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Investigating the Relationship of Serum CD163, YKL40 and VILIP-1 Levels with Autism Severity and Language-cognitive Development in Preschool Children with Autism

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Objective: This study aimed to compare serum levels of CD163, YKL-40, and VILIP-1 between children with autism spectrum disorder (ASD) and healthy controls, while also investigating their association with the severity of ASD and language development.
Methods: The study included 40 ASD-diagnosed patients (aged 18−72 months) and 40 age-matched healthy controls. Childhood Autism Rating Scale, Preschool Language Scale-4, and Ankara Development Screening Inventory were administered to children in the ASD group. Serum CD163, YKL-40 and VILIP-1 levels were measured with an enzyme-linked immunosorbent assay kit.
Results: In the ASD group compared to the control group, serum VILIP-1 levels were significantly higher ($p = 0.046$). No significant differences were observed in mean serum CD163 and YKL-40 levels between patients and controls ($p = 0.613$, $p = 0.769$). Interestingly, a positive correlation was observed between CD163 and YKL-40 levels and ASD severity ($p < 0.001$ for both). Additionally, CD163 and YKL-40 levels showed significant predictive value for ASD severity. While no significant associations were found between CD163 and YKL-40 levels and language development, a negative correlation was observed between VILIP-1 levels and language development ($p < 0.001$).
Conclusion: Our findings highlight that the levels of CD163 and YKL-40 significantly predicted ASD severity, indicating a potential role of neuroinflammation in the development of ASD.

KEY WORDS: Autism spectrum disorder; Neuroinflammation; Chitinase-3-like protein 1; Neurocalcin.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by significant deficits in social communication and interaction, repetitive behaviors, and the presence of restricted interests [1]. The prevalence of ASD in the United States was reported as 1/54 in the 2020 report published by the Centers for Disease Control and Prevention [2].

Although the aetiology of ASD has not been fully elucidated, many mechanisms, such as advanced parental age, pregnancy-related complications, drug use during pregnancy, exposure to toxins, epigenetics, oxidative stress, hypoxic damage, neurotransmitter anomalies, and neuroinflammation, have been proposed in the literature [3]. It has been shown that any disruption that affects microglial physiological function during critical developmental periods can cause defective maturation of synaptic circuits, disruptions in neuronal connections, synaptogenesis dam-

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age and neuronal damage, leading to neurodevelopmental disorders (e.g. ASD, intellectual disability and schizophrenia) in children [4]. Neuroinflammation is a process that involves the continuous activity and proliferation of glial cells (microglia and astrocytes) and leads to changes in brain functions [5]. A significant increase in neuroglial response has been identified in individuals with ASD [6]. Moreover, neuronal connectivity and impaired synaptogenesis are perhaps the most validated hypotheses that can adequately explain the pathogenesis of ASD. To increase the activity of neurons in a healthy developing brain and thus to create functional neuronal connections, non-functional, unnecessary neurons are disabled. Thus, the total number of neurons decreases, while the connectivity and functionality between the remaining neurons increase every day. There is lots of evidence that this process is impaired in children with ASD [4]. While numerous molecules have been proposed to contribute to neuroinflammation in the development of ASD, none of them have reached enough evidence to be specific diagnostic marker at yet [7,8]. Given the prevalence of ASD and the challenges in diagnostic processes, it is important to investigate new biomarkers [9] that might suggest the neuroglial response related to neuroinflammation and neurodegeneration.

Cluster of differentiation 163 (CD163) is a 130-kDa transmembrane protein belonging to group B of the scavenger receptor cysteine-rich superfamily. CD163 can be detected both as a cell surface receptor and as a serum-soluble form of sCD163 (soluble CD163). sCD163 is a marker that is expressed in microglial cells and shows microglial activation. It has been suggested that CD163 plays a role in the regulation of adaptive immune response and that its measurement in tissue or serum in inflammatory diseases might be a guide for the severity and prognosis of inflammatory diseases [10]. Given the association between ASD and alterations in immune responses [11,12], investigating sCD163 levels could be beneficial to understanding the role of neuroinflammation in ASD. This could potentially shed light on the underlying causes and development of the ASD.

