Serum Relaxin-3 and Irisin Levels in Children with Autism Spectrum Disorder: Comparison with Healthy Controls

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ABSTRACT

Objective: The incidence of Autism Spectrum Disorder (ASD) has increased in recent years. Genetic, biological and environmental factors play a role in its etiopathogenesis. The relationship between ASD and neuropeptides, one of the biological factors, is being increasingly investigated. This study aims to compare serum relaxin-3 and irisin levels of ASD cases with healthy controls.

Methods: This prospective case-control study involves a total of 68 children: 38 ASD diagnosed patients as the case group and 30 as the control group. Serum relaxin-3 and irisin levels were measured using the enzyme-linked immunosorbent assay technique.

Results: Serum relaxin-3 and irisin levels were found to be lower in the case group than in the control group. However, a statistically significant difference between groups was found only for serum irisin levels. The cut-off value for serum irisin level was determined as 3.5 ng/ml.

Conclusion: Irisin may play a potential role in the etiopathogenesis of ASD. A more extensive series of studies are needed in order to establish the use of this biomarker in the diagnosis and management of ASD.

INTRODUCTION

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and interaction, repetitive behavior patterns, and restricted interests and activities (1). ASD prevalence has been rising in recent years. While the ASD prevalence in children aged 4 years was 1.34% in 2010; this rose to 1.53% in 2012 and 1.7% in 2014 (2). Although ASD is a common disorder, its etiological causes have not been clearly elucidated. However, it is known that genetic, biological and environmental factors affect the brain during its developmental stages (3). There is a rapid increase in neurochemical, neuroendocrinological, neuro-immunological and neurophysiological studies being set out to elaborate the differences between ASD and normal development. For instance, it has been reported that serotonin, which functions as a growth factor in early neuronal development, is found to be lower in individuals with ASD than in controls, and this demonstrated a negative impact on social behavior (4). In another study, it has been shown that applying nasal oxytocin treatment to individuals with ASD between the ages of 6-12 provided an increase in social functioning levels of these individuals (5). One of the etiological factors investigated in ASD is glutamatergic receptors. Glutamate levels were found to be higher in adults with ASD (6).

In recent years, researchers have been investigating various aspects of the relationship between psychiatric diseases and neuropeptides. For instance, relaxin-3, is a highly conserved neuropeptide that is mostly secreted from four small groups of γ-aminobutyric acid (GABA) neurons (7). There are various types of relaxin in mammals. In contrast to other types, relaxin-3 is found in high proportion in the mammalian brain (8). Relaxin-3 is mostly synthesized in the nucleus incertus, pontine raphe nucleus and substantia nigra (9). Studies have shown that it is involved in cognition, arousal, motivation, anxiety, mood, pain and oculomotor control functions (10). It was stated in a study that exog-

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enous relaxin-3 injection in rats provided an anxiolytic effect. This effect is thought to be as a result of relaxin-3-mediated alteration in oxytocin level in the hypothalamus (11). Similarly, Marwari et al. (12) revealed anxiolytic and antidepressant effects of relaxin-3 in their study involving intranasal administration of relaxin-3 to rats.

Another neuropeptide factor being investigated is irisin, consists of 112 amino acids and is termed as an exercise-induced myokine. It is largely secreted from skeletal muscle, and to a lesser extent from adipose tissue, pancreas, cardiac muscle, etc. (13). The irisin has been studied in obesity and metabolic disorders due to its larger energy regulatory and thermogenetic properties (14, 15). However, in recent studies, it has been emphasized that it affects brain functions by modulating neurotransmitter release (16, 17). Furthermore, it is emphasized that irisin, by regulating brain-derived neurotrophic factor (BDNF) expression, can initiate neurogenesis in the hippocampus, reduce neuronal damage, increase expression of antioxidant enzymes, and decrease proinflammatory cytokines (18).

It is important to reveal potential biochemical differences in children with ASD in terms of both understanding the pathogenesis and determining diagnosis and treatment strategies. This study aims to compare serum relaxin-3 and irisin levels in children with ASD with a control group. Our study is the first study on these two biomarkers in individuals with ASD.

