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RESEARCH ARTICLE

Relationship Plasma Concentration of Zonulin with Severity Level of Autism Based on Criteria of Diagnostic and Statistical Manual of Mental Disorders-5 (Dsm-5)

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Abstract

Background: This study was aimed to prove whether there is relationship between plasma concentration of zonulin and severity level of autism based on criteria of diagnostic and statistical manual of mental disorders-5 (DSM-5). In Indonesia, this kind of research has never been done, considering the incidence of autism is also increasing. Methods: The participants of this research were 38 autistic (level 1=17, level 2=12 and level 3= 9) and 35 control children. The blood plasma of all participants was taken to determine the plasma concentration of zonulin. The observation sheet was based on the DSM-5 as an instrument to determine the diagnosis of autistic children. Data were analyzed using Mann-Whitney and Anova test. Results: There was a significant difference plasma concentration of zonulin between autism and control ($p = 0.002$) with 32 ± 18 vs 47 ± 22.5, respectively. There was slightly different between plasma concentration of zonulin and severity level of autisme ($p=0.239$) with 32±19, 27±18, 40±15, respectively. Cut-off point 8 ng/ mL (Sensitivity 97.4%, Specificity 2.9%). Conclusion: This finding can be concluded that plasma concentrations of zonulin were slightly related with severity, and the highest is at level 3.

Keyword: *Plasma concentration of zonulin, Autism, DSM-5, Severity.*

Introduction

Autism Spectrum Disorder (ASD) is a group of neurodevelopmental abnormalities that begin in the early life of children (although the first diagnosis can occur later in life). According to DSM-5 criteria, the main manifestation of ASD include disorders of social communication and behavior (dyadic), such as attention easily disturbed and the presence of repetitive behavior. DSM-5 replaces the multi-categorical system with a single diagnostic dimension: ASD [1, 2]. According to the Centers for Disease Control (CDC) Autism and Development Disabilities Monitoring (ADDM) Network, the prevalence of ASD increased dramatically from 4.5 in 10,000 children in 1966 to 1 in 110 in 2006 and in 2010 to 1 in 68 children [3]. In Indonesia, the prevalence of autism is believed to continue to increase from year to year, although there is no complete data on the number of autistic children, but at present it is estimated to reach 150,000-200,000 people [4]. The study Jalal [5] reported that people with autism in Indonesia were estimated at 117,000 in 2010.

In addition to disorders of the nervous system, several studies have shown that ASD also experience gastrointestinal (GI) disorders, such as abdominal pain, bloated, constipation, diarrhea and flatus which are generally comorbid from ASD. The prevalence of GI symptoms ranges from 23-70% in children with ASD [6]. Constipation is the most common symptom (85%) in children with ASD based on parent reports and

evaluations from pediatric gastroenterologist [7]. In some studies, gastrointestinal disorders were associated with symptoms of abnormal behavior and other psychiatric problems, such as: social withdrawal [8], increased anxiety [9], and irritability [10]. Mucosal barrier disorder in the form of "leaky gut" is responsible for changes in intestinal permeability.

There are several reports showing an increase intestinal permeability in autism patients, however the mechanism is not yet certain [11,12]. Gut barrier and blood brain barrier (BBB) integrity is weak in ASD, with evidence of increased levels of claudin (CLDN) -3, CLDN-5, CLDN-12, and MMP-9 which form pores and decrease in intestinal tight junction component levels (CLDN-1, occludin (OCLN), and tricellulin (TRIC)) which form a barrier in ASD compared to controls [13].

Zonulin is a physiological modulator that regulates intestinal permeability by altering tight junction interactions of proteins. Zonulin controls the travel of paracellular antigens by setting the tight junction. Some potential intestinal stimuli such as gluten and intestinal bacteria increase zonulin secretion [14].

Serum zonulin levels were significantly higher in patients with ASD compared to healthy controls [15]. In DSM-5, the main manifestations of ASD include disorders of social communication and behavior, divided into three levels of severity ranging from requiring very large support, large and support only [1,16]. Various therapies were applied ranging from behavioral therapy, medical to dietary arrangements, but not all were successful, without reference to the severity of the DSM-5 [17].

The cause of ASD is multifactorial, the role of genetics as a cause of ASD is quite significant at around 37-90%, with many genes involved [18], while manipulating genes is not easy. In this study, plasma concentration of zonulin will be measured to be used as an indicator of the severity of autism. Until now, there has been no research on the association of plasma concentration of zonulin with autism severity based on DSM-5. In Indonesia, this kind of research has never been done, considering the incidence of autism is also increasing.

Materials and Methods

Research Subject

The subjects of this study were 38 autistic children aged 6-14 years, from the PLA (Autism Service Center) and extraordinary Autism Muhammadiyah University, who lived with their parents. As a control, there were 35 elementary school children in Malang. This research has received permission from the Ethics Commission of the Faculty of Medicine, Universitas Brawijaya, No. 349 / EC / KEPK-S3 / 12/2018.

