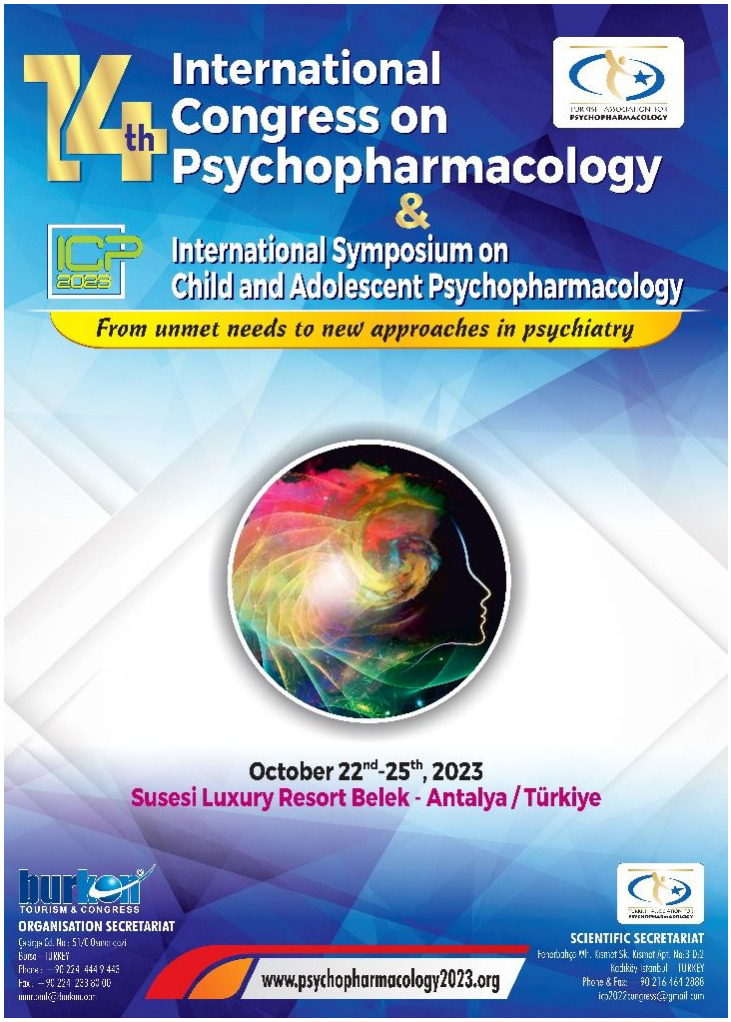

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ICP 2023 Outstanding Research Award Nominees Brief Reports

INVITATION

It is our great pleasure to announce that the Turkish Association for Psychopharmacology (TAP)'s 14th International Congress on Psychopharmacology & International Symposium on Child and Adolescent Psychopharmacology (ICP 2023) will be held on November 22-25, 2023 in Antalya, Turkey.

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Outstanding Research Award Nominees Brief Reports

14th International Congress on Psychopharmacology & International Symposium on Child and Adolescent Psychopharmacology

[Abstract:0005]

Examination of cognitive flexibility and emotion recognition in adolescent patients with eating disorders

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Indroduction
The etiology of Eating Disorder (ED) involves various factors, including developmental, genetic, neurobiological, and psychosocial aspects. Recently, research has shown interest in studying cognitive flexibility and emotion regulation in the onset of ED, particularly in individuals with Anorexia Nervosa (AN) and Bulimia Nervosa(BN).
These researches reported that emotion recognition difficulties and cognitive rigidity may play a role in the development and maintenance of ED behaviors [1].
However, despite these findings,there is a lack of studies investigating the relationship between cognitive flexibility and emotion recognition,especially in the adolescent population.This study aims to explore cognitive flexibility and emotion recognition in adolescent ED patients and their potential associations, contributing to more effective treatment approaches by understanding the underlying neurocognitive mechanisms of ED.

Methods

1.Participants:
The study included 46 participants aged 13 to 18 years seeking treatment at the Adult and Child and Adolescent Mental Health Department of Ankara University Faculty of Medicine.They were diagnosed with ED using the The Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (KSADS-PL) semi-structured clinical interview.
Healthy Control Group:The control group comprised 40 participants matched in age and education level with no psychiatric history,psychiatric illnesses,or abnormal body weight.
Inclusion Criteria:Confirmed diagnosis of ED according to DSM-5 criteria, age between 13 and 18 years, and voluntary participation with informed consent.
Exclusion Criteria:Mental retardation, acute or chronic physical illnesses affecting cognitive function,and any psychiatric diagnosis, psychotropic medication use, or abnormal body weight in the healthy control group. The study has been approved by the Ankara University School of Medicine Independent Ethics Committee (IEC).

2.Data Collection Tools:
-Socio-demographic and Clinical Data Form: A form gathering socio-demographic and clinical information from the participants.
- Stroop Test: A neuropsychological test assessing executive function, attention, and response inhibition.
- Berg Card Sorting Test (BCST): A test measuring working memory, abstract thinking, and set-shifting difficulties.
- Test of Perception of Affect via Non-Verbal Cues(TPANC): Assessing emotion recognition skills in children through nonverbal cues. It allows for separate assessments of emotions in different subdomains,such as facial expressions,vocal tones,body movements,and social stories**.**The test result is obtained as TPANC Total Score (TPANC-TS)and the reaction time for recognizing the emotional expression is calculated as TPANC Total Reaction Times(TPANC-TRT).
- Cognitive Flexibility Scale(CFS):A self-rated measurement tool evaluating cognitive flexibility.
Statistical Analysis:SPSS 23 program was used for statistical analysis. Tests like Chi-square, t-test, Mann-Whitney U test, ANOVA, Kruskal-Wallis test were used for comparisons. Pearson and Spearman correlation tests were used for relationship analyses.Statistical significance was set at $p<0.05$.

Results

1.Sample characteristics
In this study,the sample comprised 46 individuals in the patient group, with 28 individuals diagnosed with AN, 10 with BN, and 8 with Binge Eating Disorder(BED).The control group consisted of 20 females and 20 males who had no history of psychiatric illness. The median age for the ED group was 15.00, and for the healthy control group was 16.00.No statistically significant differences were observed in the sociodemographic data, including family socioeconomic status, ages, and levels of education, among the different groups.

2. Evaluation and Comparison of Clinical Data
The clinical data evaluation and comparisons are as follows:The average duration of illness for the AN group was 19.2 weeks(SD=9.2), for the BN group was 26.2 weeks(SD=15.8),and for the ED group was 14.2 weeks(SD=6.3).There were no statistically significant differences observed among the groups.

3.Cognitive Flexibility Scale Scores
The study compared CFS scores between individuals with ED and a control group.The control group had significantly higher mean CFS scores(52.85, SD=10.539)compared to the ED group(47.81, SD=8.609),indicating better cognitive flexibility in healthy individuals.

4.Cognitive Test Performances

Statistically significant differences were observed when comparing Stroop interference effects among the healthy control group,AN,BED,and BN groups.Pairwise comparisons using the Mann-Whitney U test revealed a significant difference between the BED group and the healthy control group (U: 331.00; p: 0.006).However,there were no statistically significant differences in BCST perseveration percentages among the healthy control group,AN,BED,and BN groups (Table 1).

Table 1. Cognitive Test Performances of Eating Disorder and Healthy Control Groups

	AN n=28	BN n=10	BED n=8	Control n=40	Statistics KW-χ2	P
Stroop interference[a]	99,00 (67)	95,0 (73)	111,0 (123)	63,21 (46)	9,822	,020*
BCST-percentage of perseverations[a]	11,1 (10)	14 (18)	13,5 (8)	14,8 (5)	4,173	,243

BCST: Berg Card Sorting Test
Kruskal Wallis H
[a]: Median (IQR)
**p <0,05*

5.Evaluation of TPANC

When comparing the control group with the BED and BN groups,no significant differences were found in TPANC-TS, TPANC-TRT, subdomain scores, and reaction times. However, the AN group obtained significantly higher scores in TPANC-TS and in the subdomains of "Tone of Voice Subdomain Score" and "Social Stories Subdomain Score" compared to the healthy control group (Table 2).

Table 2. Reaction Times for TPANC Subdomain Scores and TPANC Subdomain Reaction Times of the ED and Healthy Control Groups

	ED n = 46	Control n = 40	Statistics *U-t*	P value
TPANC-TS[a]	47,00 (7)	45,00 (3)	1.016[U]	0,063
Facial Expressions SS[a]	20 (3)	20 (3)	739[U]	0,439
Body Movements SS[a]	8,00 (1)	7,500 (1)	911[U]	0,334
Voice Tones SS[a]	10,00 (3)	8,50 (3)	1.108[U]	0,006**
Social Stories SS[a]	11,00 (1)	10,00 (3)	1.094[U]	0,008**
Facial Expressions S-RT[a]	68,00 (14)	68,00 (21)	906,500[U]	0,413
Body Movements S-RT[a]	32,00 (8)	29,00 (5)	908,500[U]	0,402
Voice Tones S-RT[b]	49,21 (11,97)	52,32 (10,31)	1,249[t]	0,215
Social Stories S-RT[b]	81,34 (11,57)	87,92 (18,81)	1,890[t]	0,063
TPANC -TRT[a]	231 (56)	228, (46)	778,500[U]	0,695

TS: Total Score, SS: Subdomain Scores, RT: Reaction Time, TRT: Total Reaction Time
[a]: Median (IQR), [b]: Mean (standart deviation), [U]: Mann-Whitney U, [t] Student t-test
*** p <0,01*

When comparing subgroups of ED patients and healthy controls in recognizing expressions of emotions,statistically significant differences were found for the emotions of disgust and fear. The AN subgroup showed better recognition of

disgusted expressions (U=293.500;p=0.007,corrected p-value=0.008) and fearful expressions compared to the controls(U=257.00;p=0.002,corrected p-value=0.008)(Table 3).

Table 3. Facial expression scores and reaction times for eating disorder and healthy control groups.

	ED (n=46)	Control (n=40)	Statistics U	P value
Angry[a]	9,00 (2)	8,00 (2)	1.81,00	0,650
Angry (RT)[a]	49,00 (11)	46 (13)	927,500	0,309
Sad[a]	8,00 (3)	8,00 (2)	857,000	0,722
Sad (RT)[a]	41,00 (2)	43,00 (2)	654,500	0,117
Disgusted[a]	8,00 (2)	7,00 (2)	1.81,00	0,010**
Disgusted (RT)[a]	31,00 (10)	33,00 (12)	659,500	0,119
Happy[a]	9,00 (1)	9,00 (1)	755,500	0,512
Happy (RT)[a]	38,00 (6)	37,50 (8)	823,500	0,974
Surprised[a]	7,00 (2)	7,00 (1)	889,500	0,496
Suprised (RT)[a]	23,00 (6)	34,00 (9)	767,500	0,619
Fearful[a]	8,00 (3)	7,00 (2)	1.040,000	0,034*
Fearful (RT)[a]	40,00 (10)	39,00 (11)	806,000	0,895

RT: Reaction Time
Mann-Whitney U
[a]: Median (IQR)

6.Assessment of the Relationship between Cognitive Flexibility and Emotion Recognition
In our study,no significant relationship was observed between cognitive flexibility predictors and emotion recognition in the control group.However,in the ED patients,a moderate negative correlation was found between BCST perseveration percentage and TPANC scores (r = -0.436, p = 0.003)(Table 4).

Table 4. Relationship between Cognitive Flexibility and Emotion Recognition in ED and Healthy Control Groups

GROUPS			TPANC-TS
Healthy Controls	**Stroop Enterferans**	r	-,243
		p	,166
	BCST- percentage of perseverations	r	,108
		p	,509
	Cognitive Flexibility Scores	r	-,114
		p	,483
Eating Disorders	**Stroop interference**	r	,057
		p	,729
	BCST- percentage of perseverations	r	-,436
		p	,003**
		r	-,023

	Cognitive Flexibility Scores	p	,879

*TPANC-TS: TPANC Total Score Spearman Correlation Analysis **p<0,01*

Discussion

Emotion Recognition Abilities

In our study, no significant difference in emotion recognition was found between the ED group and controls, which contrasts with previous research showing impaired emotion recognition in individuals with eating disorders, possibly due to the use of static paradigms unlike TPANC [2]. Additionally, a study reported worse emotion recognition from facial expressions in AN patients with a longer illness duration and the emotion recognition may be related to brain changes in response to hunger and irregular eating, leading to increased negative emotional intensity and impacting the understanding of others' emotions [3].Our study included AN patients with an average illness duration of 19 weeks,potentially influencing the results as neurobiological changes may not have fully taken effect yet. Although emotion recognition difficulties were not demonstrated in our adolescent ED patients, they experience challenges in social and relational domains. Investigating factors contributing to these difficulties beyond emotion recognition deficits in larger sample groups and various aspects of social cognition is needed.

Our study found that the AN group showed sensitivity to disgust, recognizing expressions of disgust and fear better than healthy controls. This finding aligns with fMRI studies indicating heightened amygdala activation and higher dorsolateral prefrontal cortex(dlPFC) hyperactivation in response to disgusted and fearful faces in AN patients. The heightened cortical activity might be associated with enhanced self-control, compensatory behaviors, and cognitive rigidity, suggesting a potential link between emotion recognition and self-control mechanisms in ED patients [4].

Cognitive Flexibility

In our study,ED patients rated themselves as more cognitively rigid,consistent with previous literature findings [5].Although they perceived themselves as rigid on self-report scales,neurocognitive tests did not show significant impairments,except for the BED group,which had a higher ADHD comorbidity.This suggests that cognitive rigidity may not impair test performance but could still impact daily life in ED patients from an early age.

Contrary to adult ED studies showing set-shifting difficulties in all ED groups [6], our study's ED patients exhibited a different set-shifting profile based on BCST percentage of perseveration.

In a study on early-onset AN and adolescent AN patients compared to healthy controls [7], our results were similar, contradicting the endophenotype hypothesis proposing cognitive rigidity in AN patients from an early age [8].

In an adolescent longitudinal study,cognitive flexibility performance did not differ significantly between healthy and AN groups [9]. Nevertheless, enhancements correlated with age manifested more prominently within the healthy control group rather than in individuals with AN. A study exploring response switching difficulties as a candidate endophenotype found no difference in set-shifting tasks between BN adolescents and healthy controls [10].Considering these studies and our study results, it is plausible that cognitive flexibility developmental trajectories in adolescent ED patients might differ from healthy controls, and as the duration of the illness extends, they could develop a more rigid cognition driven by both physiological and behavioral factors. This, in line with findings from some previous studies, underscores the potential impact of established rigid attitudes on treatment, implying the potential effects of early intervention [11].

In our study,response inhibition assessed through the Stroop Interference task showed BED patients struggling the most.Inadequate inhibitory control, reflecting uncontrollable eating behavior, might be influenced by comorbid ADHD in this group.