MATERIALS AND METHODS

PARTICIPANTS AND PROCEDURE

This prospective case-control study was conducted at Konya Training and Research Hospital between January 15 and June 15, 2020, with the approval of Ethics Committee (Protocol number 14567952-050/74 on 10.01.2020). Participants' families were informed about the study according to the Declaration of Helsinki, and written consents were obtained from those who accepted.

The case group consisted of 38 children who were followed up for or newly diagnosed with ASD according to the diagnostic criteria of DSM-5. All patients were evaluated by the same child and adolescent psychiatrist. Individuals with known additional genetic, metabolic, endocrinological, inflammatory or neurological diseases and ASD patients on psychotropic medications were not included in the study. The control group consisted of 30 individuals who visited the pediatrics department for routine examination, with no known disease or psychopathology and with no medication usage.

The psychiatric evaluation of the case and control groups was done by one child and adolescent psychiatrist. Then, families were asked to fill in a sociodemographic data form. Subsequently, blood samples were taken from the case and control groups to identify plasma relaxin-3 and irisin levels. After centrifuging the blood samples, they were preserved at -80 ° C until the time of study. Serum relaxin-3 and irisin levels were analyzed by enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Bioassay Technology Laboratory Human Irisin ELISA Kit and Human Relaxin-3 ELISA Kit).

STATISTICAL ANALYSIS

SPSS 22.0 package program was used for statistical analysis. Variables were evaluated for normal distribution using the Kolmogorov Smirnov and visual methods (histograms and probability plots). Chi square test was used to evaluate categorical data. Categorical variables are presented as frequencies and percentages. Normally and non-normally distributed data are presented as mean and standard deviation. Student t test was used to evaluate the differences in parametric data. MannWhitney U test was used to evaluate non-parametric data. The cut-off value for serum irisin level in the case group was assessed using ROC analysis. p value <0,05 was considered statistically significant.

RESULTS

In the study, there were 38 children (6 girls/32 boys) in the case group, and 30 children (6 girls/24 boys) in the control group. The average age was 74.00 ± 32.29 months for the case group and 78.60 ± 41.19 months for the control group. No statistically significant difference was found between the groups in terms of gender and age ($p = 0.651$, $p = 0.688$; respectively). The case group was analyzed in terms of ASD characteristics and ASD education process. The case group was divided as Level 1-2-3 according to the severity of ASD (n=7, n=12, n=19; respectively). Level 1 is "Requiring support." Level 2 is "Requiring substantial support." Level 3 is "Requiring very substantial support." It was found that the verbal communication of four patients (10.5%) could not be developed, and 34 patients (89.5%) had speaking skills at words and sentences level. In the case group, it was found that 33 (86.8%) ASD cases received individualized education and 22 (57.9%) went to school. The mean age of starting individualized education was 3.57 ± 1.58 years and the average duration of receiving education was 2.71 ± 1.84 hours/week. The personal features of ASD

individuals and the education processes they received are shown in Table 1. Although the relaxin-3 level was found to be lower in the case group, the difference between the groups was not statistically significant (p = 0.566). Serum irisin level was lower in the case group. The difference was statistically significant (p = 0.006). The comparison of the case and control groups in terms of serum irisin and relaxin-3 levels is given in Table 2. The cut-off value was identified as 3.5 ng/ml in the ROC analysis performed to identify the predictive cut-off value of serum irisin levels for the diagnosis of ASD. Results are provided in Table 3. When DSM-5 ASD severity levels were compared with the Kruskall-Wallis H test in terms of irisin levels, no statistically significant difference was found (p=0.443).

Table 1. *Personal and educational characteristics of the case group*

	n	%		n	%
DSM-5 classification					
Level 1	7	18.4	Reason for presentation	31	81.6
Level 2	12	31.6	Medical report	7	18.4
Level 3	19	50	Treatment/Follow up		
Speaking level			Individualized education		
No word	4	10.5	Present	33	86.8
Single words	13	34.2	Absent	5	13.2
2-word sentences	5	13.2			
3 or more words sentence	16	42.1			
Sensory perception			Inclusive education at school		
Present	31	81.6	Present	22	57.9
Absent	7	18.4	Absent	16	42.1
Sound perception			Degree of educational benefit		
Present	23	60.5	Never	5	13.2
Absent	15	39.5	Very little	4	10.5
			Moderate	18	47.4
			Substantial	6	15.8
			Very substantial	5	13.2
Smell perception					
Present	13	34.2	Information resource on ASD	26	68.4
Absent	25	65.8	Child and adolescent psychiatrist		
			Educational institution	17	44.7
			Internet/social media	27	71.1
			Other families with ASD child	5	13.2
Taste perception					
Present	8	21.1			
Absent	30	78.9			

