Original paper

EVALUATION OF BLOOD ZONULIN LEVELS, INFLAMMATORY PROCESSES AND NEURONAL CHANGES IN CHILDREN WITH AUTISM SPECTRUM DISORDER

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SUMMARY

Background: *Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by symptoms such as limited, and repetitive behavior patterns and disordered social interaction and communication. The etiology of Autism Spectrum Disorder (ASD) is not clearly known, it has been emphasized that the immune-inflammatory system may also play a role in this disease. This study aimed to evaluate in intestinal permeability, food antigen-antibody levels, inflammatory processes, and neuron damage in patients with ASD.*

Subjects and methods: *Thirty-five chıldren between the ages of 3-12 with ASD and 35 controls were included in the study. Both participants' height and weight were measured, and the parents filled the Socio-demographic Data and the Gastrointestinal Systems (GIS) Symptoms Form. Venous blood samples were collected, and serum zonulin, anti-gliadin Ig A and Ig G, IL6, TNF-alpha, TGF-ß, S100B, and NSE levels were measured by ELISA.*

Results: *Serum zonulin levels in the ASD group were found to be significantly lower. IL-6 and TGF-ß were found to be significantly higher in the ASD group. There was no difference between the two groups in terms of serum anti-gliadin Ig A and Ig G and TNF-alpha values. Also, GIS symptoms, NSE and S100B levels were found similar between two groups.*

Conclusions: *Although findings showing low zonulin levels and increased inflammatory processes in ASD were found in this study, no difference was found in the parameters of brain damage. The findings show that intestinal permeability does not decrease in ASD and that inflammatory processes may play a role in ASD.*

Key words: autism – zonulin – inflammation - neuronal damage - leaky gut

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INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by symptoms such as limited, repetitive behavior patterns and disordered social interaction and communication (American Psychiatric Assocation 2013). Although many studies suggest that genetic and environmental risk factors play a role in the etiology of ASD, the etiology of ASD has not been explained clearly (Ng Michelle et al. 2017). Advanced paternal age, problems experienced by the mother during pregnancy, neurotoxic agents, air pollution, vitamin D deficiency are among the main risk factors (Ng Michelle et al. 2017, Corrales & Herbert 2011, Geier et al. 2009, Zhu X et al. 2017).

Nutritional issues and immune-inflammatory in autism factors have been emphasised as significant risk factors in recent years. Constipation, bloating, abdominal pain and diarrhea (Fulceri et al. 2016, Loven et al. 2017, Berding & Donovan 2016) are common gastrointestinal system (GIS) symptoms in these patients, suggesting an etiopathological relationship between the intestine and ASD (Buie et al. 2010). According to the gut-brain axis hypothesis, which is considered as a possible factor in the etiology of many diseases; Changes in the intestine may affect the brain and behaviour. According to this hypothesis, when the barrier is disrupted for some cause, the permeability of the intestinal wall increases (leaky gut), and some toxic substances may easily leak from the intestinal lumen into the bloodstream (leaky gut). These substances, which enter the systemic circulation with the disruption of the intestinal integrity, may cause toxic effects in the brain directly or through immune-inflammatory changes (Forsythe & Kunze 2013).

Changes in blood zonulin levels (Wang et al. 2000), which are one of the significant indicators of deterioration in intestinal integrity and increased permeability, have been emphasized as an indication or even cause of paracellular leakage between intestinal cells (Heberling et al. 2013). For this purpose, blood zonulin levels have been investigated in psychiatric diseases (Such as ADHD, schizophrenia, bipolar disorder, obsessive-compulsive disorder, and ASD) where autoimmunity is suspected, as well as many GIS disorders (such as celiac disease, type 1 diabetes, and Crohn's disease) (Işık et al. 2020, Kılıç et al. 2020, Asbjornsdottir et al. 1982, Sturgeon & Fasano 2016, Wood et al. 2020, Usta et al. 2021). Previous research used zonulin as a marker to measure intestinal permeability and discovered that ASD patients had higher zonulin levels than controls (Esnafoğlu et. al 2017b).

