =.09ᵇ	1				
1.	CPRS. opposition subscale	.625ᵃ, <.01ᵇ	.758ᵃ, <.01ᵇ	.727ᵃ, <.01ᵇ	.786ᵃ, <.01ᵇ	.257ᵃ, =.26ᵇ	.556ᵃ, <.01ᵇ	.829ᵃ, <.01ᵇ	.383ᵃ, =.08ᵇ	.484ᵃ, <.05ᵇ	1			
1.	CPRS. social problems subscale	.506ᵃ, <.05ᵇ	.598ᵃ, <.01ᵇ	.627ᵃ, <.01ᵇ	.535ᵃ, =.01ᵇ	.234ᵃ, =.30ᵇ	.455ᵃ, <.05ᵇ	.597ᵃ, <.01ᵇ	.500ᵃ, <.05ᵇ	.431ᵃ, =.05ᵇ	.370ᵃ, =.09ᵇ	1		
1.	CPRS. restlessness-impulsivity subscale	.726ᵃ, <.01ᵇ	.856ᵃ, <.01ᵇ	.890ᵃ, <.01ᵇ	.779ᵃ, <.01ᵇ	.514ᵃ, <.05ᵇ	.617ᵃ, <.01ᵇ	.794ᵃ, <.01ᵇ	.588ᵃ, <.01ᵇ	.761ᵃ, <.01ᵇ	.614ᵃ, <.01ᵇ	.562ᵃ, <.01ᵇ	1	
1.	CPRS. emotional lability subscale	.564ᵃ, <.01ᵇ	.658ᵃ, <.01ᵇ	.650ᵃ, <.01ᵇ	.467ᵃ, <.05ᵇ	.486ᵃ, <.05ᵇ	.568ᵃ, <.01ᵇ	.536ᵃ, =.01ᵇ	.733ᵃ, <.01ᵇ	.322ᵃ, =.15ᵇ	.391ᵃ, =.08ᵇ	.670ᵃ, <.01ᵇ	.651ᵃ, <.01ᵇ	1

CTRS: Conner's Teacher Rating Scale-Revised Long Form. CPRS: Conner's Teacher Rating Scale-Revised Long Form

ᵃ: Spearman's rho correlation coefficient ᵇ: p value (p value <0.05 is bolded)

The correlation between the CTRS-CPRS subscales and zonulin levels was also evaluated in the patient group. In the CTRS scale; opposition, social problems, ADHD index, restlessness-impulsivity and emotional lability subscales were positively correlated with zonulin levels. In the CPRS scale opposition, social problems, ADHD index, restlessness-impulsivity and emotional lability subscales were also positively correlated with zonulin levels (Table 3).

We evaluated whether BMI, age and sex variables had any effect on correlation by performing partial correlation analysis. In ADHD group, CTRS (r=0.756, p<0.01) and CPRS total scores (r=0.756, p<0.01), CTRS opposition (r=0.825, p<0.01), CTRS social problems (r=0.778, p<0.01), CTRS ADHD index (r=0.592, p=0.01), CTRS restlessness-impulsivity (r=0.586, p=0.01) and CTRS emotional lability (r=0.644, p<0.05) subscales was still found to be correlated after analysis. In CPRS, ADHD index(r=0.499, p<0.05), restlessness-impulsivity (r=0.553, p<0.05) and emotional lability subscales (r=0.728, p=0.01) were also found to be correlated.