YKL-40, considered chitinase 3-like protein 1 (YKL-40) or human cartilage glycoprotein 39 (HC-gp39), is a chitin-binding lectin belonging to the glycosyl hydrolase family. YKL-40 protein is expressed by various cell types, including macrophages, chondrocytes, neutrophils, and synovial fibroblasts [13]. Its expression has been reported to increase in neuroinflammatory conditions. YKL-40 has been shown to have the capacity to regulate the plasticity or regenerative processes of neurons [14]. YKL-40 is also thought to potentially have the capacity to negatively modulate neurotrophic factor-related changes in neuronal repair and regeneration [15]. Therefore, YKL-40's contribution to regulating neuronal plasticity and its potential impact on neurotrophic factor-related neuronal repair and regeneration make it a key marker for studying the neuroinflammatory context of ASD.

Visinin-like protein 1 (VILIP-1) is a cytoplasmic protein of low molecular weight consisting of 191 amino acids. This protein is a member of the neuronal calcium sensor protein family and is involved in calcium-mediated signal transduction in neurons [16]. VILIP-1 has been described as a marker in cerebrospinal fluid and serum for some neurodegenerative and neuroinflammatory diseases [17]. It has been reported that disruption of Ca^{2+} homeostasis in neurons induces VILIP-1 to exhibit neurotoxic effects and cause damage to axons and neurons. VILIP-1 causes loss of synaptic plasticity in relation to axonal damage [18, 19]. VILIP-1 is considered a biological marker of neuronal damage due to its effects on neurons and synaptic structure [19]. For instance, VILIP-1 plays a critical role in linking pathological changes in the brain with calcium-mediated neuronal damage in Alzheimer's disease [20]. Besides, it has been reported that VILIP-1 might affect cyclic adenosine monophosphate and cyclic guanosine monophosphate-dependent neuronal processes, including neuronal differentiation, neurite outgrowth, synaptic plasticity, learning, and memory, due to these effects [21]. Therefore, investigating the potential role of VILIP-1 in ASD's pathophysiology, which comprises developmental changes in the differentiation of dendritic and axonal structures of neurons [22], illustrates the importance of obtaining a deeper understanding of its role and influence on neuronal and synaptic functions. Exploring VILIP-1 could reveal new insights and potential treatments for ASD, emphasizing the importance of investigating its various roles in neurodevelopmental disorders.

When the cellular-molecular disorders suggested in autism studies are taken together, it is believed that the synaptic transmission from other neurons cannot be regulated in the affected neurons; in other words, synaptic plasticity is impaired. For the normal functioning of the

systems, the synaptic infrastructure must be regulated according to the demands of the functional synaptic circuits. When this regulation fails, disorders in the synaptic molecular system may result in neuronal damage. Eventually, the cells respond in a way that is triggered by stress signals and does not conform to synaptic reorganization, thereby activating the glial cells. Activation of glial cells, on the other hand, leads to the production of proinflammatory signals with the participation of local defence systems and initiates neuroinflammatory processes, which is an important mechanism in ASD [23]. Based on the existing body of literature, CD163, YKL-40, and VILIP-1 have been thoroughly studied in relation to the causes of schizophrenia, depression, and other neuroinflammatory disorders [24-28]. Nevertheless, the specific functions they perform have not been extensively investigated in relation to ASD, whose aetiology are not fully understood. The choice of each of these biomarkers for the current study is based on their well-documented role in neuroinflammatory and neurodegenerative processes, which are increasingly acknowledged as important contributors to the development of ASD.

This study aims to bridge the gap in the literature by examining the serum levels of CD163, YKL-40, and VILIP-1 in children with ASD aged 1−6 years. In addition, the study aimed to investigate the correlation of these biomarkers with the language-cognitive development levels of children with ASD.

METHODS

Sample of the Study

Forty children who presented to the Gazi University Faculty of Medicine (GUTF), Department of Child and Adolescent Psychiatry with symptoms of autism spectrum disorder on an outpatient basis between September 15, 2020, and January 15, 2022 and met the inclusion criteria for the study group were included as "the ASD group." Forty children who presented to the GUTF Child Health and Diseases general pediatric outpatient clinics for various reasons and met the inclusion criteria for the control group were included as the "control group."