Changes in the intestinal microbiota, namely food antigens such as dysbiosis and gluten, are the most

important reasons that modify zonulin levels and damage intestinal integrity (Obrenovich 2018, Guttman & Finlay 2009, Fasano 2009). Studies show that ASD patients may have gluten sensitivity, even if a complete celiac disease does not occur (Croen et al. 2005, Lau et al. 2013, Onore et al. 2012). In this case, it has been suggested that in ASD, gluten and casein disrupts the intestinal integrity by triggering inflammation in the intestine, and then antigens and antibodies that enter the blood may interact with the central nervous system and cause a cross-reaction (Vojdani et al. 2004).

Likewise, it has been suggested that microbial dysbiosis may lead to an increase in inflammatory cytokines in the blood by disrupting intestinal permeability, and this may also cause neuron damage by affecting the blood-brain barrier (Alagöz 2017). There is ample evidence that inflammation and immune dysfunction are associated with a range of neuropsychiatric diseases, including depression, schizophrenia, streptococcal-associated pediatric autoimmune neuropsychiatric disorders (PANDAS) and ASD (Howes & McCutcheon 2017, Williams & Swedo 2015, Edirappuli et al. 2020). Many studies have shown a correlation between abnormal cytokine profiles and ASD, with increased IL-6, TNF-α, and decreased TGF-β levels (Thom et al. 2019, Rose et al. 2018). It has also been shown that there is a relationship between IL-6 levels and zonulin levels (Esnafoğlu et al. 2017b). S100B and Neuron Specific Enolase (NSE) levels are considered biomarkers of blood-brain barrier disruption and neuron harm. It has been demonstrated that S100B protein levels in ASD are significantly higher than in healthy controls, and there is a correlation between ASD symptom severity and S100B levels (Al Ayadhi et al. 2012). S100B, tau, NSE, active caspase-3, M30, and M65 levels were higher in autism patients in another study comparing 43 autism patients aged 3-12 years to healthy controls, and it was suggested that S100B may be a marker of apoptosis in autism (Ayaydın et al. 2020). On the contrary, 35 children with ASD and 31 healthy controls were compared in terms of S100B and NSE values in a study conducted by Esnafoğlu et al. in 2017, and it was stated that there was no significant difference between the groups (Esnafoğlu et al. 2017a).

While the etiological relationship between food allergies and inflammatory processes with ASD has been extensively examined in recent years, their neuronal effects have not been fully elucidated yet. Although previous research has addressed each area in the gut-brain axis hypothesis one by one, it is far from revealing the relationship between them. Therefore, the intestinal-brain axis hypothesis that foods and microbial factors cause neuronal damage by disrupting intestinal integrity should be investigated with a more holistic approach. In this study, zonulin levels, which are an indicator of intestinal permeability in ASD, antibody levels formed against nutrient antigens, some inflammatory processes and changes in neuron damage were compared with the control group and a holistic assessment was aimed at this issue.

SUBJECTS AND METHODS

Participants

This prospective study was conducted from December 2019 to June 2020 with 79 children aged from 3 to 12 years. The research included 40 patients who applied to the Child and Adolescent Psychiatry outpatient clinic at Inonu University Medical Faculty and were diagnosed with ASD according to DSM-5 diagnostic criteria, as well as 39 control groups who did not have ASD. Participants in the control group were selected from among children who did not have any health problems and who applied to the pediatric clinic for routine health check-ups in a way that their age and sex were similar to the ASD group. The study's aim was explained in depth to the parents, they were given time to read the informed consent form, and the parents who agreed to participate were included in the study. The Inonu University Clinical Research Evaluation Commission granted ethical approval for this study under the code 2019/201.

Conditions affecting the gastrointestinal system (malformations, inflammatory bowel disorders, history of surgery, irritable bowel syndrome, celiac disease, etc.), those with intestinal permeability or immune response issues, such as those with any autoimmune disease diagnosis, those on a special diet, those with neurological diseases, and obesity, were all ruled out of the sample. These exclusion criteria were determined using the medical records of the patients. One of the children in the ASD group was excluded from the study after presenting to a pediatric outpatient clinic with a fever one day after being included in the study, and four patients were removed from the study after hemolysis occurred after their blood was drawn. Two of the healthy control children were excluded from the research due to inadequate blood intake, and two others were removed due to blood hemolysis. As a result, the sample included 35 children with ASD and 35 healthy children.