Examination of the Relationship Between Cognitive Flexibility and Emotion Recognition

Our findings suggest that individuals with eating disorders (ED) who make fewer perseverative errors exhibit enhanced cognitive flexibility in shifting cognitive sets and selecting appropriate emotional labels. This observation is consistent

with previous studies investigating the relationship between emotion recognition and cognitive flexibility in autism and schizophrenia patients [12].
The literature on emotion recognition and cognitive flexibility in ED patients is characterized by significant heterogeneity. However, the majority of these studies have been conducted within the adult age group and with diverse sample populations. To the best of our knowledge, our study stands as the first attempt to investigate the association between cognitive flexibility and emotion recognition abilities specifically in adolescent patients with ED. It's worth noting that our study does have certain limitations, such as relying on cross-sectional assessments and including ED patients from a single center.
Our findings challenge the prevailing belief in the literature by suggesting that cognitive flexibility difficulties in ED patients may emerge during the course of the illness rather than constituting a core symptom. To shed further light on this, longitudinal studies comparing cognitive performance tests during the early diagnosis and follow-up stages are required. Furthermore, it's important to note that emotion recognition deficits alone cannot fully account for the social functioning impairments observed in these patients. Therefore, a more comprehensive investigation of the intricate neurocognitive structures responsible for processing social experiences is warranted.
Key Words:Eating Disorders, Cognitive Flexibility, Emotion Recognition

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[Abstract:0034]

Diagnostic Utility of Hippocampal Subfield Volumes in Distinguishing Early-Onset Bipolar Disorder from Early-Onset Schizophrenia: A Neuroimaging Study with Explainable Machine Learning Algorithms

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1. Introduction

Early-onset bipolar disorder (EBD) and early-onset schizophrenia (EOS) is a complex heterogeneous psychiatric disorder that is identified by onset occurring before the age of 18 years [1,2].

Schizophrenia and bipolar disorder are psychiatric disorders characterized by shared genetic factors, developmental abnormalities, neuroimaging findings, cognitive impairments, and clinical manifestations, indicating considerable overlap between the two conditions [3-7]. Accurate differentiation between EBD and EOS is crucial for appropriate treatment selection and long-term prognosis.

Twelve distinct hippocampal subfields, including the cornu ammonis (CA)1, CA3, CA4, fimbria, granule cells in the molecular layer of the dentate gyrus (GC-ML-DG), hippocampal fissure, hippocampal amygdaloid transition area (HATA), molecular layer, parasubiculum, presubiculum, subiculum and hippocampal tail, were successfully segmented using this innovative method [8]. These subfield-specific volumetric patterns may serve as potential biomarkers for differential diagnosis.

In a recent study with large sample size, the schizophrenia group reported a significantly smaller volume in left and right CA2/3, left and right CA4, left and right GC-DG, and right ML volumes compared to the bipolar disorder and healthy controls groups [9,10].

When compared to healthy control individuals, both schizophrenia and bipolar disorder patients exhibited reduced volumes in bilateral cornu ammonis, parasubiculum, subiculum, hippocampal tail, HATA, and CA4 volume [11,12].

In a recent meta-analysis encompassing the child and adolescent psychiatry literature, a notable reduction in hippocampal volume was identified in individuals with early-onset psychosis in comparison to healthy controls [13]. Conversely, a smaller-scale study reported no significant difference in hippocampal volume between patients with EOS and healthy controls [14]. Directly comparing EOS and EBD, previous studies found that have shown differences in hippocampal volume between the two disorders [15].

Recent advancements in machine learning algorithms have facilitated the development of predictive models that can analyze neuroimaging data and aid in diagnostic decision-making. By integrating neuroimaging data, advanced machine learning techniques, and interpretability methods, we aim to provide a comprehensive analysis that aids in the differential diagnosis of these challenging psychiatric conditions. To our knowledge, few studies have investigated the diagnostic value of hippocampus volume in EBD and EOS using explainable machine learning algorithms.

In summary, this study aims to explore the potential diagnostic utility of hippocampal subfield volumes in differentiating EBD from EOS. Using explainable machine learning algorithms, we aim to improve our understanding of the underlying neurobiological mechanisms and provide a reliable framework for clinical decision-making in the early stages of these disorders. Findings from this research could potentially contribute to personalized treatment strategies and improve long-term outcomes for individuals with EBD and EOS.

2. Materials and methods

2.1. Participants

The study comprised eighty-one individuals diagnosed with EOS and sixty-three individuals who met the diagnostic criteria for early-onset bipolar-I disorder (BD-I). The diagnosis of EOS and BD-I was established using the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Aged Children Present and Lifetime Version [16]. Clinical symptom severity was assessed using the Young Mania Rating Scale [17] for BD-I patients and the Positive and Negative Symptoms Scale [18] for schizophrenia patients. The daily chlorpromazine equivalent dose of total antipsychotic medications was calculated following the method outlined [19]. Individuals with recent alcohol or substance abuse, a history of traumatic head injury with loss of consciousness, epilepsy, or other neurological or chronic medical conditions such as hypertension and diabetes, were excluded from the study. The patients were longitudinally observed at a tertiary-care psychiatry-teaching hospital between January 2019 and January 2023. The study protocol obtained approval from the local ethics committee.

2.2 Data acquisition- Image processing

MRI scans of the participants were acquired using a Siemens AERA 1.5 T (Erlangen, Germany) scanner with a dedicated 8-channel head coil. The imaging protocol comprised of high-resolution T1-weighted Magnetization-Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequences. The total duration of the image acquisition process for the anatomical MRI was approximately 4.5 minutes.

Utilizing a Linux-based computing system, the standard, automated, full processing pipeline called recon-all within the FreeSurfer 7.2.0 open-source software for neuroimaging analysis was implemented to preprocess the images. The pipeline's subroutines, which have been described in detail in previous literature, include skull stripping and brain extraction, Talairach transformation, segmentation of the subcortical deep gray matter structures, intensity normalization, and initial reconstruction. The hippocampus subfield segmentation was performed using a method based on Bayesian inference [8]. The segmented hippocampal subfields are illustrated in Figure 1B. The ROI to eTIV (Estimated Total Intracranial Volume) fraction is calculated according to the proportion approach.

2.3. Supervised Machine Learning Classification Framework

Python 3.9 was utilized along with the NumPy and Sklearn libraries for conducting feature selection and constructing machine learning models. Following the proportion approach, Pearson correlation and Elastic Net regression with 10-fold cross-validation was performed for feature selection to avoid collinearity, to reduce highly correlated features and dimensionality in the selection process. Machine learning models were constructed using Support Vector Machine (SVM) linear and radial kernels, Neural Network(NN), eXtreme Gradient Boosting (XGBoost) and Random Forest (RF) algorithms. The models were evaluated using ten-fold cross-validation, and hyperparameter tuning was performed using random search. The model's performance was assessed on the train set and subsequently validated on the test set. The predictive performance was measured by computing various metrics, including the area under the curve (AUC), accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and F1 scores.

2.4. Statistical analyses of demographics and clinical characteristics

Demographic and clinical characteristics were compared between participants with EOS and EBD, as well as between the train and test sets. Independent sample t-test for continuous variables and chi-square test for categorical variables were analyzed. The statistical analysis was performed using Statistical Package for the Social Sciences, version 26 (IBM Corp., Armonk, NY, USA), with a significance level of $p < 0.05$.

3. Results

The mean age of the study sample was 16.1 ± 1.5 years and 37.8 % were female. The average chlorpromazine equivalent dose was 611.8 ± 497.4 mg/day in the whole sample (Table 1).

3.1. Performance of the Machine Learning Model

Using Elastic Net regression, a total of 3 hippocampus subfields volume fractions (Right GC-ML-DG Head, Right GC-ML-DG Body, and Left Subiculum Head) were identified to differentiate participants with EOS from EBD. The SVM-Radial kernel classifier demonstrated the best performance both in the training and test set. The AUC, accuracy, sensitivity, specificity, PPV, NPV, and F1 scores in the test set were 90.6% (95% [CI]: 0.73–0.93), 93.1%, 84.6%, 96.7%, 91.6%, 93.7%, and 88%. Table 2 presents the performance metrics of all machine learning classifiers on the test sets. Fig.2 shows the confusion matrix and the receiver operating characteristic curves in the train set and test set.

4. Discussion

This study investigated hippocampal subfield volume abnormalities in EBD and EOS patients, using ML techniques for diagnostic classification. The fundamental concept behind ML is to identify features within the existing dataset that facilitate the prediction of group membership. Several ML studies have successfully distinguished structural MRI data of schizophrenia patients from healthy control groups [20-22]. Our model, utilizing the SVM-radial classifier and incorporating three hippocampal subfield volumes selected by the elastic net regression algorithm, exhibited robust performance with a 90.6% AUC value in the test set group.

The limitations and strengths of our study should be discussed. Firstly, the cross-sectional design conducted at a single center has constrained the generalizability of our findings. Additionally, our findings cannot account for the potential effects of treatment on hippocampal subfield volumes, necessitating repeated measurements in follow-up assessments. Future studies could include first-episode patients to mitigate the confounding effects of medications. The absence of healthy controls is another limitation of the study. However, our study was a valuable contribution to the literature, demonstrating the capability of an ML model utilizing hippocampal subfield volume parameters to differentiate early-onset schizophrenia and BD. Similarly, the high diagnostic accuracy of ML appeared promising for its application in clinical practice to enhance diagnostic approaches. Furthermore, the limitation of the low MRI resolution at 1.5 T should also be considered. Lastly, external validations with larger datasets and testing the discriminative abilities of various ML algorithms in psychiatry are essential.

The results of this study have supported the notion that there could be a promising value in differentiating EOS and EBD using hippocampal subfield volumes. This could be an important aspect in the clinical practice of child psychiatry, where diagnosing newly emerging early-onset psychotic disorders can be a challenging process. Therefore, hippocampal subfield volumes might play a significant role in improving future diagnostic accuracy.

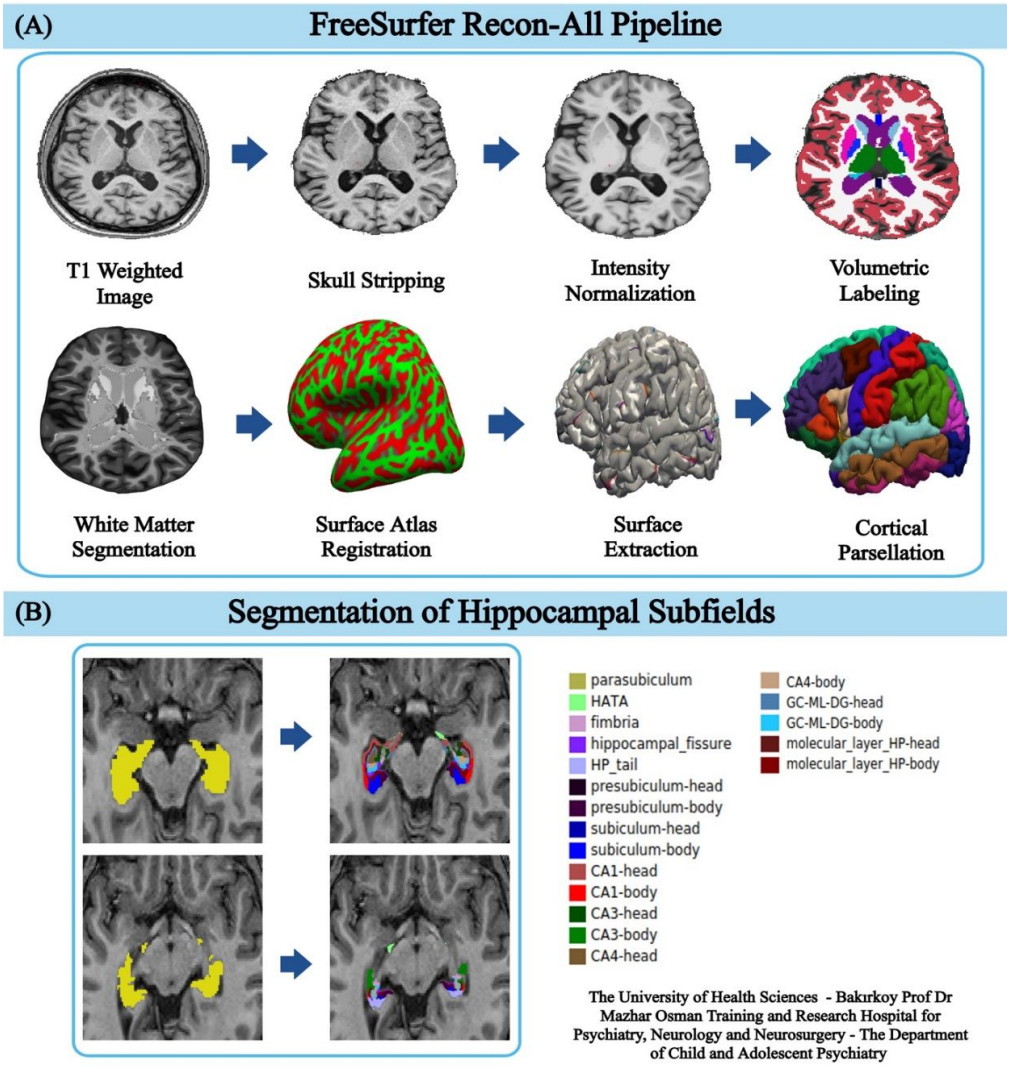

Fig.1. A detailed illustration of the step-by-step processing of fully automated FreeSurfer recon-all function in (A). A detailed representation of hippocampal subfields in (B).
CA, Cornu Ammonis; GC-ML-DG, Granuler Cell-Molecular Layer of the Dentate Gyrus; HATA, *Hippocampal*-Amygdaloid Transition Region; HP, Hippocampus.

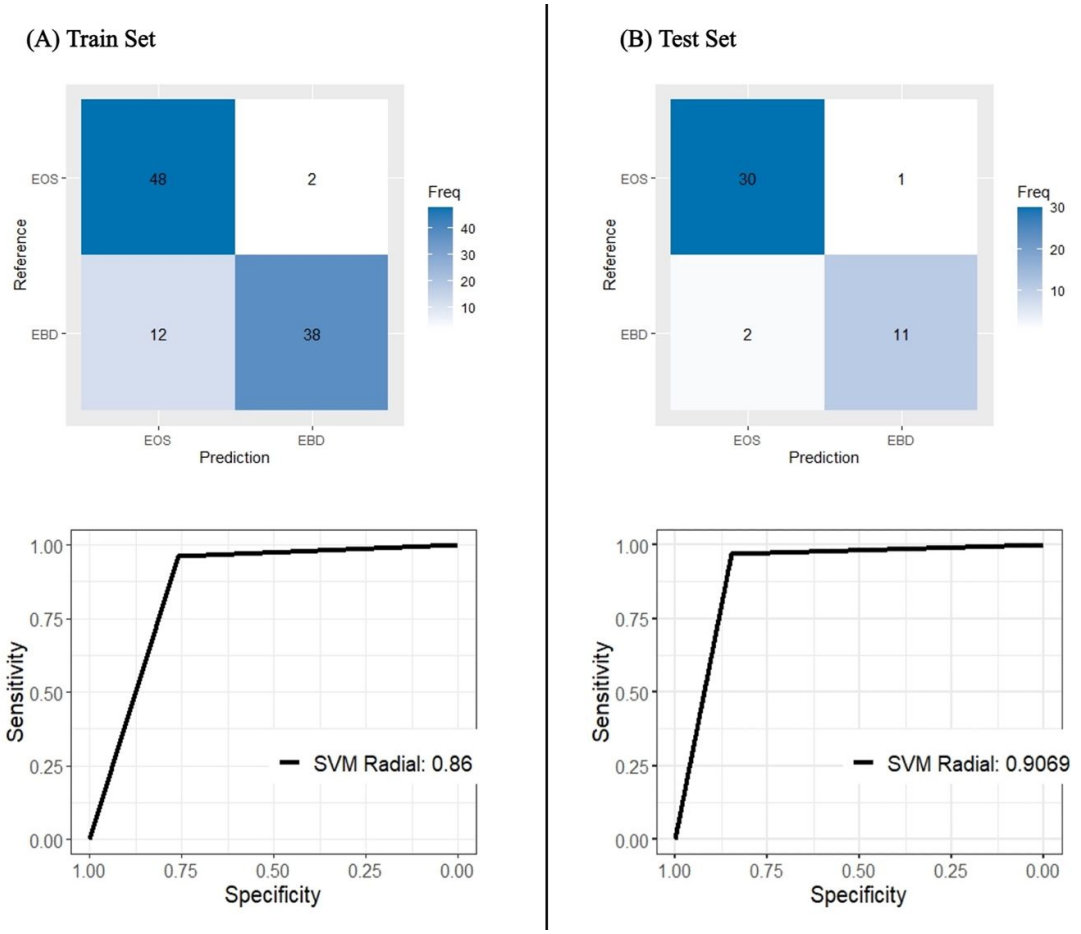

Fig. 2. Confusion matrix and receiver operating characteristics (ROC) curves in the (a) training and (b) test sets.