The inclusion criteria for the ASD group involve children who are between the ages of 18 and 72 months and have received newly diagnosed of ASD based on the criteria defined in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5). This diagnosis was independently verified by two child and adolescent psychiatrists, at least one of whom had more than 5 years of expertise in treating children with ASD. On the other hand, the control group was thoroughly chosen to consist of children who were assessed by experienced child and adolescent psychiatrists and found not to have any psychiatric disorders, thus establishing a clear separation from the ASD group.

Exclusion criteria for all subjects were having been diagnosed with an acute systemic infection or use of psychotropic drugs, having been diagnosed with chronic inflammatory, genetic, metabolic, or neurological disease or having had severe head trauma.

Measures

Sociodemographic data form

The sociodemographic data form, which was created by the researchers, includes questions both demographic and clinic features of sample such as the age, gender, educational level, educational treatments, parental relationship status, vitamin use during pregnancy monthly income, and medical and/or psychiatric disease history of the parents or other family members.

Childhood Autism Rating Scale (CARS)

The Childhood Autism Rating Scale (CARS) consists of 15 items in a separate subscale view. By completing the scale, the symptoms of ASD can be rated. Each item is rated by half scoring between 1 and 4. Here, 1 point is given for the behaviors that are considered normal according to the child's age, and 4 points are given for the behaviors that deviate the most from normal. A minimum score of 15 points and a maximum score of 60 points can be obtained from CARS, with a score between 15−29.5 points representing no ASD, 30−36.5 representing mild-moderate ASD, and 37−60 representing severe ASD [29]. The validity and reliability study of CARS in Turkey was first conducted by Sucuoğlu *et al.* [30].

Ankara Development Screening Inventory (ADSI)

Ankara Development Screening Inventory (ADSI) is a test developed for general developmental evaluation of children between the ages of 18−72 months in four different developmental domains. ADSI consists of four sub-

scales, including language/cognitive skills, fine motor skills, gross motor skills, and social/activities of daily living skills, to which are responded "Yes, No or Don't know", and 154 items. In addition to the score for the development of children in these four sub-dimensions, the general development score can also be calculated with the ADSI. ADSI has been shown to be a valid and reliable instrument for evaluating the developmental level of children in the Turkish population [31,32].

Preschool Language Scale (PLS-4)

The Preschool Language Scale was developed for the assessment of language development in children aged 1－6 years 11 months. Besides language development and other social communication domains, it also reflects the cognitive development process of the child [33,34]. PLS-4 results are provided at 2-month intervals consistent with the development of the child, with minimum and maximum values of '0－2' months, '3－5' months, '6－8' months, and '9－11' months. Scores are provided at 5-month intervals from 1 year of age, with minimum and maximum values of '12－17' months, '18－23' months, '24－29' months, '30－35' months, '36－41' months, '42－47' months, '48－53', '54－59' months, '60－65' months, '66－71' months, '72－77' months and '78－83' months. PLS-4 has been translated into Turkish and has been shown to be a valid and reliable scale in the Turkish population [35].

Biochemical Analyses

Ten milliliters (10 ml) of venous blood samples were collected from participants in the ASD and healthy control groups who met the inclusion criteria and placed into a biochemistry tube at 09:00 in the morning. The blood samples were centrifuged at 3,000 rpm for 10 minutes for the measurement of serum YKL-40, CD163, and VILIP-1 levels 30 minutes after the samples were taken into yellow top tubes with gel. The obtained serums were placed in new Eppendorf tubes and stored at －80°C until analysis.

Serum levels of YKL-40, CD163, and VILIP-1 were analyzed by the enzyme-linked immunosorbent assay method. The commercial kits used were from Bioassay Technology Laboratory (Shanghai Korain Biotech Co. Ltd.) for YKL-40 and CD163, and Cloud-Clone Corp. for VILIP-1.

