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ORIGINAL PAPER

Serum zonulin and claudin-5 levels in patients with schizophrenia

Ayşe Usta[1] · Faruk Kılıç[1] · Arif Demirdaş[1] · Ümit Işık[2] · Duygu Kumbul Doğuç[3] · Mustafa Bozkurt[3]

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Abstract

The aim of this research was to assess whether or not changes in the concentrations of serum zonulin and claudin-5 in patients with schizophrenia could have etiopathogenetic importance. In previous studies, the data regarding the relationship between intestinal and blood–brain barrier (BBB) permeability and the etiology of schizophrenia have been limited. In this study, we assumed that there may be a difference in serum zonulin and claudin-5 levels in patients with schizophrenia, which may affect the severity of the disease. Fifty schizophrenia patients and 50 healthy controls were included in this study. The patients were administered the Positive Symptoms Assessment Scale (SAPS) and Negative Symptoms Assessment Scale (SANS) to determine the severity of symptoms. Venous blood samples were collected, and the serum zonulin and claudin-5 levels were measured. The mean serum zonulin levels were significantly increased in patients with schizophrenia when compared to the control group. Serum claudin-5 levels were decreased in the schizophrenia patients when compared to the controls. The present study indicates that zonulin is increased and claudin-5 is decreased in patients with schizophrenia. These findings extend the existing knowledge on the dysregulation of intestinal permeability, especially zonulin, and BBB, especially claudin-5, and show that both proteins may be involved in the etiopathogenesis of schizophrenia.

Keywords Schizophrenia · Blood–brain barrier · Claudin-5 · Intestinal permeability · Zonulin

Introduction

Schizophrenia is a chronic psychotic disorder affecting approximately 1% of the world population [27]. Although the etiology of schizophrenia has not been clearly elucidated, studies on the neurobiology of schizophrenia are being carried out in multiple fields, including genetics, anatomy, neuron functions, neuropathology, electrophysiology, neurochemistry, neuropharmacology, and developmental neurology. These findings have already provided important information on the subject, revealing that genetic, environmental, and biochemical factors, as well as structural and functional disorders in the brain, are responsible for the etiopathogenesis of schizophrenia. However, the interaction between genetic predisposition and environmental factors is particularly important [26]. Studies conducted on this subject also show the importance of the gut–brain axis in the etiology of schizophrenia. The intestines serve as an important barrier between the external environment and the body and play an important role in the transfer of certain antigens to the body and in the development of an immune response [40].

In recent years, attention has been given to the role of increased intestinal permeability and abnormal immune responses in the etiopathogenesis of diseases, such as celiac disease, type 1 diabetes mellitus (DM), autism spectrum disorder (ASD), and schizophrenia [6, 12, 34, 41]. One important factor in intestinal permeability is zonulin. Zonulin (prehaptoglobulin) is a 47 kilodaltons (kDa) protein that increases intestinal permeability in the small intestines and contributes to intestinal innate immunity [3]. It is produced by the human small intestinal epithelium under the influence of environmental stimuli [10, 34]. Connecting to specific receptors on the epithelial surface of the jejunum and distal ileum, zonulin binds actin fibers to each other via protein kinase-c (PKC) and regulates the tight junctions

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✉ Faruk Kılıç
drfarukk33@hotmail.com

[1] Department of Psychiatry, Süleyman Demirel University Medicine Faculty, 32260 Isparta, Turkey

[2] Department of Child and Adolescent Psychiatry, Süleyman Demirel University Medicine Faculty, Isparta, Turkey

[3] Department of Biochemistry, Süleyman Demirel University Medicine Faculty, Isparta, Turkey

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(TJs) function in the cell. TJs are composed of occludin and claudin, which join epithelial cells together in the gut, thereby protecting against unwanted enterobacteria and food molecules [11]. We know that TJs are dynamic structures involved in both the physiologic and pathologic regulation of intestinal epithelial antigen trafficking [39]. The cells are opened rapidly and reversibly by zonulin secretion with different effects [10, 13]. Zonulin transactivates the receptor of the epidermal growth factor via the proteinase-activated receptor-2, resulting in a disassembly and enhanced permeability of the TJs [10]. Thus, zonulin is regarded as a non-invasive biomarker for intestinal permeability [29]. Zonulin regulates the transport of paracellular antigens by controlling TJs [37]. An increased intestinal permeability due to zonulin secretion results in the passage of some bacteria and toxins, the transport of macromolecules from the lumen to the submucosa, and triggered immune reactions [25]. Furthermore, zonulin serum concentration has been shown to be elevated in psychiatric disorders, such as attention-deficit hyperactivity disorder (ADHD), ASD, obsessive–compulsive disorder (OCD), bipolar disorder, and schizophrenia, as well as in diseases such as DM and obesity [4, 9, 18, 22, 33].