EBD, early onset bipolar disorder; EOS, early onset schizophrenia; Freq, frequence; SVM, support vector machine.

Table 1. Demographics and Illness Characteristics of Patients

Variables	EBD (n= 63)	EOS (n= 81)	*Statistics*	*p*
Age, years, M ± SD	16.3 ± 1.4	16.0 ± 1.6	t=1.4	0.243
Sex (female), n (%)	25 (40.0)	29 (35.8)	X^2=0.3	0.581
Daily chlorpromazine eq. dose, median M ± SD	591.3 ± 549.3	692.7 ± 461.2	*t* =0.4	0.761
Total PANSS scores, M ± SD	-	77.8 ± 28.9		
Total YMRS scores, M ± SD	17.5 ± 13.4	-		

EBD; early-onset bipolar disorder, EOS; early-onset schizophrenia, eq; equivalent, M; mean, SD; standart deviation, PANSS; Positive and Negative Symptoms Scale, YMRS; Young Mania Rating Scale

Table 2. Performances of predictions using five machine learning methods.

Classifier	AUC	Accuracy	Sensitivity	Specificity	PPV	NPV	F score	Kappa
SVM-radial	0.906	0.931	0.846	0.967	0.916	0.937	0.880	0.832
SVM-linear	0.801	0.863	0.923	0.838	0.705	0.963	0.800	0.699
eXreme Gradient Boosting	0.790	0.803	1.00	0.580	0.500	1.00	0.666	0.450
Random Forest	0.762	0.727	0.846	0.677	0.523	0.913	0.647	0.444
Neural Network	0.55	0.642	0.260	0.837	0.454	0.688	0.334	0.112

Note: AUC; Area Under the Curve, MCC; Matthew's Correlation Coefficient, NPV; Negative Predictive Value, PPV; Positive Predictive Value, SVM; Support Vector Machine

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[Abstract:0040]

Investigation of Chromosomal Anomalies and Copy Number Variations in Children Diagnosed with Autism Spectrum Disorder by Array CGH Method

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Introduction: Autism spectrum disorders (ASDs), a neurodevelopmental disorder, are characterized by language impairments, social deficits, and repetitive behaviors [1]. Genetics plays a major role in the etiology of ASD. Today, the genetic cause of 2.2% of the cases can be determined by conventional cytogenetic methods and only 18.2% by molecular cytogenetic methods [2]. Compelling evidence in Turkish populations suggests a role for copy-number variations (CNVs) in ASD [3,4]. The aim of this study was to identify the chromosomal anomalies and CNVs that cause the disease and provide genotype/phenotype correlations.

Methods

Study center, sampling, evaluation procedures and ethics

The files of patients who applied to the child and adolescent psychiatry outpatient clinic of Harran University Medical Faculty Hospital for any reason in between March 2021 and June 2022 were evaluated. We included patients who presented at the outpatient clinic of the Child and Adolescent Psychiatry Clinic between March 2021 and June 2022 and were diagnosed with nonsyndromic ASD through clinical interviews according to Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5). Patients were evaluated retrospectively for chromosomal anomalies, CNVs and clinical features. Our inclusion criteria were absence of syndromic features, adverse genetic consequences for Rett syndrome in female cases, and negative genetic results for Fragile X syndrome in male cases. Developmental evaluations among those <6 years old was conducted by clinical psychologists using the Ankara Developmental Screening Inventory (ADSI) while intellectual functioning of children >6 years was evaluated with Kent E.G.Y (verbal), Porteus Mazes (performance) or Wechsler Intelligence Scale for Children-Revised form (both verbal and performance). ASD symptoms were evaluated with Childhood Autism Rating Scales (for patients 4 years old). Childhood Autism Rating Scales (CARS) was developed by Schopler and Reichler in 1971 for assessment of autism among children 4–18 years and have gone through various revisions and iterations [5]. CARS consists of 15 items scored from one to four according to observations and caregiver reports. The maximum score that can be obtained is 60 and a cut-off score of 30 was suggested for the original version. The reliability and validity of the Turkish version was established by Sucuogluet al. (1996) [6]. Psychiatric diagnoses were based on DSM-5 criteria. Patient's age, gender, developmental levels/intellectual functioning, severity of ASD symptoms, comorbid neurodevelopmental/psychiatric diagnoses and accompanying medical disorders were recorded from hospital charts. Dysmorphology evaluation was carried out by a clinical geneticist.

Harran University Clinical Research Ethics Committee approved the study protocol (dated 25/07/2022 and no 14) and permission of hospital administration was also obtained for retrospective chart reviews.

Genetic analyses

Patients were evaluated by cytogenetic analysis and array-CGH (array comparative genomic hybridization) analysis. First, peripheral blood was taken from the patients in tubes with 2 ml heparin for chromosome analysis and in tubes with 2 ml EDTA for molecular analysis. Chromosome analysis was performed from heparinized peripheral blood sample

taken from our patient, phytohemagglutinin stimulated short-term lymphocyte culture, using Giemsa Trypsin banding method. Karyotype analysis was evaluated according to ISCN (International System for Human Cytogenetic Nomenculature). Genomic DNA was isolated from the peripheral blood sample using the Qiagen (GERMANY) DNeasy Blood & Tissue Kit – 50 (Cat. No:69504) in accordance with the kit protocol. Chromosomal microarray study was performed with Affymetrix CytoScan Optima (315k) chips from DNA obtained from peripheral blood. The data obtained from the CHAS 3.1 analysis program were evaluated using up-to-date databases (Pubmed, OMIM, DGV, DECIPHER). The variants detected as a result of the array analysis were searched in at least three databases and evaluated according to the American College of Medical Genetics and Genomics (ACMG) criteria. Detected variants were classified by ACMG criteria as pathogenic, possibly pathogenic, variants of uncertain significance (VUS).

Statistical Analysis

Statistical analyses were performed on IBM Statistical Package for the Social Sciences Statistics version 24.0 software (IBM SPSS Corp., Armonk, NY, USA). Descriptive statistics (number, rate, and percentage) were employed during the analysis. The independent sample t test was used for the two-way comparison of normally distributed continuous variables. The chi-square test was applied to compare the groups in terms of gender. P values <.05 were regarded as statistically significant.

Results: In our study, 12 female (22.2%) and 42 (77.8%) male, total of 54 subjects who were diagnosed as ASD were included. Mean age of the patients was 6.6 ± 3.8 years. According to CARS scores, 59.3% of the sample had mild-moderate symptoms while the rest had severe symptoms. Differences in sociodemographics, comorbid psychiatric and medical disorders according to gender groups in children with nonsyndromic/isolated Autism Spectrum Disorders were showed in Table 1.

The chromosomal anomalies and CNVs were identified in 3.8% (n=2) and 18.52% (n=10) of patients, respectively. Pathogenic changes were detected in 20% (n=2), possible pathogenic changes in 20% (n=2), and VUS in 80% (n=8) of patients with CNV. Deletion was observed in 30% (n=3) and duplication was observed in 70% of the CNVs detected in the patients. In addition, duplication was detected in two different regions in one patient. Structural and numerical chromosomal anomaly was detected in 3.7% (n=2) of the patients as a result of chromosome analysis. The demographic information, CNV regions, CNV size, aberration type, classification of CNVs and clinical diagnosis data of the patients are shown in Table 2.

Discussion: In this study, we performed cytogenetic analysis and chromosomal microarray analysis to evaluate the genetic architecture of CNVs in patients diagnosed with nonsyndromic/isolated ASD. In 2 (3.7%) out of 54 patients, pathogenic CNVs were detected, while possible pathogenic change in 2 (3.7%) and 8 (14.8%) patients had CNVs (7 duplications and 3 deletions) classified as VUS. CNVs have been found to cause or predispose to ASD. Goker et al. determined the rate of CNVs in ASD patient group as 15% [3]. In another study presenting CNVs of 23 patients with ASD were analyzed. In this study, 2 patients (8.6%) showed likely pathogenic CNVs, while 8 (34%) patients had CNVs (5 duplications and 3 deletions) classified as VUS [4]. The overall diagnostic yield in our study was similar to literature. We identified the chromosomal anomalies and CNVs in ASD risks, conferring perception to further reveal ASD etiology. Etiology can be clarified as a result of detecting the genetic changes that cause the disease with genetic analyzes and investigating their effects on the prognosis of the disease, and information about genotype-phenotype correlations can be helpful in the management of the disease.

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Table 1. Differences in sociodemographics, comorbid psychiatric and medical disorders according to gender groups in children with nonsyndromic/isolated Autism Spectrum Disorders

	Boys (n=42)	**Girls (n=12)**	**Total (n=54)**	
	n (%)	n (%)	n (%)	P
Age mean ± SD	6.59 ± 3.64	6.70 ± 4.55	6.62± 3.82	0.934*
Verbal skills				
No words	11 (26.2)	3 (25)	14 (25.9)	
Only single words	14 (33.3)	4 (33.3)	18 (33.3)	0.976**
Only a fewwords	5 (11.9)	2 (16.7)	7 (13)	
Short sentences	12 (28.6)	3 (25)	15 (27.8)	
Intellectual disability				
Yes	25 (59.5)	8 (66.7)	33 (61.1)	0.654**
No	17 (40.5)	4 (33.3)	21 (38.9)	
Autism severity				
Mild-moderate symptoms	24 (57.1)	8 (66.7)	32 (59.3)	0.554**
Severe symptoms	18 (42.9)	4 (33.3)	22 (40.7)	
Comorbid psychiatric disorder diagnosis				
Diagnosed	32 (76.2)	5 (41.7)	37 (68.5)	**0.023****
Never diagnosed	10 (20)	7 (58.3)	17 (31.5)	
Comorbid medical diagnosis				
Diagnosed	10 (25)	1 (8.3)	11 (21.2)	0.215**
Never diagnosed	30 (75)	11 (91.7)	41 (78.8)	

*Independent sample t test; **Chi-squared test. Abbreviations: CNV, copy number variations

Table 2. Summary of abnormal copy number variations detected in cases with nonsyndromic/isolated Autism Spectrum Disorders ((N:10 of 54 patient); ADHD, attention deficit hyperactivity disorder; DICCD, disruptive, impulse-control, and conduct disorders; SD, sleep disorders; ID, intellectual disability; VUS, variants of uncertain significance)

Case	**Sex / Age**	**Verbal skills**	**Autism severity**	**Comorbidity**	**CNV[a]**	**CNV size and type**	**Representative gene(s) within the CNV**	**Genes in OMIM**	**ACMG criteria**	**Inheritance**

1	F/11	Only single words	Severe symptoms	ADHD, DICCD, ID	arr[GRCh37] Xp22.12 (19302807_19523203)x3	220 kb-duplication	PDHA1, MAP3K15	PDHA1	VUS	Inheritance not known
2	M/12	Short sentences	Severe symptoms	ADHD, SD	arr[GRCh37] 10p14(9120361_9890427)x1	770 kb deletion	-	-	VUS	Inheritance not known
3	M/17	Short sentences	Severe symptoms	ADHD, SD, ID, Epilepsy	arr[GRCh37] 22q13.33(51128320_51197838)x1	70 kb deletion	SHANK3, ACR	SHANK,ACR	VUS	Inheritance not known
4	M/4	No words	Mild-moderate symptoms	ADHD, ID	arr[GRCh37] 15q11.2q12(25321028_26019936)x4	699 kb duplication	UBE3A	UBE3A	VUS/possibly pathogenic	Maternally inherited
5	M/4	No words	Mild-moderate symptoms	ADHD, SD, ID	arr[GRCh37] 15q11.2(22770421_23276605)x1	500 kb deletion	TUBGCP5, CYFIP1, NIPA2, NIPA1	TUBGCP5, CYFIP1, NIPA2, NIPA1	VUS/possibly pathogenic	Inheritance not known
6	M/8	No words	Severe symptoms	ADHD, ID	arr[GRCh37] 5q14.1(79143193_79787519)x3	640 kb duplication	THBS4, SERINC5, ZFYVE16	-	VUS	Maternally inherited
7	F/9	Only a few words	Severe symptoms	ADHD, ID	arr[GRCh37] 13q34(112712835_113306882)x3	594 kb duplication	SOX1, TUBGCP3	-	VUS	Inheritance not known
8/47,XY,+mar	M/13	Only single words	Severe symptoms	ADHD, DICCD, SD, ID, Epilepsy	arr[GRCh37] 15q13.2q13.3(22770421_32914239)x3	10.1 Mb duplication	TUBGCP5, CYFIP1, NIPA2, NIPA1, MKRN3, MAGEL2, NDN, PWRN2, PWRN1, NPAP1, SNRPN, SNHG14, PWAR5, SNORD116-1, IPW, PWA R1, SNORD115-1, UBE3A, ATP10A, GABRB3, GABRA5, GABRG3, OCA2, HERC2, APBA2, NSMCE3, TJP1, CHRFAM7A, FAN1,TRPM1,MIR211, KLF13,OTUD7A,CHRNA7, ARHGAP11A	SNRPN	Pathogenic	Inheritance not known

9/ 46, XX, add(3)(p2?)	F/3	Only single words	Mild-moderate symptoms	ID	arr[GRCh37] 3p22.3p22.2 (32994233_39335968)x3	6.3 mb duplication	CCR4, GLB1, CRTAP, FBXL2, UBP1, CLASP2, PDCD6IP, ARPP21, MIR128-2, STAC, DCLK3, EPM2AIP1, MLH1, LRRFIP2, GOLGA4, C3orf35, ITGA9,CTDSPL, MIR26A1,PLCD1, DLEC1, ACAA1, MYD88, OXSR1, SLC22A13, SLC22A14, XYLB, ACVR2B, EXOG, SCN5A, SCN10A, SCN11A,WDR48,GORASP1, TTC21A,CSRNP1,XIRP1, CX3CR1	-	Pathogenic	Inheritance not known
10	M/156	Only single words	Mild-moderate symptoms	ADHD, ID, Epilepsy	arr[hg19] 19p13.2 (7,404,656-7,813,336)x3 arr[hg19] 19p13.2 (10,772,789-11,154,043)x3	409 kb, duplication 381 kb duplication	PNPLA6, SMARCA4	PNPLA6, SMARCA4	VUS	Inheritance not known

[Abstract:0042]

Evaluation of Sluggish Cognitive Tempo symptoms in terms of motor skills in children diagnosed with Attention Deficit Hyperactivity Disorder

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Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a frequently occurring neurodevelopmental disorder during childhood, with a reported prevalence ranging from 1% to 18%. ADHD patients frequently suffer from comorbidities that impact the appearance, course, severity, and treatment response of the disease. These comorbid conditions can also negatively affect daily functioning and quality of life. Sluggish Cognitive Tempo (SCT) is an attention disorder frequently observed in childhood. Cognitive as well as motor impairments characterize the condition. The signs of motor problems are slowness and difficulty with movement, while cognitive problems can be identified by symptoms such as appearing drowsy, confused, and lost in thought. Children with ADHD have motor skill problems, which can adversely affect social competence, peer relations, and academic skills.