The intra-assay coefficient of variation (CV) values of the YKL-40, CD163 and VILIP-1 kits are $< 8\%$, $< 8\%$, and $< 10\%$, respectively, while the inter-assay CV values are $< 10\%$, $< 10\%$, and $< 12\%$, respectively. The measurement ranges of the YKL-40, CD163, and VILIP-1 kits are 1－400, 0.1－30, and 0.156－10 ng/ml, respectively. The sensitivity of the kits are 0.61, 0.052, and 0.055 ng/ml, respectively.

During the study, washing procedures were performed with a BIOTEK washer device (EL ×50 Bioelisa Washer; BioTek Instruments), and absorbance readings were made with a BIOTEK reader (EL ×800 UV Universal Microplate Reader; BioTek Instruments).

Procedure

A psychiatric interview was conducted with all participants who presented to our outpatient clinic at the Gazi University Faculty of Medicine Hospital Department of Child and Adolescent Psychiatry by an experienced clinician. According to the information received from the families of children with ASD, CARS was applied by the clinician. Psychiatric comorbidities were excluded in the DSM-5-based clinical examinations of these children, who were diagnosed with ASD by at least 2 experienced clinicians, both clinically and by considering the DSM-5 criteria.

All children with ASD between the ages of 18－72 months who met these criteria were referred to the Departments of Pediatric Neurology, Pediatric Nutrition and Metabolism, and Medical Genetics to be examined for possible neurological, metabolic, and genetic diseases. Complete blood count was routinely requested from both the case and control groups to confirm the absence of active infection. As a result of these evaluations, the families of children aged 18－72 months without any neurological, metabolic, or genetic diseases were informed about the study, and a voluntary consent form was obtained from the families. The questions in the sociodemographic data form were completed by the clinician according to the information received from the families of the participants. PLS-4 was applied to children with ASD by an audiologist with a certificate of application of the PLS-4 scale. As stated in the materials of the PLS-4 scale and to be stated in the results, the data obtained from the PLS-4 based on the calendar ages of the children were extracted, and the difference in months was recorded. Language development levels of children with ASD were determined with the PLS-4 scale. In order to evaluate the

relationship between language development level and cognitive development and general development, the ADSI was applied to children with ASD. As stated in the materials of the ADSI scale and to be stated in the results, the data obtained from ADSI based on the calendar ages of the children were extracted, and the difference in months was recorded.

The families of the children who met the inclusion criteria for the control group were informed about the study and were included after the families were asked to read the voluntary informed consent form and complete the relevant parts. It was determined that the children in the control group did not have a psychiatric diagnosis based on the histories taken by the clinician and as a result of their clinical evaluation. Based on the past medical histories of the children in the control group and the information obtained from their families, it was confirmed that they did not have any medical diseases. The questions in the sociodemographic data form were completed by the clinician according to the information received from the families of the participants.

Statistical Analysis

The data obtained from the participants in this study were analyzed using the IBM SPSS Statistics version 24.0 software package (IBM Co.). The Shapiro-Wilk test, Q-Q plot, and histogram graphs were used to determine whether the sample group showed a normal distribution in terms of dependent variables. Non-parametric tests were used when the sample group did not show a normal distribution in terms of dependent variables. The descriptive analysis method of chi-square was used to evaluate socio-demographic data; the Mann-Whitney *U* test and Kruskal-Wallis test were used in intergroup comparisons, and Spearman's correlation analysis was used to examine the association between dependent variables.

Ethics Committee Approval

The study was approved by the Clinical Research Ethics Committee of Gazi University Faculty of Medicine on August 7, 2020 with the decision number 504.

RESULTS

Table 1 presents the data regarding the age, gender, and educational levels of the groups included in the sample, as well as the scores of CARS and PLS-4 in the ASD group. No statistically significant differences were found between the mean age (U = 0.768, p = 0.758) or gender (χ^2 = 0.000, p = 1.000) of the groups. According to the total scores of CARS, 37.5% (n = 15) of the children with ASD showed severe and 62.5% (n = 25) showed mild-moderate symptoms of ASD. It was observed that the PLS-4 receptive language data of the children were a minimum of 9 months and a maximum of 59 months. It was observed that the PLS-4 expressive language data of the children were a minimum of 9 months and a maximum of 53 months.