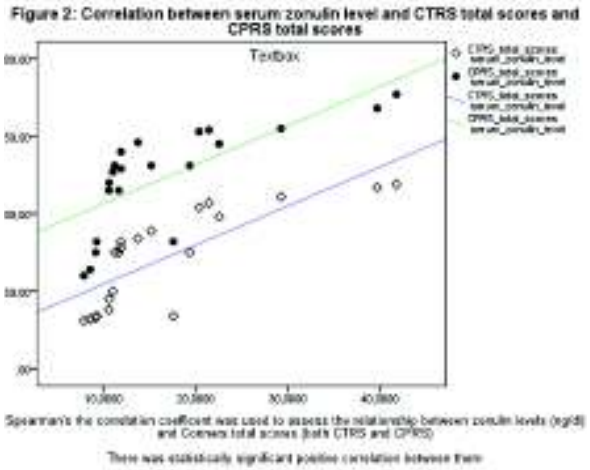


Figure 2: Correlation between serum zonulin level and CTRS total scores and CPRS total scores

We also evaluated the correlation between the CTRS and CPRS subscales and zonulin levels in the control group. The only positively correlated subscale was restlessness-impulsivity (rs = 0.501, p= 0.02) in the CTRS. On the other hand, there weren't any correlations regarding the CPRS subscales.

DISCUSSION

In this study, we investigated the serum zonulin levels among patients with ADHD and healthy controls. According to our results, there was no significant difference between patients diagnosed as ADHD and healthy controls in terms of serum zonulin levels. In addition, there was a significant correlation between CTRS/CPRS total scores and serum zonulin levels in patient group. Our study is

one of the few studies on this subject.

To our best knowledge, there are only two studies in the literature investigating serum zonulin levels in ADHD patients. Ozyurt et al. investigated serum zonulin levels in patients with ADHD and healthy controls. They found higher serum zonulin levels among children with ADHD and reported that these children have more impairment in social functioning compared to controls. Also, serum zonulin levels were found to be an independent predictor for hyperactivity and social deficit scores in regression analysis (8). In addition to this study, Aydogan Avsar et al. found significant differences between the study groups in terms of serum log-claudin-5 levels. However, according to this study there was no significant difference between the study groups in terms of serum zonulin levels. They suggested that the significant increase in serum claudin-5 levels could be evaluated as a mechanism of secondary compensation for increased blood-brain-barrier permeability and may be a marker for neuroinflammation. They mentioned some points while explaining lack of significance in serum zonulin levels such as excluding potential confounding factors such as obesity and methodological limitations (14). Therefore, in our study, we controlled BMI, age and gender variables, and yet we found that zonulin levels were still positively correlated with symptom severity.

According to our results, as the zonulin levels increased in the ADHD group, there was a more significant deterioration in opposition, social problems, restlessness-impulsivity and emotional lability sub-scores. These data were in parallel with the results found by Ozyurt et al., which can be summarized as increased zonulin levels might be related to impaired social functions (18).

Zonulin is a prehaptoglobulin which regulates intestinal permeability. Increase in zonulin levels which is induced by intestinal exposure to bacteria, gluten and gliadin could trigger opening of the paracellular pathway which is followed by an increase in intestinal permeability with the disengagement of the zonula occludens (ZO)-1 from the tight junctions. Impaired intestinal barrier permeability can lead to increased translocation of gut bacteria or of metabolic products such as lipopolysaccharide (LPS) and neuroactive peptides which could trigger an immune response that can lead to release of inflammatory cytokines and activation Inflammatory cytokines and the vagal system in turn can modulate the activity of the CNS (19). Several studies have been conducted to investigate the hypothesis that gastrointestinal permeability increased in patients with neurodevelopmental disorders and mental illnesses (20). In addition to ADHD, possible association between other psychiatric disorders and serum zonulin levels was investigated. Esnafoglu et al. found higher serum zonulin levels among patients diagnosed as autism spectrum disorders (21), Kilic et al. found higher serum zonulin levels in patients diagnosed as bipolar disorder (22) and Isik et al. also investigated serum zonulin levels among patients diagnosed as obsessive compulsive disorders and found no significant difference between patients and controls (23). As presented, there are studies investigating the association between psychiatric disorders and zonulin. Yet, studies about serum zonulin levels and ADHD are limited. We think that although a mechanic link in the gut–brain axis in ADHD has been proposed, there is still a lack of information about gut-brain axis role on ADHD etiology. In a recent review, it was suggested that there are several limitations on studies those investigating this association and little information is known whether alteration on microbiota and gut-brain axis contribute the etiology of ADHD or not. The reason we couldn't find any difference between groups may be because of this lack of association which was aforementioned by Aydogan Avsar et al (14). Another reason we didn't find any difference between groups could be the methodological limitations those mentioned by Ajamian et al. They suggest that although zonulin is a popular marker about disorders related with gut-brain axis, current commercial zonulin assays are not detecting the actual protein as prehaptoglobin-2. The researchers also recommended caution when considering serum zonulin levels as a marker of mucosal barrier integrity until assay methodology is improved (24). In a recent review, it was mentioned that studies investigating the association between ADHD and gut-brain axis have some limitations about methodological issues such as small sample sizes and standardization (25). Our sample size is