In the present study, we aimed to highlight differences in school-age children diagnosed with ADHD, SCT comorbid ADHD, and healthy control groups through a comparison of their motor skills and sociodemographic characteristics.

Methods

A total of 104 children (66 ADHD, 38 controls) were included in this study. Children were diagnosed with ADHD via the Schedule for Affective Disorders and Schizophrenia for School-Aged Children Present and Lifetime Version. The parents were asked to fill out the Conners Family Rating Scale-Revised Long Form (CPRS-R), Barkley Child Attention Scale (BCAS) and Children's Sleep Habit Questionnaire (CSHQ) to determine the symptom domains and the symptom severity. The Bruininks-Oseretsky Motor Proficiency Test (BOT-2) was used to assess motor skills.

Results

Of the 66 cases observed, 33 displayed symptoms of both ADHD and SCT, whereas the other 33 cases only presented symptoms of ADHD. Out of children with SCT comorbid ADHD, 63.6% were boys (n=21) and 36.4% were girls (n=12). The control group consisted of 19 girls and 19 boys, with a 50/50 gender split.

The mean ages of the ADHD group, SCT Comorbid ADHD group, and healthy group were 9.20±1.87, 10.00±1.47, and 9.50±1.83, respectively. There was no statistically significant difference between the mean ages of the cases and controls ($p=0.16$) (Table 1).

No significant statistical difference was observed in the sociodemographic characteristics of the groups, including time of birth, delivery method, alcohol and cigarette consumption during pregnancy, parental education and psychiatric disorders, and age of parents ($p>0.05$) (Table 1).

Motor skill parameters were evaluated using the BOT-2 test to compare ADHD cases, SCT comorbid ADHD cases, and healthy groups. The Fine Motor subscale score, which is one of the motor skill parameters assessed with the BOT-2 test, showed a statistically significant difference among the three groups ($p=0.002$). The Fine Motor subscale scores were significantly higher in the healthy group compared to the ADHD group ($p<0.05$) and the SCT comorbid ADHD group ($p<0.05$). Fine motor scores were not different between the groups with ADHD and those with comorbid SCT/ADHD. ($p>0.05$) (Table 2).

The Manual Coordination subscale score, one of the motor skill parameters evaluated with the BOT-2, showed a statistically significant difference among the groups ($p=0.0001$). Both the healthy group and the ADHD group had a similar Manual Coordination score ($p>0.05$). A significantly higher Manual Coordination subscale score was observed in the healthy group as compared to the SCT comorbid ADHD group ($p<0.05$). The ADHD group had a significantly higher Manual Coordination score than the SCT comorbid ADHD group ($p<0.05$) (Table 2).

The BOT-2 assessment revealed a statistically significant difference ($p=0.0077$) among the three groups in the Body Coordination subscale score, which is another motor skill parameter evaluated. The Body Coordination score of individuals with ADHD was not significantly different from the healthy group ($p>0.05$). In comparison to the SCT comorbid ADHD group, the healthy group demonstrated a significantly higher score on the Body Coordination subscale ($p<0.05$). However, no significant difference was found between the healthy group and ADHD group. The ADHD group showed a higher Body Coordination score compared to the SCT comorbid ADHD group ($p<0.05$) (Table 2).

The Strength and Agility subscale was one of the motor skill parameters assessed using the BOT-2 which indicated a statistically significant difference among the groups ($p=0.0028$). The healthy group had significantly higher Strength and Agility scores than the ADHD group ($p<0.05$) and SCT comorbid ADHD group ($p<0.05$). The Strength and Agility subscale scores were not significantly different between the groups with ADHD and comorbid ADHD and SCT ($p>0.05$) (Table 2).

BOT-2 assessment shows a significant difference in Total Motor score among three groups ($p=0.0009$). Healthy group showed significantly higher Total Motor score than ADHD group ($p<0.05$) and SCT comorbid ADHD group ($p<0.05$). There was no significant difference in Total Motor score between the ADHD group and the comorbid SCT-ADHD group ($p>0.05$) (Table 2).

Discussion

The study compared the motor skills and sociodemographic characteristics of children in the ADHD, SCT comorbid ADHD, and healthy control groups to highlight differences. The findings of our study reveal that motor skill tests of ADHD patients are lower compared to their normally developing peers. Furthermore, the presence of SCT in ADHD patients is associated with worse motor skills.

Children with ADHD were found to have weaker fine motor skills than the control group in a previous study [1]. Cortes et al. proposed a connection between fine motor coordination disorder and ADHD. The study assessed fine motor skills using spiral, vertical, and horizontal drawings and found that the ADHD group scored lower in all of them. They recommended that emphasis should also be given to the development of fine motor skills while treating ADHD [2]. Our study identified significantly lower scores in the Fine Motor subscale for both ADHD case groups, including those with only ADHD and those with comorbid SCT. The results are consistent with studies that showed problems with motor function in fine motor control areas in individuals diagnosed with ADHD [3]. In a study, a negative relationship was found between SCT symptoms and motor coordination and writing skills in children [4]. Since studies examining the relationship between SCT and motor skills are very limited, more research is needed on this subject.

In comparing the body coordination subscale between ADHD, ADHD with SCT comorbid, and healthy control groups, ADHD cases with comorbid SCT had notably lower scores than the other two groups. Cho et al. compared children diagnosed with ADHD and their typical peers and found that, like the findings in our study, children diagnosed with ADHD had lower scores in dexterity, bilateral coordination and balance scores (3). The prefrontal lobe plays a crucial role in executive functions, and its dysfunction may lead to motor behavior problems, impulsivity, and hyperactivity in children with ADHD [5].

Our study is the first, to the best of our knowledge, to examine motor skills in patients with ADHD and SCT. Given that SCT is a relatively new area of research, studies across different cultures are essential, and our study is likely to advance our understanding of the topic.

Our study has some limitations. The single-center nature of our study can be considered as the leading limitations. Since our study is cross-sectional, any relationships found must be evaluated carefully in terms of causality. Longitudinal studies on similar samples are needed.

In summary, individuals with ADHD performed worse than their typically developing peers in every subtest of the motor skill test. Furthermore, with the inclusion of comorbid SCT in the ADHD diagnosis, the state of motor skills worsened.

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Table 1. Sociodemographic and Clinical Characteristics of the Participants

	ADHD (N=33)	**ADHD+SCT (N=33)**	**CONTROL (N=38)**	**p**	**F Test**
Sex, n (%)					
Girl	4 (12.1%)	12 (36.4%)	19 (50.0%)	0.0032**	11.5086
Boy	29 (87.9%)	21 (63.6%)	19 (50.0%)		
Age, n (%)					
7.00	7 (21.2%)	2 (6.1%)	4 (10.5%)	0.1755	13.9463
8.00	7 (21.2%)	3 (9.1%)	11 (28.9%)		
9.00	6 (18.2%)	7 (21.2%)	5 (13.2%)		
10.00	3 (9.1%)	10 (30.3%)	4 (10.5%)		
11.00	5 (15.2%)	4 (12.1%)	7 (18.4%)		
12.00	5 (15.2%)	7 (21.2%)	7 (18.4%)		
Age					
Mean (SD)	9.2 (1.87)	10.0 (1.47)	9.5 (1.83)	0.1648	1.84
Median (Range)	9.0 (6.0, 12.0)	10.0 (7.0, 12.0)	9.0 (6.0, 13.0)		
Time of Birth, n (%)					
Term	30 (90.9%)	28 (84.8%)	38 (100.0%)	0.0538	5.8434
Preterm	3 (9.1%)	5 (15.2%)	0 (0.0%)		
Type of Birth, n (%)					
Vaginal	16 (48.5%)	11 (33.3%)	15 (39.5%)	0.4507	1.5940
Cesarean	17 (51.5%)	22 (66.7%)	23 (60.5%)		
Alcohol/Cigarette Use During Pregnancy, n (%)					
No	24 (72.7%)	31 (93.9%)	33 (86.8%)	0.0515	5.9312
Yes	9 (27.3%)	2 (6.1%)	5 (13.2%)		
Mother's Education, n (%)					
Primary school	8 (24.2%)	5 (15.2%)	11 (28.9%)	0.1954	8.6307
Middle school	6 (18.2%)	6 (18.2%)	8 (21.1%)		
High school	10 (30.3%)	13 (39.4%)	17 (44.7%)		
University	9 (27.3%)	9 (27.3%)	2 (5.3%)		
Father's Education, n (%)					
Primary school	7 (21.2%)	8 (24.2%)	9 (23.7%)	0.4156	6.0680
Middle school	9 (27.3%)	9 (27.3%)	11 (28.9%)		
High school	8 (24.2%)	12 (36.4%)	15 (39.5%)		
University	9 (27.3%)	4 (12.1%)	3 (7.9%)		

Presence of Psychiatric Disease in Mother, n (%) No Yes	 28 (84.8%) 5 (15.2%)	 30 (90.9%) 3 (9.1%)	 38 (100.0%) 0 (0.0%)	0.0538	5.8434
Presence of Psychiatric Disease in Father, n (%) No Yes	 29 (87.9%) 4 (12.1%)	 31 (93.9%) 2 (6.1%)	 36 (94.7%) 2 (5.3%)	0.5089	1.3511
Mother's Age Mean (SD) Median (Range)	 37.8 (7.09) 37.0 (26.0, 51.0)	 37.0 (6.17) 35.0 (26.0, 47.0)	 35.9 (4.92) 35.5 (25.0, 48.0)	0.1648	0.80
Father's Age Mean (SD) Median (Range)	 42.3 (8.36) 42.0 (31.0, 68.0)	 40.2 (6.35) 41.0 (28.0, 52.0)	 40.1 (6.32) 39.0 (27.0, 60.0)	0.3343	1.11
Abbreviations: N= Number of Patients, SD= Standard Deviation, SCT= Sluggish Cognitive Tempo, ADHD= Attention Deficit Hyperactivity Disorder *p<0.05 **p<0.01					

Table 2. Motor Skills Measured by BOT-2 in ADHD, ADHD+SCT and Healty Control Groups

	ADHD (N=33)	**ADHD+SCT (N=33)**	**CONTROL (N=38)**	**p**	**F Test**
Fine Manual Control					
Mean (SD)	**42.5 (6.80)**	**40.9 (8.95)**	**48.1 (10.09)**	**0.0020****	**6.62**
Median (Range)	**41.0 (30.0, 57.0)**	**41.0 (29.0, 60.0)**	**46.0 (21.0, 80.0)**		
	b	**b**	**a**		
Fine Motor Precision					
Mean (SD)	11.8 (3.99)	10.8 (4.63)	14.9 (5.00)	0.0008**	7.60
Median (Range)	11.0 (5.0, 21.0)	9.0 (3.0, 23.0)	15.0 (5.0, 28.0)		
	b	b	a		
Fine Motor Integration					
Mean (SD)	12.0 (3.28)	11.5 (3.94)	14.9 (4.51)	0.0006**	8.02
Median (Range)	13.0 (5.0, 19.0)	12.0 (6.0, 20.0)	14.5 (4.0, 26.0)		
	b	b	a		
Manual Coordination					
Mean (SD)	**40.6 (5.35)**	**34.7 (7.18)**	**43.7 (8.75)**	**0.0001****	**13.51**
Median (Range)	**41.0 (31.0, 53.0)**	**32.0 (21.0, 50.0)**	**44.0 (24.0, 62.0)**		
	a	**b**	**a**		
Manual Dexterity					
Mean (SD)	10.7 (3.56)	8.2 (3.49)	11.5 (4.43)	0.0015**	6.95

Table 2. Motor Skills Measured by BOT-2 in ADHD, ADHD+SCT and Healty Control Groups

	ADHD (N=33)	ADHD+SCT (N=33)	CONTROL (N=38)	p	F Test
Median (Range)	11.0 (5.0, 19.0) a	6.0 (4.0, 15.0) b	11.0 (4.0, 25.0) a		
Upper-Limb Coordination					
Mean (SD)	11.4 (3.09)	9.5 (4.29)	13.9 (4.27)	0.0001**	11.35
Median (Range)	12.0 (6.0, 18.0) b	9.0 (3.0, 25.0) c	14.0 (5.0, 23.0) a		
Body Coordination					
Mean (SD)	**41.4 (11.08)**	**35.6 (10.43)**	**42.6 (7.25)**	**0.0077****	**5.11**
Median (Range)	**38.0 (30.0, 79.0) a**	**34.0 (5.0, 62.0) b**	**42.0 (28.0, 60.0) a**		
Bilateral Coordination					
Mean (SD)	11.5 (4.19)	8.9 (4.95)	12.8 (3.21)	0.0006**	7.97
Median (Range)	11.0 (7.0, 24.0) a	8.0 (3.0, 21.0) b	13.0 (6.0, 19.0) a		

	ADHD (N=33)	ADHD+SCT (N=33)	CONTROL (N=38)	p	F Test
Balance					
Mean (SD)	11.1 (5.57)	9.3 (4.20)	12.0 (3.86)	0.0461*	3.17
Median (Range)	9.0 (5.0, 24.0) ab	9.0 (3.0, 24.0) b	11.0 (4.0, 20.0) a		
Strength and Agility					
Mean (SD)	**42.1 (10.23)**	**39.5 (9.28)**	**46.8 (7.09)**	**0.0028****	**6.22**
Median (Range)	**44.0 (6.0, 58.0) 2**	**38.0 (27.0, 64.0) b**	**46.5 (30.0, 62.0) a**		
Running Speed and Agility					
Mean (SD)	12.7 (3.24)	11.0 (3.95)	14.5 (3.80)	0.0005**	8.28
Median (Range)	13.0 (6.0, 18.0) b	10.0 (5.0, 21.0) b	14.0 (7.0, 25.0) a		
Strength					

Mean (SD)	12.4 (3.94)	9.9 (4.54)	13.7 (3.18)	0.0005**	8.24
Median (Range)	12.0 (5.0, 20.0)	9.0 (4.0, 20.0)	14.0 (6.0, 21.0)		
	a	b	a		
Total Motor					
Mean (SD)	**39.5 (7.67)**	**35.8 (9.46)**	**43.4 (7.67)**	**0.0009****	**7.53**
Median (Range)	**40.0 (27.0, 62.0)**	**33.0 (23.0, 56.0)**	**42.0 (33.0, 68.0)**		
	b	**b**	**a**		

*p<0.05, **p<0.01, a>b>c

Abbreviations: ADHD= Attention Deficit Hyperactivity Disorder, SCT= Sluggish Cognitive Tempo, BOT-2= Bruininks-Oseretsky Test of Motor Proficiency-Second Edition, SD= Standard Deviation

[Abstract:0055]

The Title of The Article:

IL-2RA rs2104286 and IL-2 rs2069762 polymorphisms may be associated with bipolar disorder and its clinical findings

A Short Title:
IL-2RA rs2104286, *IL-2* rs2069762, and *IL-2* rs2069763 **polymorphisms in bipolar disorder**

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Objective:

Recent researches suggest that inflammation might play a significant role in both the development and progression of bipolar disorder (BD). Studies have uncovered elevated levels of specific markers of inflammation in individuals with BD, including cytokines, CRP, and inflammatory signaling molecules. The modulation of cytokine levels during both

symptomatic and asymptomatic phases underscores this connection. Notably, a meta-analysis revealed that BD patients exhibited higher levels of IL-4, IL-6, IL-10, sIL-2R, sIL-6R, TNF-α, sTNFR1, and IL-1 receptors compared to control groups (1).