Zonulin regulates both blood–brain barrier (BBB) permeability and intestinal barrier permeability [4, 34]. The BBB acts as a protective gate between the peripheral circulation and the brain, providing its protective role through the intercellular connections of the zonula occludens type between capillary endothelial cells [31]. The transmembrane linker proteins that connect the two membranes are claudins and occludins [38]. Claudins are transmembrane proteins weighing 20–27 kDa. Claudins have at least 24 members [14]. Claudin-5, one of these members, is involved in the BBB. The claudin-5 gene that encodes the claudin-5 protein is located on chromosome 22q11 [15]. There are many studies, showing that the claudin-5 gene is associated with schizophrenia [17, 32, 36]. If the claudin-5 gene is disrupted, the brain is more frequently exposed to harmful environmental factors (less than 800 Da) [31]. In particular, claudin-5 dysfunction has been shown in psychiatric disorders, such as depression, OCD, bipolar disorder, and schizophrenia, as well as in neurodegenerative diseases, including Alzheimer's disease [18, 20, 22, 28, 36]. The relationship between claudin-5 and schizophrenia is the most important nexus of genetic and environmental factors [36]. In light of this information, abnormalities in the TJs located in the gut–brain barrier should be studied in relation to schizophrenia.

To the best of our knowledge, no studies in the existing literature have compared the serum zonulin and claudin-5 levels in a healthy control group and schizophrenia group. Thus, this study is the first to explain this relationship. In this study, we assumed that different concentrations of zonulin and claudin-5 could occur in patients with schizophrenia and that the severity of the disease could be linked to the zonulin and claudin-5 concentrations. Therefore, this study aimed to explore whether patients with schizophrenia and healthy controls had different concentrations of serum zonulin and claudin-5.

Methods

Participants and procedures

Clinically stable patients diagnosed with schizophrenia who were followed up in the psychiatry outpatient clinic of Süleyman Demirel University according to the Fifth Edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) were included in the study. All participants were given detailed information about the study, and written consent was obtained from them. After examining the records of the schizophrenia patients and taking their psychiatric histories, the patients with a history of alcohol and substance use disorder, diagnosed malignancy, inflammatory bowel disease, or active infection were excluded from the study, as were those who had used corticosteroids or a drug affecting the immune system in the last 6 months. The hospital staff was selected for the control group, and those with a history of mental disorder fitting the exclusion criteria, a history of alcohol and substance use disorder, diagnosed malignancy, inflammatory bowel disease, or active infection were excluded from the study, as were those who had used corticosteroids or a drug affecting the immune system in the last 6 months. Prior to the study, ethical committee approval was received from the Clinical Research Ethics Committee of Süleyman Demirel University Faculty of Medicine (2018/248).

The Positive Symptoms Assessment Scale (SAPS) and the Negative Symptoms Assessment Scale (SANS) were used to evaluate schizophrenia symptoms. SAPS and SANS, developed by Andreasen [1], are scales evaluating the severity and distribution of schizophrenia symptoms. The Turkish version of the scales were made by Erkoç et al. [7].

Biochemical analysis

Approximately 10 ml blood samples were taken from the antecubital vein of each individual included in the study between 08.00 and 10.00 am following overnight fasting. The samples were collected in gel-containing biochemical tubes. The serum was then obtained by centrifugation at 3000 rpm for 10 min. The serum sample was stored at − 80 °C until the study day. After collecting all samples, the zonulin and claudin-5 levels were measured with the enzyme-linked immunosorbent assay (ELISA) method.

Determination of serum zonulin and claudin-5

A quantitative measurement of the serum claudin-5 level was performed with a commercial kit (Catalog No: SEF295Hu, USCN, Wuhan, China) that used the ELISA method. The kit contained a seven-point calibration curve (31.25 pg/ml, 62.5 pg/ml, 125 pg/ml, 250 pg/ml, 500 pg/ml, 1000 pg/ml, and 2000 pg/ml), and optical density data corresponding to these concentrations were obtained by duplicating all standards. At the end of the procedure, the concentration–optical density graph of the standards was drawn, and the concentrations of all samples were calculated in pg/ml using this graph.

A quantitative measurement of the serum zonulin level was also performed with a commercial kit (Catalog No. E-EL-H5560, Elabscience, Wuhan, China) that used the ELISA method. The kit contained a seven-point calibration curve (0.78 ng/ml, 1.57 ng/ml, 3.13 ng/ml, 6.25 ng/ml, 12.5 ng/ml, 25 ng/ml, and 50 ng/ml), and optical density data corresponding to these concentrations were obtained by duplicating all standards. At the end of the procedure, the concentration–optical density graph of the standards was drawn, and the concentrations of all samples were calculated in ng/ml using this graph.