relatively small, and this could be a reason for this insignificance results between group.

Although we could not find a significant difference between patient and control groups in terms of serum zonulin levels, we found a significant correlation between serum zonulin levels and ADHD severity in patient group. In addition to this finding, when we controlled BMI and gender because of the confounding effect, positive correlation still persisted. Thus, we could suggest that serum zonulin levels does not differentiate patients with ADHD from healthy controls but could be used as a marker for determine ADHD severity. Higher serum zonulin levels could reflect increased intestinal permability (26) and this could result as more severe ADHD symptoms. If we could include more participants in our study, we think we would find some differences between the groups.

Our study has certain limitations. First, our sample size is relatively small. We did not standardize our patients' dietary habits, so dietary differences between patient and control groups could have influenced our results. This limitation is valid for most studies investigating gut-brain axis and autism spectrum disorder association. Finally, our study was cross-sectional and the patients were not followed up.

CONCLUSION

In conclusion, our study revealed the relationship between serum zonulin levels and symptom severity in patients with ADHD. The search for the use of biomarkers in psychiatric disorders continues, and studies with larger samples need to be repeated in order for zonulin to be a candidate in this regard.

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REFERENCES

1. Faraone S V., Larsson H. Genetics of attention deficit hyperactivity disorder. Mol Psychiatry. 2019;24(4):562–75.

2. Larsson H, Chang Z, D'Onofrio BM, Lichtenstein P. The heritability of clinically diagnosed attention deficit hyperactivity disorder across the lifespan. Psychol Med. 2014;44(10):2223–9.