In response to antigenic stimulation, IL-2, a pivotal signaling molecule integral to the immune system, is synthesized by specific white blood cells, primarily T cells. IL-2 regulates immune responses, fosters immune cell growth and activation, and sustains a harmonious immune system equilibrium. The *IL-2* gene is located on chromosome 4q26-q27. Its variants cause differences in functional activation. Among these variants, the -330 T/G (rs2069762) and +114 G/T (rs2069763) have been identified as modulators of IL-2 levels.

Again, IL-2RA, a transmembrane protein located on the surfaces of activated T and B cells, some myeloid precursors, oligodendrocytes, and thymocytes, has been implicated. In mice models, the depletion of regulatory T cells expressing IL-2RA and CD25 has been associated with the emergence of autoimmune disorders. The gene encoding IL-2RA resides on 10p15-p14. Notably, the minor G allele of *IL-2RA* rs2104286 has demonstrated an association with decreased soluble IL-2RA levels. Both *IL-2* and *IL-2RA* genes have been linked to multiorgan inflammation in both mice and humans (2). Given these findings, our study aims to investigate the potential correlation between the variants *IL-2RA* rs2104286, *IL-2* rs2069762, and *IL-2* rs2069763 and BD in a Turkish population.

Methods: The investigation encompassed 86 patients diagnosed with bipolar disorder (BD) who were under observation within the psychiatric ward of Malazgirt State Hospital in Turkey. The study included individuals aged 18 to 65 years who had been diagnosed with BD type I or II according to DSM-IV criteria using structured clinical interviews (SCID-I). Participants meeting these criteria were considered, provided they did not have coexisting neurological disorders, systemic conditions, a history of substance abuse, or any other medical conditions that could potentially affect cognitive function. We examined information such as clinical specifiers (psychotic features, atypical features, seasonal pattern, mixed features, rapid cycling history, peripartum onset), family history, attempted suicide, duration of disorder, age of onset, number of manic episodes, depressive episodes, and total episodes, number of hospitalizations and scale scores (HAM-D, YMRS, CGI-S, and CGI-I). The control group consisted of 100 healthy individuals who matched the age and gender characteristics and had no family or personal background of psychiatric disorders. The research protocol received approval from Istanbul University's Clinical Research Ethics Committee on January 22, 2021 (Protocol Number: 03/2021).

DNA extraction was performed from blood samples taken from the participants according to the manufacturer's instructions (Zymo Research Quick-DNA Plus Kit). Genotyping of *IL-2RA* rs2104286, *IL-2* rs2069762, and *IL-2* rs2069763 variants was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis according to the previously reported method. The software programs SPSS Statistical Program Version 20.0 and OpenEpi Info 2.2 were used to conduct all statistical analyses. The standard deviation (SD) and min/max of continuous data were displayed as the mean and median, respectively. The significance of variations in genotype distribution and clinical parameters between the two study groups was assessed using the Fisher's exact test, Pearson chi-square test, or Median testing. Calculations were made for the odds ratio (OR) and 95% confidence intervals (CIs). Statistics were deemed significant at $p \leq 0.05$.

Results:
One hundred eighty-six subjects (86 BD patients and 100 healthy controls) were evaluated for the *IL-2RA* rs2104286, *IL-2* rs2069762, and *IL-2* rs2069763 variants (Table 1). There was a statistically significant difference between patients and controls regarding the genotype distribution of the *IL-2RA* rs2104286 variant. No patients were carrying the G/G genotype. The *IL-2RA* rs2104286 A/A genotype was more frequent in the patient group than in the control group (p=0.001, OR: 4.818, 95% CI: 2.338-9.932), while the A/G genotype was higher in controls compared to patients (p=0.001, OR: 4.423, 95% CI: 2.177-8.987). The genotype distribution of *IL-2* rs2069762 and *IL-2* rs2069763 variants did not differ between the patient and control groups (p>0.05).

Then we investigated the relationship between the genotype distribution of these variants and clinical findings in patients. For *IL-2* rs2069762, the GCI-S score was higher in those with T/G and G/G genotypes. We found no significant difference between the other genotype distribution of these variants and clinical findings, including clinical specifiers, family history, attempted suicide, duration of the disorder, age of onset, number of manic episodes, depressive episodes, and total episodes, number of hospitalizations and scale scores (p>0.05) (Table 2). There was no correlation between *IL-2RA* rs2104286 A/A and AG/GG or IL-2 rs2069763 G/G and T/T-T/G genotype distribution and clinical findings (data not shown).

Discussion: Elevated levels of proinflammatory cytokines circulating in BD signify the connection between immune-mediated mechanisms and the neurobiology and progression of BD. During manic and depressive episodes of BD,

there is an upsurge in proinflammatory cytokine levels, triggering the activation of neuroinflammation pathways alongside an increase in acute phase proteins. A rat study utilizing intracerebroventricular ouabain, a substance-inducing behavior akin to mania and depression, highlighted increased IL-1β, IL-6, IL-10, TNF-α, and cytokine-induced neutrophil chemoattractant 1 (CINC-1) in the frontal cortex and hippocampus. Postmortem examinations in BD patients revealed the presence of markers indicative of excitotoxicity, neuroinflammation, and engagement of the IL-1 receptor cascade in the frontal brain region. Moreover, it's been observed that Th1 cells exhibit heightened functionality in individuals with BD.

Research has revealed that IL-2 operates as an autocrine growth factor for T cells in vitro. Its role in driving the proliferation of T cells and the generation of effector and memory T cells was swiftly demonstrated in both mice and humans. By dynamically adjusting various immune function parameters to align with the body's physiological requirements, IL-2 plays a pivotal role in maintaining immune system balance. Some of these regulatory processes occur through interactions with macrophages and monocytes, akin to CNS microglial cells in the periphery. A comprehensive analysis discovered that serum IL-2 levels were heightened in individuals with major depressive disorder compared to their healthy counterparts (3). Within the Polish population, a significant association was found between the *IL-2* rs2069762 T/T genotype and T allele, and paranoid schizophrenia (2). Furthermore, the *IL-2* rs2069763 heterozygous genotype displayed links to autoimmune conditions like rheumatoid arthritis and systemic lupus erythematosus, as reported across various studies (4). Although the rs2104286 variant located in the first intron of the *IL2RA* gene does not directly alter the structure of IL-2RA, it could impact *IL2RA* expression by influencing the function of regulatory elements, mRNA processing, and mRNA stability. In a meta-analysis, Wang et al. proposed that individuals possessing the *IL-2RA* rs2104286 A allele face an elevated risk of developing multiple sclerosis, a risk that spans both Caucasians and Asians (5).

In this present study, we investigated the *IL-2* rs2069763, *IL-2* rs2069762, and *IL-2RA* rs2104286 variants in BD. To our knowledge, this is the inaugural exploration of the connection between this variant and BD within the Turkish population. Our findings showed a higher prevalence of the *IL-2RA* rs2104286 A/A genotype within the patient group compared to controls. Upon perusing existing literature, it became apparent that a variety of studies have already been published, exploring the link between gene polymorphisms related to inflammation and BD. In a recent research endeavor, where we assessed the *IL-17F* (rs763780, 7488 A/G) gene in both BD and schizophrenia patients, we noted that the distribution of *IL-17F* genotypes in BD patients significantly different from that of healthy participants (1). The Clinical Global Impression-Severity (CGI-S) scale, utilized to gauge disease severity prior to treatment initiation, indicated that individuals carrying the *IL-2* rs2069762 T/G-G/G genotypes exhibited higher CGI-S scores in contrast to those carrying the T/T genotype. As the *IL-2* rs2069762 G allele is linked to elevated IL-2 levels, this outcome supports to the interrelation between BD and the immune system. Nevertheless, our study does carry some limitations. The sample size was relatively modest, and the population of individuals was restricted to Turkish participants. Furthermore, we did not measure IL-2 blood levels. Additionally, BD is a complex disorder influenced by a multitude of risk factors. We were unable to entirely rule out every factor's potential influence on the emergence of BD. Therefore, further investigations utilizing larger and more diverse sample sizes are imperative.

In conclusion, the outcomes of this investigation highlighted a correlation between *IL-2* and *IL-2RA* variants and both BD susceptibility and clinical presentations. These genetic variations could potentially play a role in initiating the inflammatory processes witnessed during the disorder's progression. This insight could offer a promising avenue for the development of novel treatment approaches. Consequently, we propose that further examination of these variants within BD patients be undertaken on a broader scale, encompassing diverse ethnic groups.

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Tables:

Table 1. Genotype distributions of *IL-2RA* rs2104286, *IL-2* rs2069762, and *IL-2* rs2069763 variants in groups

	Patients	**Controls**	**OR**	**95%CI**	**p**
***IL-2RA* rs2104286**	**n:86 (%)**	**n:100 (%)**			
Genotypes					
A/A	36 (40.7)	13 (13)	4.818[&]	2.338-9.932[&]	**0.001[&]**
A/G	50 (58.2)	86 (86)	4.423[&]	2.177-8.987[&]	**0.001[&]**
G/G	0 (0)	1 (1)	0.990[&]	0.971-1.010[&]	1.000[&]
***IL-2* rs2069762**	**Patients**	**Controls**	**OR**	**95%CI**	**p**
Genotypes					
G/G	13 (15.1)	10 (10)	0.679*	0.213-1.174*	0.427*
G/T	9 (12.2)	12 (12)	3.678*	0.993-13.630*	0.051*
T/T	1 (1.4)	78 (78)	1.805[&]	0.877-3.713[&]	0.144[&]
***IL-2* rs2069763**	**Patients**	**Controls**	**OR**	**95%CI**	**p**
Genotypes					
G/G	45 (52.3)	59 (59)	4.624[&]	0.908-23.548[&]	0.065[*]
G/T	34 (39.6)	39 (39)	4.128*	0.790-21.568*	0.093*
T/T	7 (8.1)	2 (2)	4.342*	0.877-21.487*	0.083*

$p < 0.05$, *: OR (95%CI) was adjusted by age and sex, [&]Fisher's Exact Test. The results that statistically important were shown in bold.

Table 2. *IL-2* rs2069762 T/T and T/G-G/G genotype distributions according to clinical characteristics

***IL-2* rs2069762**	**T/T n:70 (%)**	**T/G-G/G n:16 (%)**	**OR**	**95%CI**	**p**
Rapid cycling history	18 (25.7)	5 (31.2)	0.705*	0.209-2.370*	0.571*
Atypical features	13 (18.6)	4 (25)	0.705*	0.189-2.626*	0.603*
Psychotic mania	38 (54.3)	8 (50)	1.230*	0.411-3.685*	0.712*
Psychotic depression	9 (12.9)	4 (25)	0.390*	0.099-1.534*	0.178*
Peripartum onset	6 (8.5)	2 (12.5)	0.799*	0.131-4.864*	0.808*
Seasonal pattern	34 (48.6)	6 (37.5)	1.662*	0.513-5.384*	0.397*
Mixed features	13 (18.6)	4 (25)	0.630*	0.171-2.324*	0.488*
Family history	42 (67.1)	6 (37.5)	2.629*	0.839-8.232*	0.097*
Attempted suicide	18 (25.7)	3 (18.7)	1.701*	0.420-6.887*	0.456*
Duration of disorder (year)	15 (5-40)	15 (1-40)			0.830[#]
Age of onset (year)	25 (10-52)	20 (13-45)			0.466[#]
Number of hospitalizations	2 (0-21)	2 (0-21)			0.593[#]
Manic episodes (number)	2 (0-21)	2 (1-25)			0.862[#]
Depressive episodes (number)	1 (0-12)	1 (0-9)			0.604[#]
Total episodes (number)	4 (1-23)	4 (2-27)			0.921[#]
HAM-D	9 (0-31)	10.5 (7-34)			0.299[#]
YMRS	6 (0-37)	7.5 (0-38)			0.782[#]
CGI-S	5 (2-7)	5.5 (4-6)			**0.044[#]**
CGI-I	2 (1-4)	2.5 (1-4)			0.217[#]

$p < 0.05$, *: OR (95%CI) was adjusted by age and sex, [&]Fisher's Exact Test. [#]Median Test
The results that statistically important were shown in bold.

HAM-D - Hamilton depression rating scale; YMRS - young mania rating scale; CGI-S - clinical global impression scale-severity; CGI-I - clinical global impression scale improvement. $p < 0.05$, *: OR (95%CI) was adjusted by age and sex, [&]Fisher's Exact Test. [#]Median Test. The results that statistically important were shown in bold.

[Abstract:0132]

Unraveling the pivotal role of autistic traits in misophonia: A preliminary investigation of the interrelationships between misophonia, sensory sensitivity and anxiety

Running Title: The pivotal role of autistic traits in misophonia

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Objective

Misophonia, considered as a psychiatric disorder (1), has not been defined in diagnostic classification systems such as the Diagnostic Manuel of Mental Disorders-5 (DSM-5) and the International Classification of Disease-10 (ICD-10). However, it has been reported that approximately half of the individuals with misophonia has accompanying psychiatric diagnoses (2,3), including anxiety disorder (4), obsessive-compulsive disorder (OCD) (1,5,6) and major depressive disorder (MDD) (7).

The relationship between misophonia and autistic traits has recently been addressed in a few studies. A recent study has reported a positive correlation between the severity of misophonia and autistic traits (8), while contradictory results were identified that autism spectrum traits had no significant relation to the severity of the misophonia symptoms (3).

Lately, another focus on the psychiatric research field of misophonia is the possible association with general sensory sensitivities, and an obvious finding is that children with misophonia showed greater sensory hypersensitivity than those of others without misophonia not only in the auditory domain but also more widely across multiple senses (7,9). The profound association between autism and misophonia has prompted researchers to explore the possibility of misophonia as a sensory manifestation of autism spectrum disorder (ASD). However, the precise nature of the association between elevated autism characteristics and increased sensory sensitivity in individuals with misophonia have not yet been conclusively established.

The current study investigates the comorbid psychiatric disorders, co-occurrence of internalizing symptoms (anxiety, OCD and depressive symptoms), sensory profiles and autistic traits among female adolescents with misophonia in a comparison with a control group without misophonia. The first aim of this study was to determine the accompanying psychiatric disorders using a psychiatric interview. The second aim was to investigate specific psychological profiles associated with misophonia, including autism-like traits, sensory profiles, and anxiety symptoms. The third aim was to clarify the relationship between misophonia and these psychiatric features.

Methods

The sample consisted of 44 adolescent females (14-18-year-old), newly diagnosed with misophonia (*n*=22), and age-matched healthy controls (*n*=22) without any psychiatric diagnosis. The misophonia group comprised clinical cases who applied to the child and adolescent psychiatry outpatient clinic. The control group was selected from among the patients who applied to the pediatric outpatient clinics for minor acute illnesses (eg, common cold, coughs). Exclusion criteria for both groups had no previous psychiatric, neurological or other medical chronic diseases and/or uncorrected visual and hearing impairments. The research protocol was approved by the local ethics committee of the hospital (Ethics approval reference number: E-21/06-195). Written informed consent was obtained from all participants and their parents.

Psychiatric assessments were made by experienced child and adolescent psychiatrists who are certified in the application of the Schedule for Affective Disorders Schizophrenia for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL). The Amsterdam Misophonia Scale Revised (AMISOS-R) was used to evaluate the presence of misophonia. The following questionnaires were administered after participants had given informed consent: Revised Children's Anxiety and Depression Scale Child Version (RCADS-CV), the Youth Self Report (YSR), and the Adolescent/Adult Sensory Profile (AASP).