Statistical analysis

The "SPSS for Windows 20.0" package program was used for the data analysis. The distribution of the data was evaluated by Kolmogorov–Smirnov test. Data with normal distribution were evaluated by Student's *t* test and those without normal distribution were evaluated by Mann–Whitney *U* test. Chi-square test was used for categorical data. The data obtained were presented as the mean and standard deviation. The relationship between the data was analyzed by Pearson and Spearman correlation. The results were evaluated with a 95% confidence interval. $P < 0.05$ was considered statistically significant.

Results

Descriptive statistics regarding the sociodemographic data of the patients are given in Table 1. There was no difference between the two groups in terms of age and sex, but there was a difference in terms of their BMIs (Body Mass Index). The BMI score of the patient group (29.77 ± 6.19) was higher than the control group (25.86 ± 3.59) ($p < 0.001$).

As a result of this analysis, the mean serum zonulin level was found to be 166.55 ± 61.43 ng/ml in the schizophrenia patient group and 130.11 ± 55.72 ng/ml in the control

Table 1 Demographic and clinical characteristics of patients with schizophrenia and controls

	Schizophrenia ($n=50$)	Controls ($n=50$)	$z/x^2/t$	p
Age (years)	38.3 ± 10.7	40.1 ± 8.5	-0.555[a]	0.559
Sex				0.198
Male/female	37/13	31/19	1.654[b]	
Marital status				**< 0.001**
Single/married	33/17	11/39	19.643[b]	
Education				0.104
Primary/high/university	17/17/16	14/27/9	4.523[b]	
Smoking				**0.045**
Smoker/nonsmoker	31/19	21/29	4.006[b]	
BMI	29.7 ± 6.19	25.8 ± 3.5	3.863[c]	**< 0.001**
Zonulin (ng/ml)	166.5 ± 61.4	130.1 ± 55.7	3.107[c]	**0.002**
Claudin-5 (pg/ml)	959.8 ± 583	1230.7 ± 692.4	-2.116[c]	**0.037**
Duration of illness (year)	14.2 ± 9.6			
Duration of hospitalization (month)	2.3 ± 2.5			
Duration of drug use (year)	13.4 ± 9.4			
History of suicide attempt				
Yes/no	16/34			
SAPS	23.4 ± 23.6			
SANS	46.6 ± 26.9			

BMI body mass index, *SAPS* scale for the assessment of positive symptoms, *SANS* scale for the assessment of negative symptoms

[a]Mann–Whitney *U* test; [b]Chi-square test; [c]Student *t* test; Values in boldface are statistically significant ($p < 0.05$)

group (Fig. 1). The serum zonulin levels in the schizophrenia patients were higher than in the healthy controls ($t=3107$, $p=0.002$) (Table 1).

The mean serum claudin-5 level was 959.87 ± 583.07 pg/ml among the schizophrenia patients and 1230.79 ± 692.40 pg/ml in the control group (Fig. 1). The serum claudin-5 levels in the schizophrenia patients were lower than in the healthy controls ($t=-2116$, $p=0.037$) (Table 1).

Among the schizophrenia patients, the correlation between their age, BMI, duration of disease, and zonulin and claudin-5 levels was examined, and a statistically significant positive correlation was found between BMI and zonulin ($r=0.413$, $p=0.003$); however, no significant correlation was found between age, disease duration, and zonulin level. There was no significant correlation between claudin-5 level and age or BMI and disease duration ($p>0.05$).

There was no significant correlation between the serum zonulin concentration and the total SANS score ($r=-0.259$, $p=0.070$); however, there was a significant negative correlation between the serum zonulin concentration and the positive schizophrenia symptoms evaluated using the SAPS ($r=-0.306$, $p=0.031$). In the patient group, no significant correlation was found between the serum claudin-5 levels, SAPS scores, or SANS scores ($p>0.05$).

Discussion

In our study, the serum zonulin and claudin-5 levels of schizophrenia patients and healthy controls were examined. The main finding of our study was that the serum zonulin levels were significantly higher and the serum claudin-5 levels were significantly lower in the schizophrenia patients when compared to the healthy controls.