3. Ercan ES, Polanczyk G, Akyol Ardıc U, Yuce D, Karacetın G, Tufan AE, Tural U, Aksu H, Aktepe E, Rodopman Arman A, Başgül S, Bılac O, Coşkun M, Celık GG, Karakoc Demırkaya S, Dursun BO, Durukan İ, Fidan T, Perdahlı Fiş N, Gençoğlan S, Gökçen C, Görker I, Görmez V, Gündoğdu ÖY, Gürkan CK, Hergüner S, Tural Hesapçıoğlu S, Kandemir H, Kılıç BG, Kılınçaslan A, Mutluer T, Nasiroğlu S, Özel Özcan Ö, Öztürk M, Öztop D, Yalın Sapmaz S, Süren S, Şahin N, Yolga Tahıroglu A, Toros F, Ünal F, Vural P, Perçinel Yazıcı İ, Yazıcı KU, Yıldırım V, Yulaf Y, Yüce M, Yüksel T, Akdemir D, Altun H, Ayık B, Bilgic A, Hekim Bozkurt Ö, Demirbaş Çakır E, Çeri V, Üçok Demir N, Dinç G, Irmak MY, Karaman D, Kınık MF, Mazlum B, Memik NÇ, Foto Özdemir D, Sınır H, Ince Taşdelen B, Taşkın B, Uğur Ç, Uran P, Uysal T, Üneri Ö, Yilmaz S, Seval Yılmaz S, Açıkel B, Aktaş H, Alaca R, Alıç BG, Almaidan M, Arı FP, Aslan C, Atabay E, Ay MG, Aydemir H, Ayrancı G, Babadagı Z, Bayar H, Çon Bayhan P, Bayram Ö, Dikmeer Bektaş N, Berberoğlu KK, Bostan R, Arıcı Canlı M, Cansız MA, Ceylan C, Coşkun N, Coşkun S, Çakan Y, Demir İ, Demir N, Yıldırım Demirdöğen E, Doğan B, Dönmez YE, Dönder F, Efe A, Eray Ş, Erbilgin S, Erden S, Ersoy EG, Eseroğlu T, Kına Fırat S, Eynallı Gök E, Güler G, Güles Z, Güneş S, Güneş A, Günay G, Gürbüz Özgür B, Güven G, Çelik Göksoy Ş, Horozcu H, Irmak A, Işık Ü, Kahraman Ö, Kalaycı BM, Karaaslan U, Karadağ M, Kılıc HT, Kılıçaslan F, Kınay D, Kocael Ö, Bulanık Koç E, Kadir Mutlu R, Lushi-Şan Z, Nalbant K, Okumus N, Özbek F, Akkuş Özdemir F, Özdemir H, Özkan S, Yıldırım Özyurt E, Polat B, Polat H, Sekmen E, Sertçelik M, Sevgen FH, Sevince O, Süleyman F, Shamkhalova Ü, Eren Şimşek N, Tanır Y, Tekden M, Temtek S, Topal M, Topal Z, Türk T, Uçar HN, Uçar F, Uygun D, Uzun N, Vatansever Z, Yazgılı NG, Miniksar Yıldız D, Yıldız N. The prevalence of childhood psychopathology in Turkey: a cross-sectional multicenter nationwide study (EPICPAT-T). Nord J Psychiatry [Internet]. 2019 Feb 17;73(2):132–40.

4. Cortese S, Coghill D. Twenty years of research on attention-deficit/hyperactivity disorder (ADHD): looking back, looking forward. Evid Based Ment Heal [Internet]. 2018 Nov;21(4):173–6.

5. Leffa DT, Torres ILS, Rohde LA. A review on the role of inflammation in attention-deficit/hyperactivity disorder. Neuroimmunomodulation. 2019;25(5–6):328–33.

6. Dunn GA, Nigg JT, Sullivan EL. Neuroinflammation as a risk factor for attention deficit hyperactivity disorder. Pharmacol Biochem Behav. 2019;182(503):22–34.

7. Fasano A. Leaky gut and autoimmune diseases. Clin Rev Allergy Immunol. 2012;42(1):71–8.

8. Galiatsatos P, Gologan A, Lamoureux E. Autistic enterocolitis: fact or fiction? Can J Gastroenterol. 2009 Feb;23(2):95-8.

9. Boonchooduang N, Louthrenoo O, Chattipakorn N, Chattipakorn SC. Possible links between gut–microbiota and attention-deficit/hyperactivity disorders in children and adoles-

cents. Eur J Nutr [Internet]. 2020;(0123456789). Available from: https://doi.org/10.1007/s00394-020-02383-1

10. Fasano A. Intestinal Permeability and Its Regulation by Zonulin: Diagnostic and Therapeutic Implications. Clin Gastroenterol Hepatol [Internet]. 2012 Oct;10(10):1096–100.

11. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K, Rallabhandi P, Shea-Donohue T, Tamiz A, Alkan S, Netzel-Arnett S, Antalis T, Vogel SN, Fasano A. Gliadin Induces an Increase in Intestinal Permeability and Zonulin Release by Binding to the Chemokine Receptor CXCR3. Gastroenterology. 2008; Jul;135(1):194-204.e3.

12. Sapone A, De Magistris L, Pietzak M, Clemente MG, Tripathi A, Cucca F, Lampis R, Kryszak D, Cartenì M, Generoso M, Iafusco D, Prisco F, Laghi F, Riegler G, Carratu R, Counts D, Fasano A. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes. 2006;55(5):1443–9.

13. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y Acad Sci. 2012;1258(1):25–33.