ASD: Autism Spectrum Disorder
DSM-5: The Diagnostic and Statistical Manual of Mental Disorders-5

Table 2. *Comparison of the case and control groups in terms of serum relaxin-3 and irisin levels*

	Case group (n=38) Mean±Std. Dev.	Control group (n=30) Mean±Std. Dev.	z score	p value
Serum relaxin-3 level	220.20±28.66	232.66±42.00	-0,57	0.566
Serum irisin level	4.40±0.74	7.28±1.58	-2,76	0.006*

MannWhitney U test
* statistical significance

DISCUSSION

In this prospective case-control study, plasma irisin and relaxin-3 levels of individuals with ASD were compared with control group, and were found to be lower in those diagnosed with ASD compared to the control group as an outcome of our study. However, statistical significance between the groups was found only for plasma irisin level.

There are many neuropeptides that have different functions in the human body. One of these is relaxin, which has been widely researched in recent years. Although there are many types of relaxin in humans, relaxin-3 is more involved with the central nervous system (7, 8). Due to its role in fasting, food intake, etc., it has been studied more commonly in obesity and metabolic diseases (19). However, anxiolytic and antidepressant effects of relaxin-3 have been revealed in studies conducted in rats. In a study conducted by Ryan et al. (20) on rats, relaxin / insulin-like family peptide receptor 3 (RXFP3) selective agonist was administered to rats whose anxiety model was created experimentally. At the end of the experiment, it was found that symptoms of anxiety and depression in rats decreased. As a result of the study, it was stated that relaxin-3/RXFP3 signals play a role in the modulation of depression and anxiety, and that RXFP3 agonists can be used in future in the management of anxiety and depression. Zhang et al. (21) similarly demonstrated the anxiolytic effects of RXFP3 agonists on mice. It was suggested that modeling possible therapeutic potential of these drugs is important in revealing how well their actions are conserved. In contrast to these studies, Rytova et al. (22) indicated that chronic

Table 3. *Diagnostic value of irisin in predicting autism spectrum disorder*

Variable	AUC (95% CI)	Cut-off value	Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
Plazma irisin level	0,696 (0,571-0,822)	3,5 ng/ml	65,8	60,0	67,6	58,1

AUC: Area under the curve
CI: Confidence interval

activation of RXFP3 promoted anxiety and social avoidance in rats. Nakazawa et al. (11) in a study found that relaxin-3 has an anxiolytic effect via a signaling pathway involving oxytocin. In addition, it is known that oxytocin has effects on basic symptoms and brain structures in individuals with ASD (23).

In our study, plasma relaxin-3 level was found to be low in patients with ASD. However, the difference with the control group was not statistically significant. Considering the association of oxytocin and relaxin-3, relaxin-3 level is expected to be low. However, due to the small sample size, this difference may not be significant.

In recent years, it has been shown that skeletal muscle also functions as a secretory organ in the body. Cytokines and other peptides produced by muscle cells and released into the circulation are referred to as myokines (24). Irisin is one of these myokines. The irisin can affect many systems in the body. Mostly, research has focused on the metabolic effects of irisin. Tentolouris et al. (25) in their study involving comparison of diabetic patients and controls found lower serum irisin levels in the case group. Similarly, Yüksel et al. (26) found low serum irisin levels in pregnant women with gestational diabetes. In addition, the effects of irisin on the central nervous system were investigated on mice. Li et al. (27) investigated the effect of irisin on cerebral ischemia caused by middle cerebral artery occlusion in mice. Researchers found that plasma irisin concentrations decreased after ischemia, and that plasma irisin level had a negative correlation with brain infarct volume and neurological deficits. Additionally, they found that irisin protects neuronal cells during ischemia, and irisin treatment in mice causes regression in brain damage, neurological deficits and cerebral edema. Moon et al. (28) investigated the role of irisin in the hippocampus in mice and determined that irisin at physiological concentration (5-10 nmol/L) did not cause alteration in cell proliferation compared to controls, but irisin at pharmacological concentration (50-100 nmol/L) caused increased cell proliferation. Children with ASD have some abnormalities in their brain structures compared to other children with normal development. Sparks et al. (29) in a neuroimaging study compared ASD children with developmentally delayed and typically developed children and found an increase in amygdala and hippocampus volumes in children with ASD. In a study conducted by Xu et al. (30) indicated that hippocampus volumes of individuals with ASD were larger than controls and this abnormal pattern continued into adolescence.