First, a psychiatry interview with the children and their parents was conducted. Later, the children were observed in the observation room and psychological examinations based on the DSM-5 were conducted. The Sociodemographic Data Form and the GIS Assessment Form were filled by the parents. Following that, the children's weights were measured using floor scales sensitive to 100 g, and their heights were measured using a portable height gauge by the same individual, and their Body Mass Index (BMI) was calculated.

After the interview, a sterile syringe without a plastic structure on the posterior end was used to take 5 ml of venous blood samples from the children in the ASD and control groups. Without waiting, blood samples were centrifuged at 1500 rpm for 5 minutes and their serum was removed. Each of the serum samples obtained was divided into propylene Eppendorf tubes to form 3 separate batches and stored at -80°C until analysis. All serum samples were thawed at room temperature and

serum zonulin, anti-gliadin IgA, anti-gliadin IgG, IL6, TNF-, TGF-, NSE, and S100B levels were analyzed using the Enzyme-Linked Immunabsorbant (ELISA) method when the number of samples predicted for the analysis and control groups had been reached.

Measures

Sociodemographic Data Form

It is a questionnaire developed by the research team that inquires about the child's gender, age, education, and special education status, as well as whether the child has a physical condition that necessitates on-going care, medication use, family history, and income level (low: less than 1000 TL, moderate: 1000-3000 TL, high: more than 3000 TL). It is filled in together with the parents.

GIS Symptoms Assessment Form

It is a form developed by the research team that consists of 2 parts, namely in infancy and the present, and evaluates whether there are frequent abdominal pain, vomiting, diarrhea, constipation.

Statistical Assessment

Statistical analyses were performed with SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). Descriptive data related to the quantitative variables are given as the median and minimum-maximum, while data related to the qualitative variables are given as a number (n) and percentage (%). The Pearson and Fisher chi-square test was used for the analysis of qualitative variables. Mann-Whitney U test was used to analyze the quantitative variables. Values of $p<0.05$ were accepted as statistically significant.

RESULTS

Clinical Features

Sociodemographic Characteristics of the Participants

The ASD group comprised 35 children - 80.0 % male (n=28) and 20.0 % female (n=7) - while the 35 children in the control group were 68.6% male (n=24) and 31.4% female (n=11) The research group had a median age of 8 (min-max: 3-11) and the control group

Table 1. Clinical Features

Sociodemographic characteristics	Research Group n (%)	Control Group n (%)	χ^2	p[a]
Gender			1.19	0.412
Male	28 (80.0)	24 (68.6)		
Female	7 (20.0)	11 (31.4)		
Education level			10.21	*0.017*
No school	8 (22.9)	5 (14.3)		
Kindergarten	8 (22.9)	5 (14.3)		
Primary school	18 (51.4)	14 (40)		
Secondary school	1 (2.9)	11 (10.0)		
Medication use			15.96	*<0.001*
Yes	13 (37.1)	0 (0.0)		
No	22 (62.9)	35 (100.0)		
Special training			49.51	*<0.001*
Yes	29 (82.9)	0 (0.0)		
No	6 (17.1)	35 (100.0)		
Residental area			3.21	0.239
Village	3 (8.6)	4 (11.4)		
Town	7 (20.0)	2 (5.7)		
City	25 (71.4)	29 (82.9)		
Family history of psychiatric disease			0.00	1.000
Yes	6 (17.1)	6 (17.1)		
No	29 (82.9)	29 (82.9)		
Parents			0.00	1.000
Together	33 (94.3)	33 (94.3)		
Divorced	2 (5.7)	2 (5.4)		
Family income			3.73	0.179
Low	2 (5.7)	1 (2.9)		
Middle	21 (60.0)	14 (40.0)		
High	12 (34.3)	20 (57.1)		

	Research group n=35		Control group n=35		Z	p[b]
	Median	Min-Max	Median	Min-Max		
Age	8.00	3-11	9.00	3-12	-0.79	0.427

[a] chi-square test p-value; [b] Mann- Whitney U test p-value

had a median age of 9 (min-max: 3-12). The ages and genders of both groups were statistically similar (p value of gender=0.412, p value of age=0.427).