Data on Blood Parameters, Scales and Age in the ASD Group

Comparison of serum CD163, YKL-40, and VILIP-1 levels between the ASD and control groups is presented in

Table 1. Data on the age, gender, and educational status of children in ASD and control groups

Demographic characteristics	ASD group (n = 40)		Control group (n = 40)		U	χ^2	p value
Age (mo)	44.28 (18−70)	16.52 (19−71)	42.65	14.2	0.768		0.758
CARS	39.35	7.89					
PLS-4 receptive language lower value	21.45	14.08					
PLS-4 receptive language upper value	25.55	14.94					
PLS-4 expressive language lower value	19.35	12.71					
PLS-4 expressive language upper value	23.15	13.76					
Sex						0.000	1.000
Girls	9 (22.5)		9 (22.5)				
Boys	31 (77.5)		31 (77.5)				
Educational status						4.073*	0.044
Not attending kindergarten	23 (57.5)		14 (35.0)				
Attending kindergarten	17 (42.5)		26 (65.0)				

ASD, autism spectrum disorder; CARS, Childhood Autism Rating Scale.
*$p < 0.05$.

Table 2. There was a significant difference in the groups regarding VILIP-1 (p = 0.046) levels, with no significant different observed for CD163 (p = 0.613) and YKL-40 (p = 0.769) levels. The children in the ASD group exhibited notably higher VILIP-1 levels compared to the control group.

Table 3 demonstrates the relationship between children's age with ASD and serum biomarker levels, total CARS scores, and PLS-4 data. There was a strong positive correlation between CARS total scores and CD163 (r = 0.969, $p < 0.01$) and YKL-40 (r = 0.966, $p < 0.01$) values, but no significant correlation between CARS total scores and VILIP-1 (r = 0.009, p = 0.954) values. While there was a strong correlation between VILIP-1 values and various PLS-4 language differences, no significant difference was detected among the other variables. There was a strong positive correlation between VILIP-1 values and the ADSI general development upper value difference (r = 0.701, $p < 0.01$), as well as the ADSI general development lower value difference (r = 0.655, $p < 0.01$). A strong positive correlation was found between CD163 and YKL-40 parameters (r = 0.960, $p < 0.01$). There was no significant correlation between VILIP-1 and the other two parameters.

Table 4 shows the results of hierarchical multiple regression analysis. A two-step hierarchical regression analysis was performed to determine the predictors of severity of ASD. From the correlation analysis age as a demo-

Table 2. Comparison of serum CD163, YKL-40, and VILIP-1 levels of the groups

Blood values	Group	N	Mean	Total	U	p value
CD163	ASD group	40	39.19	1,567.5	747.5	0.613
	Control group	40	41.81	1,672.5		
YKL-40	ASD group	40	39.74	1,589.5	769.5	0.769
	Control group	40	41.26	1,650.5		
VILIP-1	ASD group	40	45.69	1,827.5	592.5*	0.046
	Control group	40	35.31	1,412.5		

*$p < 0.05$.

Table 3. Correlation between variables in the ASD group

Variables	1	2	3	4	5	6	7	8	9
1. Age	-								
2. CD163	−0.353*	-							
3. YKL-40	−0.379*	0.960**	-						
4. VILIP-1	0.134	0.030	−0.080	-					
5. CARS total score	−0.389*	0.969**	0.966**	0.009	-				
6. PLS-4 expressive language upper value difference	0.566*	−0.020	−0.105	0.660**	−0.050	-			
6. PLS-4 expressive language lower value difference	0.664*	−0.079	−0.153	0.598**	−0.107	0.978	-		
8. PLS-4 receptive language upper value difference	0.450*	−0.023	−0.090	0.716**	−0.011	0.934	0.904	-	
9. PLS-4 receptive language lower value difference	0.531*	−0.043	−0.132	0.668**	−0.068	0.946	0.943	0.985	-

ASD, autism spectrum disorder; CARS, Childhood Autism Rating Scale; -, not available.
*$p < 0.05$, **$p < 0.01$.