To the best of our knowledge, no other studies have compared the serum zonulin levels of schizophrenia patients and healthy controls. Thus, this is the first study to explain this relationship. In a recent study conducted by Barber et al. involving 98 schizophrenia patients, 42 patients had significantly higher serum zonulin levels than cut-off values [4]. The limitation of that study was the lack of a healthy control group. Studies have also examined serum zonulin levels in patients with ASD, a neurodevelopmental disorder like schizophrenia. For example, in one study conducted by Esnafoğlu et al., the serum zonulin levels of 32 patients with ASD and 33 healthy controls were studied; the zonulin levels of the patient group were found to be significantly higher than those of the control group [9]. In another similarly designed investigation, we assessed the serum zonulin and claudin-5 levels in patients with bipolar disorder. We demonstrated that zonulin and claudin-5 concentrations increase in patients with bipolar disorder, which may contribute to the role of intestinal permeability or BBB in the pathogenesis of bipolar disorder [22]. In the present study, the zonulin levels of the patients with schizophrenia were found to be significantly higher than those of the control group. In gut-barrier disorders, intestinal luminal contents pass through the mucosal layer, affecting the enteric ganglions, and then enter the systemic circulation and affect the central nervous system [9]. In addition, intestinally sourced neurotransmitters, inflammatory cytokines, and some mediators are released [19]. We know that zonulin regulates tight junctions in intestinal and blood–brain barriers and it has been assumed as a marker of systemic inflammation [25, 34]. In our study, increased serum zonulin levels suggest that intestinal permeability and blood–brain barrier permeability

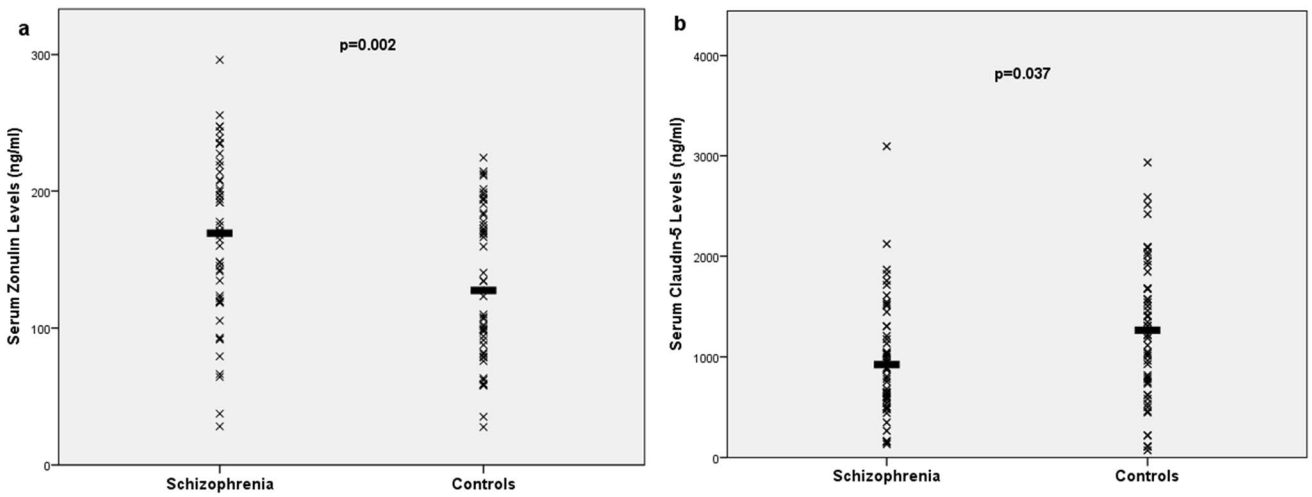


Fig. 1 Dot plots representing the distribution of serum: **a** zonulin and **b** claudin-5 levels in patients with schizophrenia and controls

may be impaired in patients diagnosed with schizophrenia. Impaired permeability of the intestinal and blood–brain barrier may influence the factors that cause the development of schizophrenia. On the other hand, the causality of changes in zonulin levels may be unclear and those results may be epiphenomena.

In studies evaluating the relationship between obesity and zonulin, it was shown that the zonulin levels were higher in obese subjects when compared to control groups and were positively correlated with BMI [29]. In a study of 143 patients with type 2 DM, 124 patients with impaired glucose intolerance, and 124 healthy subjects, the relationship between serum zonulin levels and BMI was investigated, and a positive correlation was found between these two parameters [43]. Esnafoğlu et al. also showed that there was a positive correlation between BMI and zonulin levels in an ASD group [9]. Similarly, a positive correlation was found between the BMI and zonulin levels in the schizophrenia patients in our study, and the obese patients had significantly increased zonulin levels when compared to the normal and overweight patients. These findings support the previous studies in the literature on the relationship between obesity and zonulin.