It was found that 37.1% (n=13) of the children in the ASD group used a regular medication. None of the children in the control group had regular drug use. 11.4% medication users' (n=4) only stimulant, 14.2% (n=5) only antipsychotic, 5.7% (n=2) antipsychotic and stimulant, 2.8% (n=1) antipsychotics and mood stabilizers 2.8% (n=1) was using SSRIs and antipsychotics. A regular medication use of children in the ASD group was found to be significantly higher than in the control group (p<0.001).

A chronic physical illness was present in 8.6% (n=3) of the children in the ASD group and 2.9 % (n=1) of the children in the control group. In terms of chronic physical illness, both groups were found to be similar (p=0.614).

Sociodemographic Characteristics of Families

The research group and the control group each had 94.3% (n=33) of their children's parents living together, and there was no difference in the rate of divorce between the two groups (p=1.00).

When comparing families based on where they lived, both groups were found to be similar (p=0.239). Also, when families were compared in terms of income levels, no significant difference was found (p=0.179).

Table 1 shows the sociodemographic features of the children and their families.

Findings Related to the Anthropometric Measurements of the Participants

In terms of height, weight, and BMI, there was no significant difference between the ASD and control groups (p values 0.070, 0.573, 0.648, respectively). Table 2 shows the data of the participants' anthropometric measurements.

Findings Related to GIS Symptoms of Participants

When autism and control groups were compared in terms of GIS complaints such as diarrhea, constipation, vomiting and abdominal pain in infancy, it was observed that 17 (48.6%) of the autism group had GIS complaints in infancy, and 14 (40.0%) of the control group had GIS complaints in infancy. In terms of GIS complaints in infancy, there was no significant difference between the two groups (p=0.630).

When children in the ASD and control groups were compared in terms of GIS complaints such as diarrhea, constipation, vomiting and abdominal pain, it was observed that 11 (31.4%) of both groups had GIS complaints at present. In terms of GIS grievances, it has been decided that the two classes are similar (p=1.000). Table 3 shows the findings of the participants' GIS complaints.

Table 2. Children's BMI, Weight and Height scores

	Research group N=35		Control group N=35		Z	p
	Median	Min-Max	Median	Min-Max		
Height (cm)	126	91-157	135	100-167	-1.81	0.070
Weight (kg)	27	13-56	32	16-77	-0.56	0.573
BMI	17	13-29	17	13-30	-0.45	0.648

Table 3. GIS symptoms of the participants

GIS Symptoms	Research group N (%)	Control group N (%)	χ^2	p
Infancy			0.52	0.630
Yes	17 (48.6)	14 (40.0)		
No	18 (51.4)	21 (60.0)		
At the present time			0.00	1.000
Yes	11 (31.4)	11 (31.4)		
No	24 (68.6)	24 (68.6)		

Table 4. Serum zonulin, Antigliadin IgA, Antigliadin IgG, IL-6, TNF-α, TGF-β, S100B, NSE levels of the participants

	Research group n=35 Median (Min-Max)	Control group n=35 Median (Min-Max)	Z	p
Zonulin (ng/ml)	4.51 (2.24-34.91)	7.62 (2.47-32.33)	-2.44	*0.014*
Antigliadin IgA (U/ml)	221.20 (58.30-1160.30)	209.30 (53.10-1807.00)	-0.57	0.569
Antigliadin IgG (U/ml)	11.50 (4.10-84.40)	13.80 (3.60-67.20)	-0.20	0.837
IL6 (ng/l)	18.75 (7.96-90.10)	12.59 (5.86-75.15)	-2.53	*0.011*
TNF-α (ng/l)	38.42 (13.39-351.37)	43.48 (10.19-447.23)	-0.36	0.716
TGF-β (ng/ml)	4.85 (0.94-18.03)	2.88 (0.51-32.63)	-3.28	*0.001*
NSE (ng/ml)	4.27 (1.35-39.71)	5.28 (1.58-48.47)	-1.04	0.299
S100B (ng/l)	262.28 (73.50-936.38)	260.45 (54.03-706.38)	-0.17	0.865

Findings Regarding the Biochemical Values of the Participants

The serum values of "zonulin, anti-gliadin IgA, anti-gliadin IgG, IL-6, TNF-, TGF-, S100B, NSE" were compared between the research and control groups.