Statistical Analysis

IBM SPSS (Statistical Program for Social Sciences) 22.0 was used for statistical analyses. Prior to the analyses, the Shapiro-Wilk test was used to determine the normality of the data distribution. For continuous clinical variables,

independent *t*-test or Mann-Whitney *U* test was used according to distribution characteristics within the group comparisons. Fisher's exact test was used for the categorical variables. Spearman correlations were used to determine the relationships between scale scores. After examing the possible factors that might be associated with the misophonia, structural equation modeling (SEM) was used to further explore the causal relationship between variables. IBM AMOS was used for path analysis. All statistical tests were two-tailed with a threshold for significance of $\alpha = 0.05$.

Results

Both groups were found to be similar in terms of age, parental age, education level, and family characteristics ($p > 0.05$). Thirteen (59%) out of 22 girls with misophonia had psychiatric disorders, while the girls in the control group did not receive any psychiatric diagnosis.

Girls with misophonia had significantly higher scores of depression, anxiety, and obsessive-compulsive symptoms compared with controls. According to the RCADS-CV and AASP scores, significant differences were found between the two groups (Table 1). Comparing the autistic traits between the two groups, Student's *t test* was performed. Girls in the misophonia group had significantly higher AT scores than controls on the YSR [mean (SD)=185.72 (15.96) vs 170.00 (14.76); $t(42)=-3.39$, $p=.002$]. While investigating the associations between total anxiety and total internalizing scores, autistic trait scores, and sensory profile scale scores, statistically significant correlations were found in the whole sample (Table 2). According to the results from previous studies and significant correlations that we found, we tested structural equation modeling (SEM). Figure 1 displays the path analysis. All measures indicated a excellent fit model ($\chi 2=6.68$, df=2, $p=0.03$, NFI=0.901, IFI=0.929, CFI=0.919, RMSEA=0.021).

Discussion

Numerous studies investigating the phenomenology and clinical correlates of misophonia have revealed associations between misophonia and internalizing symptoms, including OCD, anxiety, and depressive symptoms (4,7,10). Consistently, while conducting a symptom-level examination, the present results align with previous findings, indicating higher levels of OCD, anxiety across all subcategories, and MDD symptoms on the RCADS-CV in the misophonia group than controls. However there are also studies that have failed to establish these significant relationships (2).

Recent research investigations have demonstrated that individuals with misophonia exhibit heightened levels of autistic traits (9), and a strong positive association has been observed between the severity of misophonia symptoms and the presence of these autistic traits (8). Higher YSR-AT scores indicating increased autistic traits in the misophonia group than controls confirmed these findings. However, our study did not include the assessment of specific autistic subscales, such as attention-switching and communication, which have been previously examined in relevant studies (8,9).

According to the current results, female adolescents with misophonia also demonstrated greater sensory sensitivity using AASP; not only in the auditory domain but also more widely across multiple senses (auditory, visual, taste/smell, touch, movement, and activity) (11). The strong correlations observed between misophonia and general sensitivities suggest a potential link between selective sound sensitivities and increased prevalence of other forms of sensory hypersensitivity (7). On the other hand, it has been suggested that misophonia is not so narrowly defined as a selective sound sensitivity; it is a part of a broader syndrome of sensory intolerance (12). Another related issue is whether misophonia is different from sensory over-responsivity (SOR), which is a clinical condition seen in childhood and is associated with ASD (13). SOR is characterized by intense distress (e.g., irritability or anger outbursts) by sensory stimulation, such as a particular auditory stimuli (e.g., sirens). Since the trigger stimuli associated with SOR are not the same as those associated with misophonia (e.g., chewing, breathing), it can be argued that SOR and misophonia are a discrete entity (12).

Path analysis revealed that misophonia significantly increased the likelihood of exhibiting anxiety symptoms, with sensory sensitivity act as a mediator in this relationship. This finding underscores the potential role of heightened sound sensitivities in the development of elevated anxiety symptoms, with auditory triggers directly inducing internal distress upon encountering the sounds (7). This evidence holds potential implications for interventions as it unveils an underlying mechanism that indirectly influences the outcomes associated with misophonia.

It is widely recognized that individuals with ASD are at an increased risk of anxiety disorders (14). The emerging evidence on the association between SOR, anxiety and stress in ASD has spurred theoretical speculation, with one model proposing that ASD contributes to SOR, leading to perceived stress and subsequent anxiety (15). In line with these suggestions, our mediation analysis demonstrates the direct and indirect impact of autistic traits on anxiety, providing support for the model in which the relationship between autistic traits and anxiety is mediated by sensory sensitivity. In other words, defining autism-related traits, particularly their prevalence among children with misophonia, may constitute a primary research emphasis within the realm of misophonia.

This cross-sectional study has several limitations. First, the cross-sectional design did not allow for causal relationships to be established between misophonia and psychiatric comorbidities. Second, the study relied on self-reported measures of psychiatric symptoms and sensory profiles, which may be subject to response bias and social desirability effects. Additionally, because the severity of misophonia was not assessed, it was not possible to examine the relationship between psychiatric symptoms and misophonia severity. Furthermore, the study sample consisted exclusively of adolescent girls, which may limit the generalizability of the findings to other populations. Lastly, the sample size was relatively small, which may impact the statistical power of the analysis and generalizability of the results. Despite these limitations, by highlighting the potential key role of autism-like symptoms in misophonia, particularly in mediating the relationship between sensory sensitivity and anxiety, this research contributes to a deeper understanding of the complex interplay between these factors. These findings have important implications for both clinical practice and future research for developing targeted interventions that address autistic traits to mitigate the impact of misophonia-related sensory sensitivity and anxiety outcomes.

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Table 1. Comparisons of scale scores across the groups

	Misophonia (n=22)	Control (n=22)		
	Mdn (IQR)	Mdn (IQR)	Z/U	*p*

RCADS-CV				
GAD	55 (47.5-62.5)	49 (41-57)	-2.07/154	**0.038***
SAD	57 (46.5-67.5)	48 (39-57)	-2.00/157	**0.045***
PD	72 (63-81)	46.5 (36-57)	-3.26/103.5	**0.001****
OCD	64 (57-71)	50.5 (41.5-59.5)	-3.18/106.5	**0.001****
SP	54 (44.5-63.5)	44.5 (35.5-53.5)	-2.31/143.5	**0.021***
MDD	73 (61.5-84.5)	41.5 (30-53)	-4.22/62.5	**<0.001*****
Total Anxiety	65 (55.5-74.5)	47 (39-55)	-3.33/100	**0.001****
Total Internalizing	66 (55.5-76.5)	45 (36.5-53.5)	-3.86/77.5	**<0.001*****
AASP				
Low registration	33 (28-38)	29 (23-35)	-1.89/161.5	0.058
Sensory seeking	42 (38.5-45.5)	44.5 (40.5-48.5)	-1.77 (166.5)	0.075
Sensory sensitivity	47 (42.5-51.5)	35 (30.5-39.5)	-3.85/78	**<0.001*****
Sensory avoiding	44.5 (40.5-48.5)	36.5 (32.5-40.5)	-3.33/100	**0.001****

Note: Medians are shown with inter-quartile range in parantheses.
Mdn: Median; IQR: Inter-quartile range; RCADS-CV: Revised children's anxiety and depression scale-child version; GAD: Generalized anxiety disorder; SAD: Separation anxiety disorder; PD: Panic disorder; OCD: Obsessive-compulsive disorder; SP: Social phobia; MDD: Major depressive disorder; AASP: Adolescent/adult sensory profile

Mann-Whitney U Test
*p <0.05, ** p <0.01, *** p < 0.001

Table 2. Correlation anaylses of scale scores

	RCADS-CV Internalizing	**YSR-AT**	**AASP Low registration**	**AASP Sensory seeking**	**AASP Sensory sensitivity**	**AASP Sensory avoiding**
RCADS-CV Anxiety	0.974***	0.609***	0.502**	-0.022	0.632***	0.673***
RCADS-CV Internalizing		0.646***	0.523***	-0.031	0.654***	0.685***
YSR-AT			0.479**	-0.048	0.581***	0.610***
AASP Low registration				0.207	0.551***	0.372*
AASP Sensory seeking					-0.136	-0.212
AASP Sensory sensitivity						0.786***
AASP Sensory avoiding						

RCADS-CV: Revised children's anxiety and depression scale-child version; YSR: Youth Self Report; AT: Autistic trait; AASP: Adolescent/adult sensory profile

Spearmen Correlation Test

*p <0.05, ** p < 0.01, *** p < 0.001

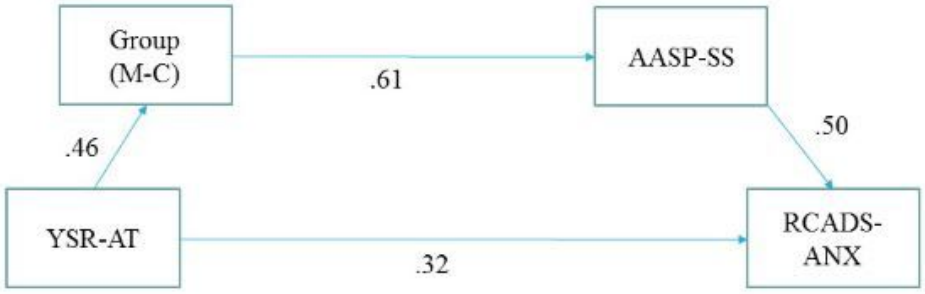

Figure 1. Path diagram of the relation between variables

YSR-AT: Youth self report-autistic traits; M: Misophonia; C: Control; AASP-SS: Adolescent/adult sensory profile-sensory sensitivity; RCADS-ANX: Revised children's anxiety and depression scale-child version.

[Abstract:0136]

Altered PE in TPJ of Depressed Patients During Social Interaction

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Introduction: Depression is associated with impairments in social cognitive functions, resulting in difficulties in interpersonal relationships and social interactions[1]. Understanding the neural systems underlying these issues is a complex task, and research in this area is ongoing. One prominent brain network that has been studied in relation to depression and social cognition is the Default Mode Network (DMN)[2]. Several studies have suggested that the DMN is involved in processing social information and regulating social interactions. It is thought to be related to the internal representation of the self, others, and the expected state of the environment during social interactions. These internal representations are essential for performing advanced cognitive functions, such as perspective-taking, empathy, and theory of mind, which are often impaired in individuals with depression. The DMN plays an important role in internal representations and comparing this representation with the actual sensory input from the external world. However, if there is a discrepancy or prediction error (PE) between the expected and actual situation, the DMN becomes deactivated, and other brain networks become more engaged[3]. The PE is a crucial factor in enabling the brain networks to switch from default mode to task-positive mode[4]. The Temporoparietal Junction (TPJ) process of PEs is related to social situations[5]. It is considered a "switcher" between different brain networks. We propose that abnormal PE signaling may be linked to difficulties in transitioning between the DMN and other brain networks, as well as the persistent activation of the DMN in certain pathological conditions.

Method: We conducted a study with 29 participants diagnosed with Major Depressive Disorder (MDD) and 28 age-gender matched controls. After the volunteers were given basic information about the research, their compliance with

the research criteria were evaluated. The common selection/inclusion criteria of the participants in the study are to be between the ages of 18-60, to be right-handed, to have completed at least 8 years of education without any disruption/delay. Furthermore, another criterion is the participation of the healthy volunteers without any diagnosis of axis 1 psychiatric disease according to the SCID-5 (Structured Clinical Review DSM Disorder). Other searched criteria for the patients are to be diagnosed with MDD and not to be diagnosed with an axis 1 other than MDD. This study was supported by Scientific Research Project Coordination Unit of Ege University (project ID no: 21-3T/35). We designed a modified trust game with 60 trials, which consists of 60 trials based on interpersonal trust, and requires the participant

to predict the other player's behavior and make a decision as an fMRI assignment. In the game model shown and each trial follows the procedure given below, consisting of 6 consecutive screens given in Fig. 1.

In the first phase of the game, the participant receives a return on investment that creates a positive sense of confidence (DMN active). In the second phase, this relationship will be negative (Salience Network (SN) and Dorsal Attention Network (DAN) active). In the final phase, the participant will receive the returns on the investments that will increase the confidence again (DMN active). We employed a mathematical model to estimate PE and learning rate parameters for each by each trial. This mathematical model is based on the Rao and Ballard's predictive coding model. It is preferred due to its suitability for the game-theory based fMRI task, where it is able to receive the feedbacks and in which the predictions are updated based on the information. In this model, the process that plays an important role in the higher cognitive processes of the brain and shows the effect of feedback on the next decision which is added to the model as a variable that represents the learning parameter.

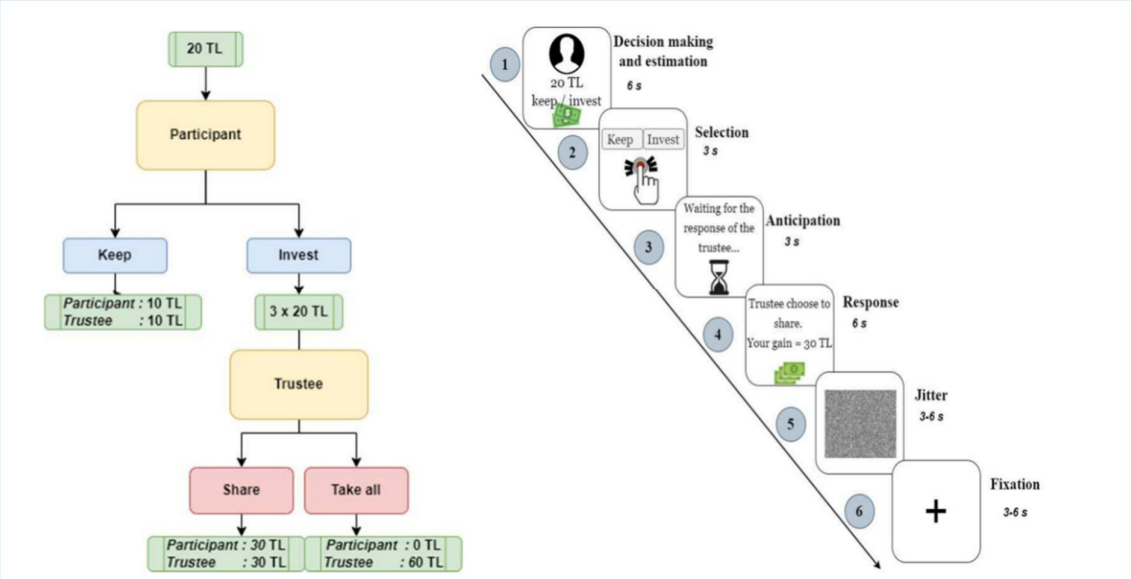

Fig. 1. Trust game flow model and fMRI task model for each trial

In the fMRI model, a statistical analytical design is applied for each participant with the GLM. The subject-specific estimated PE values were normalized between [-1,1] and obtained for each trial by regressing them against expected outcomes in GLM, yielding spatial coordinates for PE. The screens where the PE is seen in the fMRI

model are the difference between the anticipation (screen 3) where the participant waits for the trustee's response and the response (screen 4) where the he sees the response (error =|expectation-actual|). Thus, learning model parameters are included in the model in addition to the fMRI task, separately for each subject.

Results: Despite different participant patterns, the evaluation results demonstrated strong convergence with low root means square errors (RMSE). In both depression and control groups, social PEs activated the right TPJ (rTPJ) (Fig. 2.). PE signal in the controls mostly associated with supramarginal gyrus but in the MDD mostly associated with angular gyrus (posterior part of TPJ) of DMN. PE signal located in angular gyrus might be responsible for persistent DMN in depression. Group differences were observed in the right pTPJ through GLM and t-test analysis for control-depression comparison (Fig. 2.).