Our study is the first to investigate plasma irisin levels in individuals with ASD. In our study, plasma irisin levels were found to be lower in the case group than in the control group. This finding supports the norm that there are some abnormalities in the brain of ASD patients at cellular level due to many factors. This can be attributed to the fact that irisin serves a healing purpose in brain conditions such as ischemia and anoxia. It is thought that brain damage that may occur in prenatal, natal and postnatal periods in these individuals may be related to low serum irisin levels. In addition, the fact that a cut-off value was determined for plasma irisin level and that low irisin levels support the diagnosis of ASD stand out as the strengths of our study.

LIMITATIONS

Our study is strong in terms of pioneering the investigation of irisin and relaxin-3 levels in individuals with ASD and determining a relationship between low irisin levels and ASD. Despite all its strengths, it has some drawbacks. The small sample size, being a cross-sectional study, and not evaluating other neuroendocrinological variables that may affect irisin and relaxin-3 levels can be considered as limitations.

CONCLUSION

In conclusion, the etiopathogenesis of ASD is still not fully elucidated. Many genetic, endocrinological and environmental factors are being studied. It is important to identify the possible biochemical abnormalities in children with ASD in terms of understanding the pathogenesis and determining the diagnosis and treatment strategies. Our study investigated plasma relaxin-3 and irisin levels in individuals with ASD. A statistically significant lower plasma irisin levels in individuals with ASD than the control group was found. Identifying biological abnormalities in ASD is also important for the reliability of the diagnosis of ASD, which is a clinical diagnosis. As an outcome of the study, the predictive cut-off value of plasma irisin level in the diagnosis of ASD was identified as 3.5 ng/ml. Studies on this subject with larger series are needed in order to clearly demonstrate the association of ASD and irisin. Our study will be a guide for future studies.

Key Points

Statistically significant difference between the groups was found only for serum irisin levels.