Research Design

This type of research was a case control study with an epidemiological approach to determine the relationship between plasma cconentration of zonulin and autism severity. Besides that, it also determines children's characteristics based on age, gender, nutritional status and gastrointestinal disorders.

Age, Gender, and Gastrointestinal Disorders Determination

Determination of age, gender and intestinal disorders based on a questionnaire was given to their parents.

Determination of Nutritional Status

Determination of nutritional status (BMI/ age) based on WHO [19] by first measuring height using microtoise and weight using standardized scales.

Determination of Autism Severity

The observation sheet based on the DSM-5 as an instrument to determine the diagnosis of autistic children was performed by Psycholog.

Determination Plasma Concentration of Zonulin

Plasma concentration of zonulin was measured using Human HP / Haptoglobulin Elisa KIT (Elisa Sandwich) (LS Bio/ Life Span Bio Science, Inc.), with a sensitivity of <1,044 ng / mL.

Statistical Analysis

The data obtained were tabulated and analyzed. Data on the general characteristics of the subject, the severity of autism and plasma concentration of zonulin were analyzed with descriptive statistics.

The cut-off point was analyzed by Curve Receiver Operating Characteristics (ROC). The difference plasma concentration of zonulin between controls and autism was analyzed by Mann-Whitney test. The relationship between plasma concentration of zonulin and autism severity was analyzed by the Anova test, using SPSS version 20.0.

Results

General Characteristics of Subjects

The subjects in the study were 38 autism and 35 controls. Most of autism were male (86.8%), while in control were female (62.9%). The gender was a significant difference between autism and control (p= 0.000). Nutritional status was almost the same between autism and control, most with normal nutritional status (68.4% and 74.3%).

Gastrointestinal disorders (diarrhea, constipation, bloating) were "rarely" also almost the same between autism and control, ranging from 81.6% - 94.7%. The results of the calculation of subject characteristics that completed the study were presented in Table 1.

Table 1: General Characteristics of Subjects (Autism and Control)

No.	Variable	Autism (n=38)		Control (n=35)		P
		Total	%	Total	%	
1.	Gender					
	- Male	33	86.8	13	37.1	0.000[a)*)]
	- Female	5	13.2	22	62.9	
2.	Nutritional Status					
	- Thin	2	5.3	4	11.4	0.671[b)]
	- Normal	26	68.4	26	74.3	Ref
	- Overweight	10	26.3	5	14.3	0.254[a)]
3.	Diarrhea					
	- Rarely	36	94.7	32	91.4	
	- 1x/month	0	0	2	5.7	0.576[b)]
	- 2-3x/month	2	5.3	1	2.9	
4.	Constipation					
	- Rarely	31	81.6	31	88.6	
	- 1x/month	2	5.3	0	0	0.404[a)]
	- 2-3x/month	1	2.6	4	11.4	
	- >3x/month	4	10.5	0	0	
5.	bloating					
	- Rarely	32	84.2	31	88.6	
	- 1x/month	2	5.3	2	5.7	0.588[a)]
	- 2-3x/month	3	7.9	2	5.7	
	- >3x/month	1	2.6	0	0	

*) significant (p<0.05); a) Chi Square test; b) Fisher's Exact Test

Differences of Age, Plasma Concentration of Zonulin in Autism and Controls

The age of autism and control spread evenly (p=0.224), with average age 9-10 years. The plasma concentration of zonulin was significant difference between autism and control (p = 0.002) with 32 ± 18 vs 47 ± 22.5, respectively. The plasma concentration of zonulin in control was higher than autism. The results were presented in Table 2.

Table 2: Differences of Age, Plasma Concentration of Zonulin in Autism and Control

Variable	Mean± SD		Med (min-max)		p
	Autism	Control	Autism	Control	
Age (year)	9 ± 3	10 ± 2	8.6(6-14)	9.9(7-13)	0.224[a)]
Zonulin Plasma (ng/mL)	32 ± 18	47 ± 22.5	27(7.7-89.3)	40(4.1-99)	0.002[b)*)]

*) significant (p<0.05); a) T-test independent; b) Mann-Whitney

Differences of Age, Plasma Concentration of Zonulin Based on Level Severity of Autism

Age results differ significantly based on the severity of autism, where at level 3, the age of autism was much younger than levels 1 and 2, which were relatively the same age. The Plasma concentration of zonulin not differ significantly based on severity, but zonulin concentrations were slightly related with severity, and the highest was at level 3. The results were presented in Table 3.