Table 4. Summary of the results of hierarchical regression analysis predicting severity of autism spectrum disorder

Predictor	B	Beta	t	p value	F	R	R^2
Step 1					F(1.38) = 4.30	0.32	0.12
Age	−0.15	−0.32	−2.07	0.046			
Step 2					F(2.36) = 505.22	0.98	0.96
CD163	0.31	0.23	2.16	0.037*			
YKL-40	0.51	0.01	6.93	0.001**			

*$p < 0.05$, **$p < 0.01$.

graphic variable was controlled for within the first step. In the first step, age of children with ASD significantly contributed to the model, suggesting that children with higher age have lower ASD severity. Controlling for the effects of CD163 and YKL-40 levels of children with ASD, the effect of CD163 and YKL-40 levels on severity of ASD becomes statistically significant in the final step. As such, it can be suggested that CD163 and YKL-40 accounted for a significant amount of variance in ASD severity among children with ASD over and above the effects of age.

Data on the Sociodemographic and Clinical Characteristics of the Families of Children with ASD and the Control Group and The Relationship Between ADSI and PLS-4 Data in the Group with ASD are given in Supplementary Tables 1, 2 (available online).

DISCUSSION

In this study, the serum CD163, YKL-40, and VILIP-1 levels of children with ASD aged 18−72 months were compared with those of the control group, which consisted of typically developing children of similar age and socioeconomic characteristics. Serum VILIP-1 levels were found to be higher in the ASD group compared to the control group, while there was no significant difference in serum YKL-40 and CD163 levels between the groups. The correlation between serum CD163, YKL-40, and VILIP-1 levels, age, ASD severity, and language and cognitive development levels of children with ASD was examined. Serum YKL-40 and CD163 levels of children with ASD were found to be significant predictors of ASD severity according to the CARS scores after controlling for age. However, no significant correlation was found between serum VILIP-1 levels and ASD severity. A significant correlation was found between serum VILIP-1 levels and PLS-4 scores. According to the ADSI scores, VILIP-1 levels increase similarly as the deficiency in general developmental level and language development level increase (Supplementary Tables 1, 2; available online). There was no significant correlation between YKL-40 and CD163 and language development level.

Evaluation of Data on Serum CD163

Our study demonstrated no significant difference in sCD163 levels between the patient group and healthy controls. There is no study examining serum levels of CD163 in children and adolescents with ASD. However, unlike our results, a study of patients diagnosed with schizophrenia reported significantly higher CD163 levels in the "high inflammatory biotype" of schizophrenia patients with high increases in cytokine transcripts compared to the control group [24]. Studies conducted in patients diagnosed with both Parkinson's disease and Alzheimer's disease reported significantly higher CSF CD163 levels in both disease groups compared to the control group [36,37]. The absence of a significant difference between the patient group and the control group was thought to be due to the fact that sCD163 levels are affected by biochemical parameters related to body mass index, lipid metabolism, and liver functions [38].

Our study revealed a positive correlation between the symptoms and severity of the disease and serum CD163 levels, in line with the literature [24,37,39,40]. In addition, serum CD163 level was found to be a predictor of ASD severity, even after controlling for age. This result shows that the level of serum CD163 increases with the increase in the severity of symptoms in ASD and is supported by literature data reporting that the severity of ASD becomes more pronounced with the increase in the level of neuroinflammation in ASD [11]. The results of this study suggest that monitoring serum CD163 levels in the clinical follow-up of children with ASD may provide prognostic benefits. However, our study is the first to investigate the relationship between CD163 and ASD, and these results should be supported by clinical studies with larger samples.

Evaluation of Data on Serum YKL-40

The results of the study showed no significant difference between the ASD group and the control group in terms of serum YKL-40 levels. Our result is consistent with the results of studies in the literature reporting no significant relationship between CSF or serum YKL-40 levels in diseases in which neuroinflammation plays a role in the etiopathogenesis [41-44] but is inconsistent with the results of studies that found a significant relationship [45-49]. This may be related to non-neurodegenerative diseases such as obesity and asthma that affect YKL-40 levels [13,50,51]. In this study, positive correlation was found between the levels of YKL40 and CD163 levels in serum. Some biomarkers directly reflect the process of microglial/macrophage activation [52]. YKL-40, is secreted

by activated macrophages and microglia [53] a microglial biomarker, has recently been reported to play a role in downstream anti-inflammatory and antioxidant responses by inhibiting the proliferation and activation of T lymphocytes [54]. Our data contribute to the growing recognition of the role of microglia/macrophages in ASD. Furthermore, this finding suggests that YKL-40 and sCD163 serum concentrations, can be used as biomarkers to assess the severity of ASD and may serve as markers to monitor the effects of treatment and predict disease prognosis.