ASD serum zonulin level was detected to be significantly lower than the control group (p=0.014). Serum IL-6 level was found significantly higher in the ASD group than in the control group (p=0.011). The ASD group had significantly higher serum TGF-β levels than the control group (p=0.001). Serum Anti-gliadin IgA, Anti-gliadin IgG, TNF-α, S100B, NSE values were similar between groups (p=0.569, 0.837, 0.168, 0.716, 0.299, 0.865, respectively). Table 4 shows the distribution of the participants' serum zonulin, Anti-gliadin IgA, Anti-gliadin IgG, IL-6, TNF-α, TGF-β, S100B, and NSE values.

DISCUSSION

Serum zonulin levels, inflammatory parameters (IL-6, TNF-α, TGF-β), anti-gliadin antibody levels (anti-gliadin Ig-A, anti-gliadin Ig-G), and neuron damage parameters (S100B, NSE) were compared in 35 children diagnosed with ASD between the ages of 3 and 12 to a control group of children in the same age range in this study. The ASD group had lower serum zonulin levels than healthy controls, but higher IL-6 and TGF-β levels. No statistically significant difference was observed between the ASD and control groups when serum anti-gliadin Ig-A, anti-gliadin Ig-G, and TNF-α levels were compared. Serum NSE and S100B levels, which are thought to be indicators of neuronal damage, were also found to be similar between the two groups.

There are conflicting data on ASD and serum zonulin levels in previous studies. The serum zonulin levels of 32 ASD patients and 33 healthy controls were compared by Esnafoğlu et al., and the ASD group's serum zonulin levels were detected to be significantly higher than the control group's (Esnafoğlu et al. 2017a). However, zonulin levels were measured in both the autism and control groups in another ASD study, and no significant difference was detected between the groups (Karagözlü et al. 2021). In contrast to previous research, zonulin levels were found to be lower in ASD patients relative to controls in our study. Low zonulin level in ASD may be a consequence rather than an etiological factor, or it may be associated with different clinical phenomena such as GIS disorders. Esnafoğlu et al. (2017b) found that serum zonulin levels were high in the autism patient group in general, but they also found high zonulin levels in some control cases and stating that other factors that zonulin alone may not affect the pathogenesis of ASD should be evaluated. Also, in this study, all participants in the autism patient group had a significant correlation between GIS complaints and zonulin levels. This indicates that high zonulin levels could be related to GIS complaints in these patients caused by dietary problems, rather than a direct etiological link to autism. In the study conducted by Rose et al. in a group of patients with ASD; prehaptoglobulin-2 levels thought to reflect serum zonulin levels in these children, showed no change (Rose et al. 2018). The precursor of zonulin, prehaptoglobulin 2, was evaluated in this study and prehaptoglobulin 2 levels were found to be higher only in patients with severe GIS symptoms (Rose et al. 2018). This is consistent with the idea that zonulin levels may vary depending on the frequency of GIS complaints in these patients rather than the disease.

Ohlsson et al. (2019) found lower zonulin levels in patients with MDD who recently attempted suicide, compared to both patients with MDD who did not attempt suicide and healthy controls. They suggested the hypothesis that the underlying mechanism for low zonulin levels in the patient community is unknown, but that further intestinal epithelial cell death or dysfunction may decrease zonulin expression, and that lower plasma zonulin levels may be an indication of greater intestinal permeability (Ohlsson et al. 2019). It might be thought that the low zonulin levels found in our research group were caused by epithelial cell death through a similar mechanism.