Table 3: Differences of Age, Plasma Concentration of Zonulin Based on Level Severity of Autism

Variable	Mean±SD			Med (min-max)			p
	1	2	3	1	2	3	
Age (year)	10±3	10±3	7±1	10 (6-14)	10 (6-13)	7 (6-9)	0.022*)
Zonulin Plasma (ng/mL)	32±19	27±18	40±15	27 (7.7-89.3)	22 (8.3-64.8)	43 (20.5-62.7)	0.239

*) significant (p<0.05); Anova test; 1= requiring support only; 2= requiring large support; 3= requiring very large support

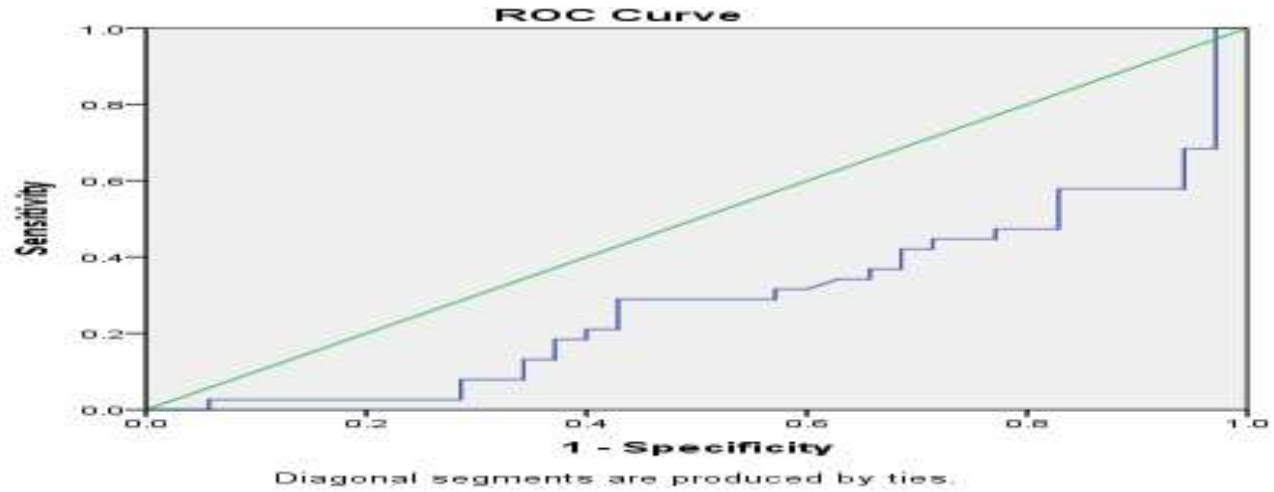

Figure 1: ROC Curve of the Plasma Concentration of Zonulin between Autism and Control

The area under the curve (AUC) was 28.5%. If the plasma concentration of zonulin that was desired only high sensitivity (97.4%), without taking into account the specificity, the cut-off point was 8. If the sensitivity was 82%, the cut- off point was 16 with the same specificity 2.9%.

Discussion

In the subject characteristics, there was a significant difference in gender between autism and control, while other variables (nutritional status, gastrointestinal disorders, age) were spread evenly. This was consistent with the literature which says that the highest prevalence of autism was male [20, 22]. Age results differ significantly based on the severity of autism, where at level 3, the age of autism was much younger than levels 1 and 2, which were relatively the same age.

Another study showed that there was related factors with earlier diagnosis included greater symptom severity, high socioeconomic status, and greater parental concern about initial symptoms. Family interactions with the health and education systems prior to diagnosis also influenced age at diagnosis [23]. According to Ohlsson [24], gastrointestinal symptoms had not any influence on zonulin plasma concentration symptoms, but the increasing of zonulin plasma concentration were associated with higher waist circumference, diastolic blood pressure, fasting glucose, and increased risk of metabolic diseases. Nutritional status and gastrointestinal symptoms in our results did not differ significantly between autism and control because the highest distribution of subjects was normal. The average plasma concentration of zonulin in autism was significantly lower than control.

However, the results of the average plasma concentration of zonulin in control were almost similar with level 3 autism, if the overall severity of autism were differentiated. The results of the ROC curve showed a cut-off point of plasma zonulin levels was 8 ng / mL (Sensitivity 97.4% and Specificity 2.9%) or 16 ng / mL (Sensitivity 82% and Specificity 2.9%).

This means that both autism and control have plasma zonulin levels that were higher than the cut-off point. Based on our results, indicated that zonulin was the basic protein that modulates tight junctions to regulate intercellular passage. Demir [25] reported that Zonulin is secreted mainly from the liver, but also from enterocytes, adipose tissue, brain, heart, immune cells, lungs, kidney, and skin. Fasano [26] also showed that gliadin and bacteria induce zonulin secretion, which increases intestinal permeability, introducing foreign antigens to the immune system and triggering inflammation.

Conclusion

This finding can be concluded that zonulin concentrations were slightly related with severity, and the highest is at level 3. High plasma concentration of zonulin in autism and control, that need to be aware as a trigger for the inflammatory process.

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