Zonulin regulates both BBB permeability and intestinal barrier permeability [4, 34]. In a study conducted by Rahman et al., they found that intestinal epithelial barrier exposure to zonulin for 24 h decreased claudin-5 at the TJs. The authors showed that zonulin can rapidly enhance the permeability of the BBB and small intestinal epithelial barrier in vitro [34]. Claudin-5 is a member of the claudin family of proteins, a major component of TJs, and a regulator of the BBB [36]. Claudin-5 is the most important component of endothelial TJs in the BBB and prevents the passage of external pathogenic substances to the brain [32]. If claudin-5 is disrupted, the brain is more often exposed to harmful environmental factors [16]. In a study conducted in 2003, size-dependent passage (less than 800 Da small molecules) was observed in mice with a claudin-5 deficiency in the BBB [31]. The relationship of claudin-5 to schizophrenia is the most important meeting point of genetic and environmental factors [36].

Genetic studies have described the relationship between schizophrenia and 22q11 deletion [2, 5], which is relevant, because the claudin-5 gene that encodes the claudin-5 protein is also located on chromosome 22q11 [36]. In addition, many studies have determined that the claudin-5 gene is associated with schizophrenia [17, 32]. For example, in a study conducted in the Iranian population in 2014, it was shown that the claudin-5 gene is associated with schizophrenia [32]. In another study conducted in China in 2004, claudin-5 was shown to be one of the most important genes associated with schizophrenia [36]. In 2017, Nishiura et al. also demonstrated with a quantitative immunohistochemical analysis that claudin-5 protein expression was significantly decreased in the prefrontal cortex gray matter microvessels of schizophrenia patients when compared to their control group [30]. Our group also carried out investigations into the serum zonulin and claudin-5 levels in children with OCD. In that study, we found that serum claudin-5 levels were found to be significantly higher in OCD patients than in controls and concluded that high claudin-5 levels might be involved in the etiology of OCD [18]. In the present study, serum claudin-5 levels were found to be significantly lower in schizophrenia compared to the control group. It is possible that the presence of a defect in the claudin-5 gene may have reduced the serum claudin-5 levels. Although our research was unable to provide any evidence of the putative role of claudin-5 in schizophrenia, the relationship between claudin-5 levels and schizophrenia must be assessed through additional research.

In our study, the relationships between serum claudin-5 levels and age, sex, BMI, disease duration, and clinical scales (SAPS, SANS) were examined in schizophrenia patients, and no statistically significant relationship was found between them. However, a significant negative correlation was found between zonulin levels and SAPS scores in the schizophrenia patients. A possible explanation for this finding might be that changes with the varying cytokine levels in schizophrenia. A recent paper reported that no relationship exists between zonulin or cytokines and psychiatric symptoms. However, the authors found an interaction for IL-1β and a trend for TNF-α associated with higher total Brief Psychiatric Rating Scale (BPRS) scores [4]. Also, zonulin levels increased during psychosocial stress and were partly related to changes in inflammatory markers [24]. Thus, increases and decreases in cytokine concentrations associated with schizophrenia symptoms may explain the connection that we found in our research between serum zonulin concentrations and symptoms in schizophrenia. We also think that serum changes of zonulin and claudin-5 levels may be correlated with the molecules of the brain. As a result, we suggest further studies of the autopsy material of the brain and intestines of schizophrenia patients and healthy controls to prove that there really is an alteration of zonulin and claudin-5 expression in the brain and intestines.

The present study has some limitations, including its cross-sectional design and small-sample size. Consequently, new longitudinal studies should be conducted in larger sample groups. In addition, our study lacked an assessment of individual differences, such as economic status, eating habits, and other psychosocial factors, potentially affecting the intestinal permeability between the groups. Our sample also consisted of non-drug-naive patients with schizophrenia. In our study, all of the patients had been using medication, though various studies have shown that the administration of antipsychotic drugs is associated with an increase in

claudin-5 levels [15]. Thus, drug use may have affected the biochemical parameters results. Another important limitation is that the intestinal microbiota were not evaluated in the patient and control groups.

Conclusion

In conclusion, our results show that in patients with schizophrenia, zonulin levels were found to be high and claudin-5 levels low. Regardless of aforementioned limitations, our results assume that zonulin and claudin-5 may be involved in the etiopathogenesis of schizophrenia. To the best of our knowledge, no prior study in the literature has compared the serum zonulin and claudin-5 levels in schizophrenia patients and healthy controls. Thus, this is the first study to explain this relationship. Further studies into this matter will contribute to the literature by explaining the relationship of zonulin and claudin-5 in schizophrenia and will allow for the development of new treatment modalities.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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