Previous studies into the relationship between obesity and zonulin levels have reported that zonulin levels in obese patients are higher than in control groups and that BMI and zonulin levels are positively correlated (Ohlsson et al. 2017, Küme et al. 2017). The levels of zonulin were detected to be significantly higher in obese patients with high waist circumference, diastolic blood pressure, and fasting glucose cases in a study published by Ohlsson et al. in 2017 (Ohlsson et al. 2017). Esnafoğlu et al. showed that in the group with ASD, there is a significant positive correlation between BMI and zonulin levels (Esnafoğlu et al. 2017b). This led to the idea that higher zonulin levels are linked to a higher BMI rather than the disease itself. Obese patients were removed from this study, and the BMI values of the ASD and control groups were comparable. Thus, possible zonulin changes caused by obesity and BMI between the ASD and control groups were prevented. With the exclusion of a confounding factor such as obesity, it can be thought that zonulin levels in the ASD group can be evaluated more efficiently.

In a current study conducted in 2020, in a large sample study comparing 398 ASD and 379 healthy controls, the researchers reported that the group with ASD carried less HP-2 gene, which synthesizes zonulin, compared to the control group (Cupaioli et al. 2020). The fact that zonulin levels are low in ASD patients in our study may add to the body of evidence supporting the genotyping study conducted with such a large sample.

In recent years, one of the most significant research areas of ASD etiology has been the potential impact of food and intestinal microbiota on the disease. Generally, anti-gliadin IgA and IgG levels are evaluated to investigate the effect of antibodies against food antigens on intestinal permeability. However, when the literature is reviewed, it is seen that the studies generally reveal inconsistent results. Patients without GIS symptoms and controls were compared in a study with 66 ASD patients and 101 healthy controls to rule out the effects of GIS comorbidities in autism on serology, and anti-gliadin IgA levels were detected to be lower in the ASD group (Abdel Maksout et al. 2020). However, Pavone et al. reported that anti-gliadin levels were normal in 11 patients with ASD (Pavone et al. 1997). Gluten intolerance was examined in ASD patients in another study, and abnormal anti-transglutaminase IgA levels were found in only 1 (0.68%) of 147 patients with ASD, whereas abnormal anti-gliadin IgA levels were reported in 5 (3.4%). Furthermore, when celiac disease was confirmed by biopsy, it was reported that the prevalence of ASD was not higher in this study group than in the general population (Batista et al. 2012). Anti-gliadin IgA and IgG levels were not found to be different between the two groups in our study, which is similar to recent literature.

Another aim of the present study is to assess inflammatory processes in patients with ASD. Serum levels of IL 6, a systemic predictor of inflammation, TNF-α, another proinflammatory cytokine, and TGF-β, an anti-inflammatory cytokine, were compared for this reason. As a result of the study, serum levels of IL-6 and TGF-β were found to be high in the research group, but TNF-α levels were similar between the two groups. These findings are important in terms of studies showing the effectiveness of anti-inflammatory therapy in psychiatric diseases (Müller 2013).

In a study comparing children with ASD, healthy siblings and a control group with developmental delay, it was found that IL-1β, IL-6, IL-8 and IL-12p40 increased in children with autism. Besides, increased cytokine levels have also been related to the regressive form of ASD, communication difficulties, and abnormal behaviours (Ashwood et al. 2011). It has also been suggested that mast cells in ASD cause brain inflammation by producing IL-6 and TNF-α (Theoarides et al. 2016). Higher levels of TNF-α levels were observed in the ASD group in a study comparing plasma cytokine levels of patients with autism aged 4-7 years, and high TNF-α concentrations were positively correlated with the severity of ASD symptoms (Xie et al. 2017). On the other hand, in a meta-analysis published in recent years, he reported that there is no significant change in TNF-α levels between individuals with ASD and controls (Masi A et al. 2015). No significant difference was detected in TNF-α levels between the autism and healthy control groups in our research too.

While increased IL -6 levels are detected in chronic inflammation, the lack of a significant difference in TNF-α levels, which are an indicator of acute inflammation, may be related to the fact that autism is a chronic condition.

TGF-β is an inhibitory cytokine produced by T cells and some other cell types. One study found that while plasma pro-inflammatory cytokines were increased in the ASD group, anti-inflammatory cytokines and TGF-β were decreased and this decrease was associated with behavioural problems (Ashwood et al. 2011). Plasma cytokine levels of 87 children with ASD aged 2 to 6 years were compared to 41 healthy controls in another study, and TGF-β levels were detected to be higher in ASD children (Hu et al. 2018). TGF-β levels in the ASD group were found to be significantly higher than healthy controls in our research. This can be interpreted as an increase in TGF-β as an anti-inflammatory cytokine to compensate for the patients' increased pro-inflammatory activity.