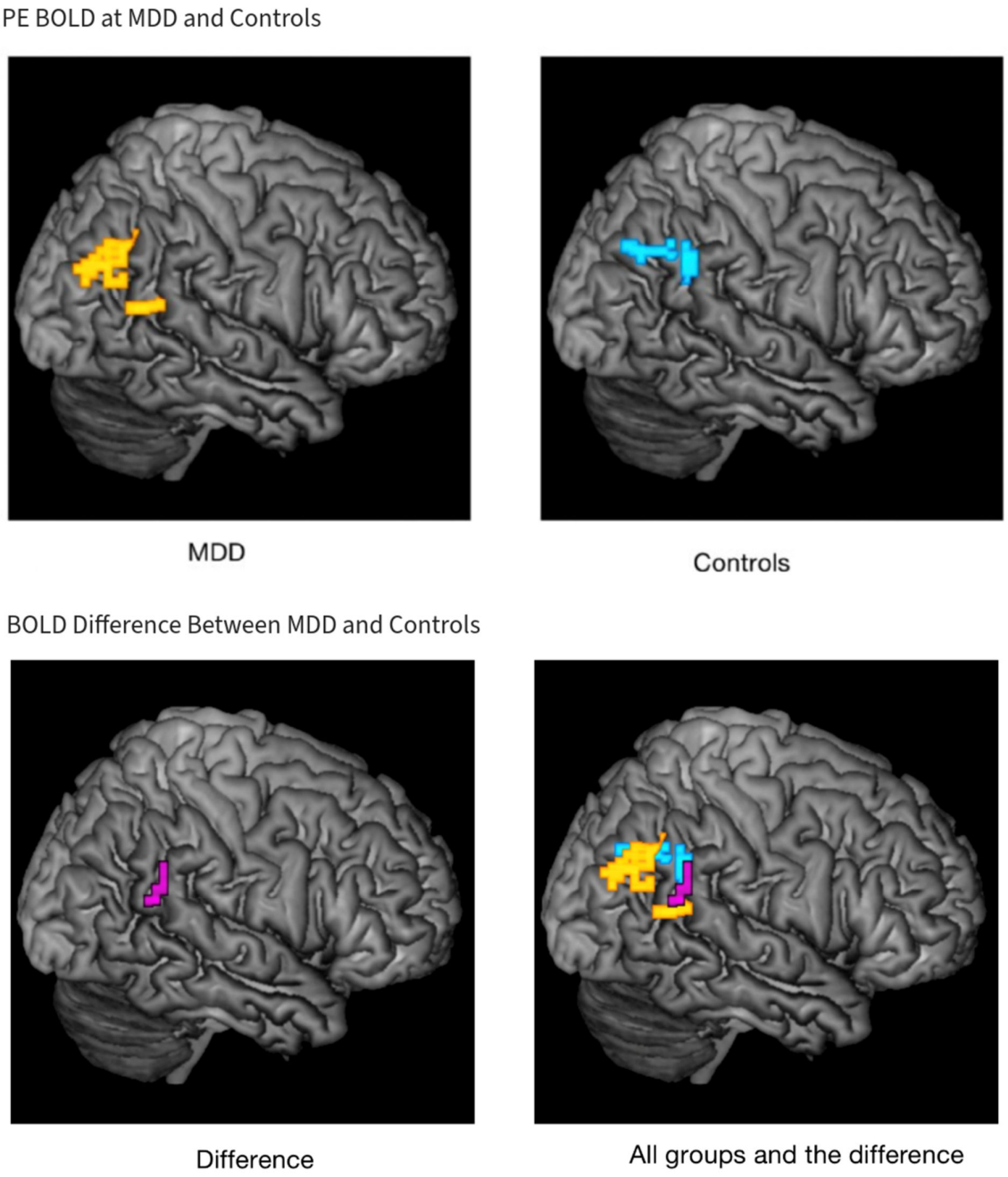

Fig. 2. fMRI Results

Discussion: The right posterior temporoparietal junction (pTPJ) is a region of the brain that has been extensively studied and is known to play a crucial role in social cognition. One of its primary functions is to become significantly activated when individuals engage in activities that involve adopting the mental perspectives of others. This ability to understand and empathize with the thoughts, beliefs, and intentions of other people is a fundamental aspect of our social interactions. In our research study, we conducted observations on individuals diagnosed with depression. Within this group, we identified notable abnormalities in how social PEs were encoded within the TPJ. This finding raises important questions about potential pathologies related to social cognition in individuals with depression. When individuals are tasked with understanding and empathizing with the thoughts and intentions of others, the pTPJ in a healthy brain is notably activated. However, in our study involving individuals with depression, we noticed deviations in the way social PEs were encoded within this region. The concept of social PEs is crucial here. These errors essentially represent the discrepancies between what an individual expects to happen in a social context and what actually

transpires. It's akin to a mental "mismatch" between one's social predictions and reality. These errors, when encoded properly, help us adapt and fine-tune our social interactions. However, in depressive subjects, we observed irregularities in the encoding of these social prediction errors at the TPJ. The PE signal located in the pTPJ might be a contributing factor to the persistent dysfunction of the DMN often associated with depression. The DMN is a network of brain regions that is active when the mind is at rest and involved in self-referential thinking. Its persistent activity in depression is linked to rumination and negative self-focus. Therefore, the abnormalities we identified in encoding social prediction errors within the pTPJ could potentially shed light on why the DMN remains active and overactive in individuals with depression. This, in turn, suggests that there are indeed significant pathologies in social cognition processes among individuals struggling with depression.

In summary, our study underscores the critical role of the pTPJ in social cognition and highlights the intriguing possibility that aberrations in the encoding of social prediction errors within this brain region may be linked to the persistence of the DMN in depression. These findings have the potential to deepen our understanding of the neural mechanisms underlying social cognition in this fundamental hub (TPJ) can guide the development of different approaches for depression treatment, including correction attempts (e.g., TMS, chip) targeting central areas. By shedding light on the TPJ's role in social cognition, this project may offer valuable insights for future studies and therapeutic interventions.

Keywords: Depression; Prediction Error; Social Cognition; Functional Magnetic Resonance Imaging; Brain Networks; Temporoparietel Junction

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[Abstract:0138]

The Effect of Exposure Via Media to the 6 February Kahramanmaraş Earthquake on Traumatic Stress

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Background and Objectives
Post-traumatic stress disorder is the most researched disorder among the psychopathologies seen after natural disasters. Mental symptoms seen in people who do not directly experience the traumatic event but are in close relationships with the victims (such as mental health workers, emergency response team personnel) are called secondary traumatic stress. Studies dealing with secondary exposure to a particular stressor have concluded that participants show high rates of secondary traumatic stress symptoms. In a recent study with emergency nurses, the Secondary Traumatic Stress Scale was used and it was reported that 94% of the participants experienced secondary traumatic stress of varying severity.

The relationship between watching a mass disaster on television or on social media and the development of post-traumatic stress disorder is not fully known and there are few studies addressing the issue. A study examining the effects of television images on psychological symptoms after the September 11 terrorist attacks showed that exposure to trauma images was associated with post-traumatic stress disorder symptoms. A study put forward in Pakistan examining the effect of watching news of violence on the development of post-traumatic stress disorder suggested that the severity of symptoms may be related to the time spent following the news.

Post-disaster psychological problems are affected by the factors that occur before, during and after the disaster, and by personal and socio-cultural characteristics. In a study conducted with people who developed acute stress disorder after

an earthquake, it was concluded that acute stress disorder was more common in those with high anxiety sensitivity. A study examining post-disaster psychopathologies in adolescents showed that comorbidities of major depressive disorder and anxiety disorder were higher in those who developed post-traumatic stress disorder.

In this study, our aim is to investigate the effects of exposure to the Kahramanmaraş Earthquake through the media on the traumatic stress symptoms and anxiety, depression and stress levels of physicians and medical students.

Methods

Study data were collected using self-report questionnaires completed cross-sectionally between April 2023 – June 2023. Snowball sampling method was used. The forms were created using Google Forms (Google, California, USA) and delivered to physicians and medical students via WhatsApp and Facebook groups. The Depression Anxiety Stress Scale (DASS-21), the Impact of Events Scale (IES) were used as assessment instruments. Approval for the study was granted by the Clinical Research Ethics Committee of Ondokuz Mayıs University (2023000092-1, date:02.04.2023).

Study data were extracted from Google forms, converted to Excel format, and then analysed using IBM SPSS Statistics 21.0 package program (IBM Corp., Armonk, Chicago IL, USA). Descriptive statistics were reported as mean (±) standard deviation, frequency, and percentage. The suitability of variables to normal distribution was examined using analytical methods (KolmogorovSmirnov/Shapiro-Wilk tests). Spearman correlation coefficient was used to analyze the association between the sociodemographic data and the scales.

Results

In our study, the data of 182 participants were examined. When the sociodemographic data were analyzed, it was concluded the average age was 28.1, 64.3% of the participants were female (n:117), 28.6% of them had a friend or relative injured, 57.1% were exposed to news or images about the earthquake for more than 12 hours a week, 14.3% were exposed to 4-12 hours a week, and 28.6% were exposed to less than 4 hours a week.

According to the IES-R scores, more than half of the participants (53.8%) had normal scores; however, it was concluded that 14.8% experienced mild, 7.1% moderate and 24.2% severe traumatic stress symptoms. The association between IES-R and DASS-21 scale data was examined. A significant association was found between trauma score and symptoms of anxiety (r=0.680, p=0.001), depression (r=0.586, p=0.001) and stress (r=0.664, p=0.001).

The link between sociodemographic data of participants who were considered to have experienced traumatic stress using IES-R and their IES-R scores was analyzed by logistic regression analysis. According to the results of the analysis, it was concluded that the male gender was protective in terms of traumatic stress, those whose relatives or friends were injured in the earthquake, and those who were exposed to traumatic images for more than 12 hours a week experienced significantly more traumatic stress symptoms.

Discussion

A study examining the effect of television images related to the traumatic event on traumatic stress by using IES-R concluded that traumatic stress symptoms were encountered at a rate of 29.4%. In a study conducted among survivors of the September 11 terrorist attack, the participants were grouped according to the images they watched most frequently (buildings collapsing, people fleeing, plane crashing into towers), in the group with the highest score, post-traumatic stress disorder was found to be 11.2%. In our study, which we conducted with a sample of physicians and medical school students, it was concluded that 46.2% of the participants experienced traumatic stress symptoms after exposure to the Kahramanmaraş Earthquake through the media, and 23.2% of them had severe symptoms. This rate, which is high compared to other studies, is particularly striking. This difference may be due to the relatively small sample of our study compared to other studies. In addition, may indicate how damaging the earthquake disaster we experienced is even on individuals in our country who did not directly experience the disaster.

A review of post-traumatic psychiatric disorders noted that although the most frequently examined mental disorder among trauma victims is post-traumatic stress disorder, it is usually accompanied by symptoms of depression and anxiety, fears of stigma, and suicidal thoughts. In a study conducted with disaster victims after the Fani Cyclone, which occurred in Odisha, India in 2019, it was concluded that 40.9% of the sample had a diagnosis of post-traumatic stress disorder, and that anxiety, depression and stress symptoms were higher in the group with post-traumatic stress disorder. In our study, a positive and significant relationship was found between traumatic stress scores and depression, anxiety and stress levels, in line with the literature.

When the literature is examined, there are studies showing that the duration of exposure to traumatic images through television increases the symptoms of traumatic stress. In our study, it was found that male gender was protective in terms of traumatic stress, the injury of a relative or friend in the disaster, and exposure to traumatic images for more than 12 hours a week increased traumatic symptoms. However, participants whose relatives or friends were injured in the earthquake followed the earthquake news more frequently than the media, and traumatic stress symptoms may be related to the loss of friends and relatives rather than exposure to earthquake news. We hope that future studies will provide more illuminating findings on the effect of media exposure on traumatic stress symptoms.

Table 1: Sociodemographic Data

		Mean±SD (%)	n

Age		28,1±6,2	182
Gender	Male	64,3	117
	Female	35,7	65
Traumatic Events	0	71,4	130
	1	18,7	34
	2-3	8,8	16
	More than 4	1,1	2
Did your friend or relative injured?	Yes	28,6	52
	No	71,4	130
Have you been involved in providing social or medical support?	Yes	26,9	49
	No	73,1	133
How long have you been exposed to relevant news or images via television or social media?	More than 12 hours in per week	57,1	104
	Between 4-12 hours	14,3	26
	Less than 4 hours in per week	28,6	52
Have you had a significant stress factor in the last 1 year?	1	28,0	51
	2 or more	9,3	17
	No	62,6	114
Do you have a diagnosis of psychiatric illness?	Yes	17,6	32
	No	82,4	150

SD: Standart Deviation

Table 2. DASS-21 Correlation analysis with Trauma Score

			Trauma
DASS-21	Anxiety	r	0,680
		p	0,001
	Depression	r	0,586
		p	0,001
	Stress	r	0,664
		p	0,001

DASS-21: Depression Anxiety Stress Scale-21

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[Abstract:0138]

Investigation of the Neuroprotective Effect of Co-administered Probiotics and Vitamin D Against Neurotoxicity: an *in vitro* example

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Objective

The aim of this study was to investigate the neuroprotective effects of co-administered probiotics and calsitriol on rotenone induced SH-SY5Y cells. In addition, cell viability and antioxidant enzyme activities were investigated.

Methods

Cell culture

The human neuroblastoma cell line SH-SY5Y was obtained from the American Type Culture Collection (ATCC, CRL-2266). Cells were grown in Dulbecco's Modified Eagle Medium: Ham's F12 (1:1 mixture) (Sigma Aldrich-D6429) supplemented with 10% fetal bovine serum (Capricorn Scientific-FBS11A), 100 units/ml penicillin/streptomycin (Sigma Aldrich-P4333) in a 5% CO_2 incubator at 37 °C (Figure 1A).

Reagents

Rotenone (Sigma-Aldrich, St Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) and stored at a stock solution of 400 nM. A concentration of 150 nM was applied to SH-SY5Y cells to test viability. Calcitriol was purchased from Cayman (Ann Arbor, Michigan, USA) and dissolved in DMSO (Sigma, USA) to prepare a 428 µM stock solution. Calcitriol was diluted to the appropriate concentration (40, 20, 10, 5, 2.5, 1.25 ve 0.65 µM) in the culture medium to treat cells. SLAB51 probiotics were prepared as described previously (1). The stock solution was prepared as 100 mg/ml, and diluted in the culture medium to the appropriate concentration (0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mg/ml).

MTT assay

Cell viability was measured by a quantitative colorimetric assay using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Cells ($1x10^4$ cells/well) were seeded in two different 96-well plates. After 24 hours of incubation cells were treated with probiotics, calcitriol and rotenone simultaneously. After 24 hours of incubation, MTT assay protocol was administered (2, 3).

Measurement of intracellular protein concentrations

Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, USA).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to measure antioxidant enzyme activities (i) Protein Tyrosine Kinase (PTK); ii) Superoxide Dismutase (SOD); iii) Glutathione Peroxidase (GSH); iv) Glutathione Reductase (GSR); v) Catalase (CAT)) in the treatment groups. Analyzes were performed using a commercial ELISA kit (Shanghai Sunred Biological Technology, China) according to the manufacturer's protocol. All experiments were duplicated.

Statistical analysis

The GraphPad Prism 9.5.0 software was used for all statistical analyzes. Data are presented as mean±standard error of the mean (SEM), and our data were considered statistically significant if $p<0.05$. Comparisons between groups were performed with a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to compare amongst treatment groups.