14.bAydoğan Avşar P, Işık Ü, Aktepe E, Kılıç F, Doğuç DK, Büyükbayram Hİ. Serum zonulin and claudin-5 levels in children with attention-deficit/hyperactivity disorder. Int J Psychiatry Clin Pract. 2021 Mar;25(1):49-55.

15. Ünal F, Öktem F, Çetin Çuhadaroğlu F, Çengel Kültür SE, Akdemir D, Foto Özdemir D, Çak HT, Ünal D, Tıraş K, Aslan C, Kalaycı BM, Aydos BS, Kütük F, Taşyürek E, Karaokur R, Karabucak B, Karakök B, Karaer Y, Artık A.. [Reliability and Validity of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5 November 2016-Turkish Adaptation (K-SADS-PL-DSM-5-T)]. Turk Psikiyatri Derg. 2019;30(1):42–50.

16. Kaner S, Büyüköztürk Ş, Işerī E, Özaydin L. Conners Anababa Dereceleme Ölçeği Yenilenmiş Uzun Formu: Faktör Yapısı, Geçerlik Ve Güvenirlik Çalışması. Çocuk ve Gençlik Ruh Sağlığı Derg [Internet]. 2006;18(1):45–58.

17. Şener Ş, Dereboy Ç, Dereboy F, Sertcan Y. Conners Öğretmen Dereceleme Ölçeği Yenilenmiş Uzun Formu: Faktör Yapısı, Geçerlik Ve Güvenirlik Çalışması. Çocuk ve Gençlik Ruh Sağlığı Derg. 1995;2(3):131–41.

18. Özyurt G, Öztürk Y, Appak YÇ, Arslan FD, Baran M, Karakoyun İ, Tufan AE, Pekcanlar AA. Increased zonulin is associated with hyperactivity and social dysfunctions in children with attention deficit hyperactivity disorder. Compr Psychiatry. 2018;87(2018):138–42.

19. Yarandi SS, Peterson DA, Treisman GJ, Moran TH, Pasricha PJ. Modulatory Effects of Gut Microbiota on the Central Nervous System: How Gut Could Play a Role in Neuropsychiatric Health and Diseases. J Neurogastroenterol Motil [Internet]. 2016 Apr 30;22(2):201–12.

20. Asbjornsdottir B, Snorradottir H, Andresdottir E, Fasano A, Lauth B, Gudmundsson LS, et al. Zonulin-Dependent Intestinal Permeability in Children Diagnosed with Mental Disorders: A Systematic Review and Meta-Analysis. Nutrients [Internet]. 2020 Jul 3;12(7):1982.

21. Esnafoglu E, Cırrık S, Ayyıldız SN, Erdil A, Ertürk EY, Daglı A, Noyan T.. Increased Serum Zonulin Levels as an Intestinal Permeability Marker in Autistic Subjects. J Pediatr [Internet]. 2017 Sep;188:240–4.

22. Kılıç F, Işık Ü, Demirdaş A, Doğuç DK, Bozkurt M. Serum zonulin and claudin-5 levels in patients with bipolar disorder. J Affect Disord. 2020;266:37–42.

23. Işık Ü, Aydoğan Avşar P, Aktepe E, Doğuç DK, Kılıç F, Büyükbayram Hİ. Serum zonulin and claudin-5 levels in children with obsessive–compulsive disorder. Nord J Psychiatry. May-Jul 2020;74(5):346-351.

24. Ajamian M, Steer D, Rosella G, Gibson PR. Serum zonulin as a marker of intestinal mucosal barrier function: May not be what it seems. D'Auria S, editor. PLoS One [Internet]. 2019 Jan 14;14(1):e0210728.

25. Hiergeist A, Gessner J, Gessner A. Current Limitations for the Assessment of the Role of the Gut Microbiome for Attention Deficit Hyperactivity Disorder (ADHD). Front Psychiatry [Internet]. 2020 Jun 26;11.

26. Fasano A. Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011;91(1):151–75.