According to the gut-brain axis hypothesis, altered permeability in the gut may affect the blood-brain barrier through inflammatory cytokines in the blood. Participants' S100B and NSE levels were assessed to analyze neuronal damage in this study, and S100B and NSE levels were found to be similar in both groups. It was seen that there were different data in the results regarding S100B and NSE, which are considered to be indicators of brain damage and CSF permeability in neuropsychiatric disorders like autism (Ayaydın et al. 2020, Lv et al. 2016, Al-Ayadhi et al. 2012). It was observed that S100B, tau, NSE, active caspase-3, M30, and M65 levels were found to be higher in autism patients in a study comparing 43 ASD patients aged 3-12 years to healthy controls, and S100B was thought to be a marker of apoptosis in autism (Ayaydın et al. 2020). In a study conducted by Esnafoğlu et al. In 2017, 35 children with ASD and 31 healthy controls were compared in terms of serum S100B and NSE values, and it was reported that there was no statistically significant difference between the groups (Esnafoğlu et al. 2017a). The presence of brain damage and associated high biomarkers such as S100B and NSE in the early years of life in patients with autism may be one of the reasons for this situation. The average age was similar in both the study conducted by Esnafoğlu et al. and in this study. The age of 7-8 may be considered advanced in terms of observing this harm. The relationship between neonatal asphyxia and S100B levels was investigated in a study, and it was found that neonatal asphyxia increased S100B levels (Beharier et al. 2012). Neurological conditions such as epilepsy and cerebral palsy were also excluded in the study conducted by Esnafoğlu et al. and in our study. As a result, the fact that S100B and NSE values in autism and healthy controls in our study are similar can be related to their similar asphyxia histories.

Limitations

The current study, like all researches, has some limitations and strengths. First, although the GIS findings and anthropometric measurements are similar across the groups in this sample, individual variations may be a limitation. Other limitations of the study are cross-sectionality of the study, the fact that no scale examining the symptoms of autism were used, and the biochemical parameters were measured only from blood. Another limitation is that our patients' medication use could not be excluded. Also, the fact that serum zonulin levels, which we use to assess intestinal permeability, may be released from organs other than the intestine, such as the brain and lungs, may have influenced our findings. The evaluation of zonulin levels in stool samples as well as serum zonulin levels in future studies, taking this issue into account, may give more clear information about this issue.

In addition to all these limitations, our study also has its strengths. First of all, this study is one of the few studies investigating the gut-brain axis, which is a very new area of research in a neurodevelopmental disease like ASD, whose etiology is not fully understood. Furthermore, this axis was examined holistically in terms of intestinal permeability, inflammatory processes in the blood, and the existence of neuronal injury, rather than from a single point. The similarity of demographic data in both groups eliminated the effect of these features on the variables studied. Another strength of the research is the absence of these influencing variables, as the patient and control groups were similar in terms of age, gender, and GIS findings. Considering that obesity is one of the important factors affecting zonulin level and it is a common problem in ASD, not including obese patients in this study is one of the strengths of the study. Another advantage of the study is that it excludes neurological disorders such as epilepsy and cerebral palsy, which are common in autism.

CONCLUSION

As a result, although findings showing low zonulin levels and increased inflammatory processes in ASD were found in this study, no difference was found in the parameters of brain damage. The findings show that intestinal permeability does not decrease in ASD and that inflammatory processes may play a role in ASD. The findings of this analysis, however, may not be generalized considering the current limitations. The current study, unlike other autism studies, it will make a contribution to the literature in terms of being a study that deals with the gut-blood-brain with a holistic approach. To fully understand this, longitudinal studies with large samples, including ASD patients with and without high GIS findings, are needed.

Contribution of individual authors:

Gülşen Kartalci: study design, data collection, statistical analysis.
Arzu Çalişkan Demir: study design, first draft.
Sukru Kartalci: study design.
Nuray Uremis: data collection.
Yusuf Turkoz: study design, data collection.
All authors approval of final version.

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