The cut-off value for the serum irisin level was determined as 3.5 ng/ml.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association, 2013.
2. Christensen DL, Maenner MJ, Bilder D, et al. Prevalence and characteristics of autism spectrum disorder among children aged 4 years - Early autism and developmental disabilities monitoring network, seven sites, United States, 2010, 2012, and 2014. MMWR Surveill Summ 2019;68:1-19.
3. Hodges H, Fealko C, Soares N. Autism spectrum disorder: Definition, epidemiology, causes, and clinical evaluation. Transl Pediatr 2020;9:55-65.
4. Chugani DC. Neuroimaging and neurochemistry of autism. Pediatr Clin North Am 2012;59:63-73.
5. Parker KJ, Oztan O, Libove RA, et al. Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism. Proc Natl Acad Sci USA 2017;114:8119-8124.
6. Fatemi SH, Halt AR, Stary JM, et al. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. Biol Psychiatry 2002;52:805-810.
7. Tanaka M, Iijima N, Miyamoto Y, et al. Neurons expressing relaxin 3/INSL 7 in the nucleus incertus respond to stress. Eur J Neurosci 2005;21:1659-1670.
8. Bathgate RAD, Halls ML, Van Der Westhuizen ET, et al. Relaxin family peptides and their receptors. Physiol Rev 2013;93:405-480.
9. Ma S, Bonaventure P, Ferraro T, et al. Relaxin-3 in GABA projection neurons of nucleus incertus suggests widespread influence on forebrain circuits via G-protein-coupled receptor-135 in the rat. Neuroscience 2007;144:165-190.
10. Ma S, Smith CM, Blasiak A, Gundlach AL. Distribution, physiology and pharmacology of relaxin-3/RXFP3 systems in brain. Br J Pharmacol 2017;174:1034-1048.
11. Nakazawa CM, Shikata K, Uesugi M, et al. Prediction of relaxin-3-induced downstream pathway resulting in anxiolytic-like behaviors in rats based on a microarray and peptidome analysis. J Recept Signal Transduct Res 2013;33:224-233.
12. Marwari S, Poulsen A, Shih N, et al. Intranasal administration of a stapled relaxin-3 mimetic has anxiolytic- and antidepressant-like activity in rats. Br J Pharmacol 2019;176:3899-3923.
13. Aydin S. Three new players in energy regulation: Preptin, adropin and irisin. Peptides 2014;56: 94-110.
14. Moreno-Navarrete JM, Ortega F, Serrano M, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. J Clin Endocrinol Metab 2013;98:769-778.
15. Polyzos SA, Anastasilakis AD, Efstathiadou ZA, et al. Irisin in metabolic diseases. Endocrine 2018;59:260-274.
16. Mattson MP. Energy intake and exercise as determinants of brain health and vulnerability to injury and disease. Cell Metab 2012;16:706-722.
17. Erickson KI, Weinstein AM, Lopez OL. Physical activity, brain plasticity, and Alzheimer's disease. Arch Med Res 2012;43:615-621.
18. Korta P, Pocheć E, Mazur-Biały A. Irisin as a multifunctional protein: Implications for health and certain diseases. Medicina (Kaunas) 2019;55:485.
19. Calvez J, De Ávila C, Timofeeva E. Sex-specific effects of relaxin-3 on food intake and body weight gain. Br J Pharmacol 2017;174:1049-1060.
20. Ryan PJ, Büchler E, Shabanpoor F, et al. Central relaxin-3 receptor (RXFP3) activation decreases anxiety- and depressive-like behaviours in the rat. Behav Brain Res 2013;244: 142-151.
21. Zhang C, Chua BE, Yang A, et al. Central relaxin-3 receptor (RXFP3) activation reduces elevated, but not basal, anxiety-like behaviour in C57BL/6J mice. Behav Brain Res 2015;292: 125-132.
22. Rytova V, Ganella DE, Hawkes D, et al. Chronic activation of the relaxin-3 receptor on GABA neurons in rat ventral hippocampus promotes anxiety and social avoidance. Hippocampus 2019;29:905-920.
23. Yamasue H, Domes G. Oxytocin and autism spectrum disorders. Curr Top Behav Neurosci 2018;35: 449-465.
24. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: Skeletal muscle as a secretory organ. Nat Rev Endocrinol 2012;8:457-465.
25. Tentolouris A, Eleftheriadou I, Tsilingiris D, et al. Plasma irisin levels in subjects with Type 1 diabetes: Comparison with healthy controls. Horm Metab Res 2018;50:803-810.
26. Yuksel MA, Oncul M, Tuten A, et al. Maternal serum and fetal cord blood irisin levels in gestational diabetes mellitus. Diabetes Res Clin Pract 2014;104:171-175.
27. Li DJ, Li YH, Yuan HB, et al. The novel exercise-induced hormone irisin protects against neuronal injury via activation of the Akt and ERK1/2 signaling pathways and contributes to the neuroprotection of physical exercise in cerebral ischemia. Metabolism 2017;68: 31-42.
28. Moon HS, Dincer F, Mantzoros CS. Pharmacological concentrations of irisin increase cell proliferation without influencing markers of neurite outgrowth and synaptogenesis in mouse H19-7 hippocampal cell lines. Metabolism 2013;62:1131-1136.
29. Sparks BF, Friedman SD, Shaw DW, et al. Brain structural abnormalities in young children with autism spectrum disorder. Neurology 2002;59:184-192.
30. Xu Q, Zuo C, Liao S, et al. Abnormal development pattern of the amygdala and hippocampus from childhood to adulthood with autism. J Clin Neurosci 2020;78: 327-332.