Our study showed a positive and significant correlation between total CARS scores showing the symptoms and severity of ASD and serum YKL-40 levels. Our results are consistent with the results of other studies that reported a positive correlation between YKL-40 levels and clinical severity in diseases in which neuroinflammation plays a role in etiopathogenesis [46,47,55]. Considering the literature data reporting that the severity of ASD becomes more pronounced with the increase in the level of neuroinflammation in ASD [11] along with these results, it is suggested that YKL-40, a neuroinflammatory marker, may be a promising marker that can be used in the clinical follow-up of children with ASD.

Considering the studies showing that there is a relationship between the language-cognitive levels of children with ASD and the severity and prognosis of the disease [56,57]; we expected that serum YKL-40 and serum CD163 levels, which we found to be correlated with ASD severity, would also be correlated with delays in children's language cognitive levels. However, in our study, no significant relationship was found between the PLS-4 data showing the language development level and the ADSI data showing the cognitive development level in the ASD group, and serum YKL-40 and CD163 levels. As the reason for this; It can be explained by the relatively limited number of our sample, the presence of many different factors affecting language-cognitive development in children, and the fact that YKL-40 levels were not examined in CSF.

Evaluation of Data on Serum VILIP-1

The most remarkable result of our study is significantly higher serum VILIP-1 levels in the ASD group compared to the control group. A study involving patients with schizophrenia reported a correlation between VSNL1 gene expression and abnormalities in neuronal cell architecture [25]. It has been reported that disruption of Ca^{2+} homeostasis in neurons induces VILIP-1 to exhibit neurotoxic effects and cause damage to axons and neurons [18,19]. The high level of VILIP-1, a marker of neuronal damage, in the ASD group supports the literature results indicating impaired neuron plasticity and pointing out neurodegenerative processes in the etiology of ASD [4, 58,59].

Another important result of our study is the significant correlation between serum VILIP-1 levels and PLS-4 data showing the level of language development and ADSI data showing the level of general development in the ASD group. The results of our study revealed that as the delay in language development and general development increased, serum VILIP-1 levels increased. Considering that language development in preschool children reflects the cognitive level [60], this result can be interpreted as a potential association between the degree of intellectual disability in individuals with ASD and serum VILIP-1 levels. In parallel to the studies presenting results on neuronal cell loss and damage in the etiology of ASD and emphasizing that cognitive destruction is associated with neuronal cell damage and cell loss, the correlation between mental disability and VILIP-1 levels showing neuronal damage in our study suggests that our results are consistent with those reported in the literature [4,58].

Our study showed no significant correlation between total CARS scores showing the symptoms and severity of ASD and serum VILIP-1 levels. In the literature, there are studies reporting that VILIP-1 is correlated with disease severity in neurodegenerative diseases [61-63], while our study is inconsistent with the literature data. However, it is believed that the absence of a significant correlation between total CARS scores and VILIP-1 levels could be explained by the fact that factors [3] other than neuronal damage are also effective in the etiopathogenesis of ASD.

Conclusions

While VILIP-1 levels were found to be significantly higher in the ASD group compared to the control group, there was no significant difference between the groups in terms of serum CD163 levels and YKL-40 levels. Serum YKL-40 and CD163 levels were determined to be significant predictors of disease severity in children with ASD. These results suggest that VILIP-1 may be associated with the etiopathogenesis of ASD independent of symp-

tom severity, while CD163 and YKL-40 may be associated with symptom severity of ASD. These findings need to be supported by large-scale prospective studies.

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■ **Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

■ **Author Contributions**

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