Results

Effects of Probiotics, Calcitriol, and Rotenone in SH-SY5Y Cells

In the probiotics treatment groups, cell viability was significantly increased in all groups compared to the control group ($p<0.05$). In particular, at doses of 0.01, 0.05 and 0.1 mg/ml, these levels were considered the most effective doses, as the increase in cell viability was over 150% (Figure 1B).

Concentrations of 0.65, 1.25, 2.5 and 5 µM significantly increased cell viability ($p<0.05$) in comparison with control group. The concentrations of 1.25, 2.5, and 5 µM were determined as the most effective calcitriol concentrations since the increase in cell viability was greater than 0.65 µM (Figure 1C).

The concentration that reduces viability to approximately 50% is considered the most effective concentration for clear cytotoxicity and reversibility. Therefore, rotenone was adjusted to 150 nM in further studies investigating the potential neuroprotective effects of different treatment groups (Figure 1D).

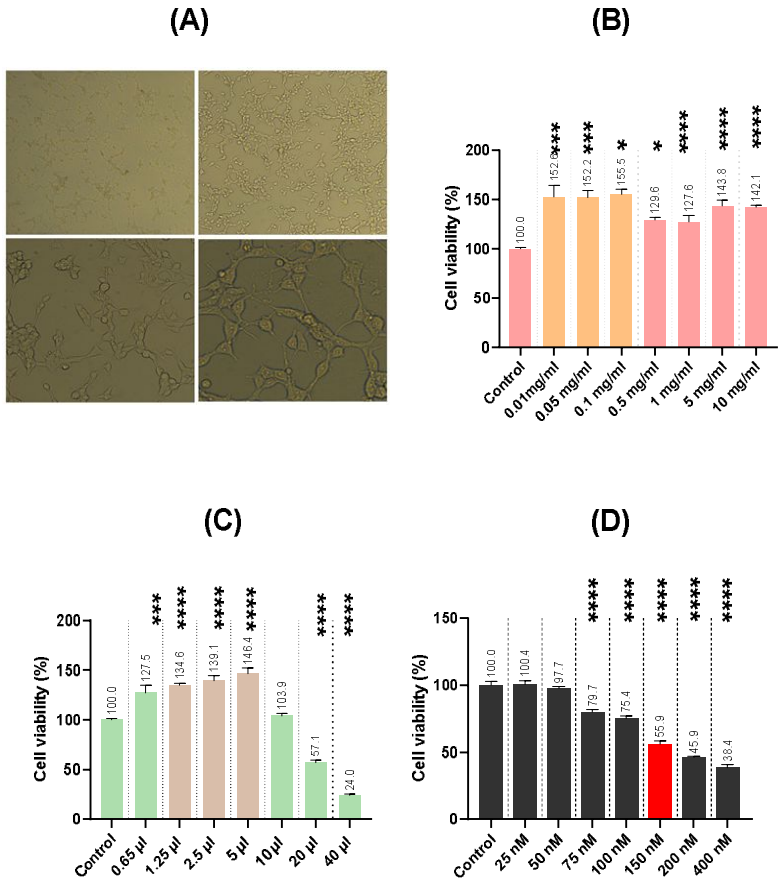

Fig. 1 Dose-response relationships **(A)** Photomicrograph of SH-SY5Y cell line (5X, 10X, 20X, 40X) **(B)** SH-SY5Y cells were treated with probiotics. Data represent mean±SEM (n=8)(*p<0.05, ***p<0.001, ****p<0.0001) **(C)** SH-SY5Y cells were treated with calcitriol. Data represent mean±SEM (n=8)(***p<0.001, ****p<0.0001) **(D)** SH-SY5Y cells were treated with rotenone. Data represent mean±SEM (n=8)(****p<0.0001)

Accordingly MTT results, rotenone alone significantly decreased cell viability by 53.8% compared to the control group (p<0.0001). The increase in all experimental groups was statistically significant, except for the groups receiving 1.25 µM vitamin D, 2.5 µM vitamin D+0.01 mg/ml probiotic, and 1.25 µM vitamin D+0.1 mg/ml probiotic (p<0.05).

Investigation of the Antioxidant Properties of Probiotics and Calcitriol by ELISA Tests

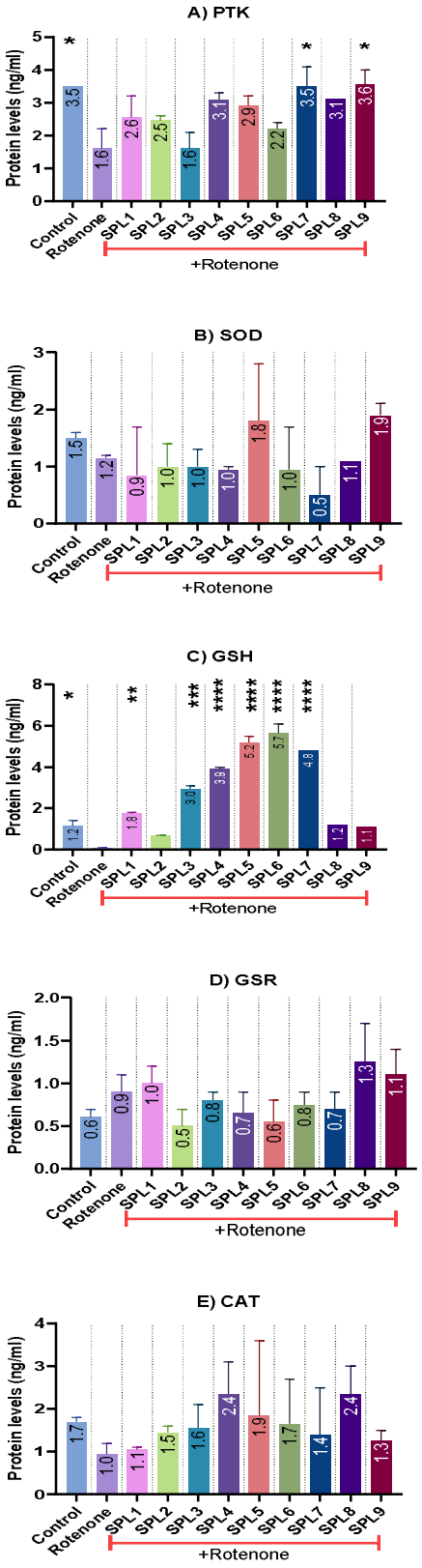

Fig. 2 ELISA results. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

PTK activity in the rotenone group was significantly lower than the control group (p<0.05). PTK activity increased significantly in the 5 μM vitamin D+0.01 mg/ml probiotic and 1.25 μM vitamin D+0.1 mg/ml probiotic groups (p<0.05). SOD activity tended to decrease in rotenone group compared to control group. SOD activity tended to increase in 2.5 μM vitamin D and 1.25 μM calcitriol+0.1 mg/ml probiotics. GSH activity in the rotenone group was significantly lower than the control group (p<0.05). GSH activity increased significantly in all treatment groups (except 0.05 mg/ml

probiotics, 2.5 µM calcitriol+0.1 mg/ml probiotics, 1.25 µM calcitriol+0.1 mg/ml probiotics)($p<0.05$). In contrast to other antioxidant enzymes, GSR activity tended to increase in the rotenone group compared with the control group. GSR levels tended to increase in 0.01 mg/ml probiotics, 2.5 µM calcitriol+0.1 mg/ml probiotics, 1.25 µM calcitriol+0.1 mg/ml probiotics. CAT activity tended to decrease in rotenone group compared to control group. CAT activity tended to increase in all treatment groups.

Discussion

Neurotoxicity refers to the direct or indirect effects of chemicals that disrupt the nervous system of humans and animals. Numerous chemicals can cause neurotoxic diseases in humans. Rotenone is a naturally occurring insecticide, pesticide and fish killer extracted from the roots of plants of the genera *Lonchocarpus* and *Derris*. It is very lipophilic and therefore readily penetrates all biological membranes, including the blood-brain barrier (4). In an *in vitro* study, it was observed that cell viability decreased, reactive oxygen species (ROS) increased, and intracellular signaling proteins related to cell viability decreased after SH-SY5Y cells were treated with rotenone (5). Another study reported that apoptosis was induced by the exposure to 10 µM rotenone for 24 hours in serotonergic and dopaminergic neurons in SH-SY5Y cells (6). In this study, it was found that cell viability decreased with increasing rotenone concentration. The toxic effect began at 75 nM and persisted at concentrations of 100 nM, 150 nM, 200 nM, and 400 nM. Rotenone was adjusted at 150 nM in this study since the concentration that reduces viability by half is considered the most effective concentration (Figure 1D).

Probiotics, which play a leading role to improve gut microbial balance, are beneficial microorganisms that have a positive effect on host health when ingested in sufficient amounts and play a role in ensuring gut microbial homeostasis (7). In our study, the SLAB51 formulation of probiotic was used, and it was found that all tested concentrations (0.01, 0.05, 0.1, 0.5, 1, 5, and 10 mg/ml) significantly increased cell viability without toxic effects. Since the increase in cell viability was more than 150%, especially at the concentrations of 0.01, 0.05, and 0.1 mg/ml, these concentrations were accepted as the most effective group. Castelli et al. (2018) reported that none of the concentrations of the same probiotic showed toxic effect in their study (1). The findings prove that probiotics can reduce the production of ROS by showing an antioxidant effect and that they have a neuroprotective effect by modulating the gut-brain axis in various ways.

In our study, calcitriol was found to maintain cell viability up to 10 µM and to have a toxic effect above 20 µM. At the concentrations of 0.65, 1.25, 25, and 5 µM, a significant increase in cell viability was observed. Since the increase in cell viability was greater at the concentrations of 1.25, 2.5, and 5 µM than at 0.65 µM, these concentrations were determined to be the most effective calcitriol concentrations. In another study found that calcitriol significantly reduced cell viability higher than 15 µM (7).

Cell viability in the experimental groups to which only probiotics and rotenone were added increased by 67.5% compared to the group to which rotenone was added. When evaluated in terms of antioxidant enzymes, PTK, GSH, GSR (only 0.01 mg/ml) and CAT activities increased compared with the rotenone group alone. These results show that the probiotic used in our study increased cell viability but did not act strongly enough to increase the levels of all antioxidant enzymes. The increase in PTK, SOD (only 2.5 µM concentration), GSH, and CAT activity in the groups to which only calcitriol was added suggests that calcitriol plays an active role in preventing oxidative stress. Among the groups in which probiotic and calcitriol were added together in co-incubation, PTK, SOD (only 1.25 µM vitamin D + 0.1 mg/ml probiotic added group), GSH, GSR (except for 5 µM vitamin D + 0.01 mg/ml probiotic added group), CAT activity increased detected.

Probiotics and calcitriol have shown independent neuroprotective effects on neurotoxicity in many studies. However, there are very few studies that have examined the protective effects of taking both substances simultaneously. That's why, this study is one of the limited numbers of studies showing that the combination of probiotics and calcitriol significantly increases cell viability and antioxidant enzyme capacity. To obtain more accurate results, experimental groups and concentrations need to be diversified, different probiotic species used, immunohistochemical experiments performed, and *in vivo* and clinical studies conducted to take the study on this topic to the next level. We have suggested that our promising results will establish a base for further studies.

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[Abstract:0138]

Turkish Adaptation Study of The Turkish Form of the Metacognition Assessment Scale-Abbreviated (MAS-A)

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OBJECTIVE
Metacognition can be defined as the ability to understand and reflect on the mental states of both oneself and others, and use mental knowledge to cope with the difficulties of social life. For this reason, developing metacognitive abilities is to improve one's perception of one's and others, to understand that one's can be mistaken from time to time, to identify new strategies about these situations and to coping especially mis-judges caused by psychiatric diseases. As a result of all these, metacognitive abilities are a treatment goal in order to cope with life's difficulties and improve social skills. If the improvement of metacognitive abilities is to be a treatment goal, there is a great need for a reliable scale measuring this ability.

There are various scales related to metacognition in the Turkish literature, but these scales are self-report scales, as well as scales that are about learning or anxiety and obsessional meta congitive believes and thoughts. There is not any scale that evalute metacognitive abilities in psychotic patients who are known to use metacognitive therapy effectively. Although self-report scales are valuable, it is more valuable for the reliability of the research that the researcher conducts a psychiatric interview and scores metacognitive skills according to this interview.

In order to overcome these limitations, we aimed to introduce the Metacognition Assesment Scale- Abbreviated (MAS-A) to the Turkish literature. MAS-A is used quite frequently in international literature to evaluate metacognition, it was previously used in a study conducted in Turkey. However, Turkish validity and reliability studies were'nt conducted.

While applying this scale, the Indiana Psychiatric Illness Interview (IPII), which is a semi-structured interview, is conducted by the interviewer. During the interview, the participant's voice is recorded and metacognitive areas are scored according to MAS-A scale by listening to the recordings.

The developers of scale focused on synthetic metacognition and divided metacognition into 4 separate areas: 1) Self-Reflectivity 2) Awareness of Others Mind 3) Decentration 4) Mastery. Self-Reflectivity can be defined: as realizing that one's own mind is a separate representation from others, being able to describe emotions (happiness, anger, etc.) and mental processes (understanding, thinking, etc.), being able to realize one's thoughts are fallible, being able to distinguish between dreams and reality and connect emotions and thoughts causally. Participant gets points between 0 to 9 in this area. *Awareness of Others Mind* can be defined: as the ability to understand that others have a separate mental world from oneselves, to connect the feelings and thoughts of others causally, and to evaluate the other's world holistically with its causes and consequences. Participant gets points between 0 to 7 in this area. Decentration can be defined: as the ability to not see oneself in the center of the world, to understand that other people have a separate world and occupations, and to consider reasonable that others may think differently even the opposite of one's own. Participant gets points between 0 to 3 in this area. Mastery can be defined: as being able to find healthy ways of coping with the difficulties encountered in life, recognizing the fallibility of coping strategies with their own and others' situations and developing them. Participant gets points 0 to 9 in this area. Higher subscale scores predict better metacognitive capacity.

METHODS
70 healtyh control and 70 patients with schizophrenia spectrum disorders according to DSM-5 diagnostic criteria were included in the study.

With permission and assistance of the scale developers, MAS-A Codebook and IPII semi-structured interview questions were translated into Turkish and three researcher was trained. IPII interviews were conducted with participants and was audio recorded. Then, all researchers listened to the audio recordings separately and scored metacognitive domains according to MAS-A to test the reliability between researchers.

In order to evaluate the convergent validity, Metacognition Questionnaire (MCQ-30), Beck Cognitive Insight Scale (BCIS), Reading the Mind İn The Eyes Test (RMET), Facial Emotion Identification Test (FEIT), Facial Emotion Discrimination Test (FEDT), Global Assessment of Functioning Scale (GAS) was applied.

Statistical Analysis: Continuous variables in this study were given as mean±standard deviation or median and interquartile range according to distribution characteristics. Categorical variables were expressed as frequency and %. While Student's-t test or Mann-Withney-U test were used in pairwise comparisons, again according to distribution characteristics, Chi-Square test was used in comparisons between categorical variables.

Inter-rater reliability and compliance were evaluated with intra-class correlation coefficients (ICC). A two-way mixed model was used in ICC analyses. Spearman correlation analysis was used for convergent validity. Threshold value for statistical significance was accepted as $p<0.05$.

RESULTS

Sociodemographic and clinical characteristics of all participants have been shown in Table 1.

Table 1. Sociodemografic and Clinical Features of the Participants

		Psychosis group	**Healthy group**	**p**
Age (mean±SD)		34,8±12	34,9±11	
Gender n(%)	female	31(%44)	31(%44)	>0,05
	male	39(%56)	39(%56)	
Education Status	primary school	11(%15,7)	12(%17,1)	>0,05
	middle school	10(%14,3)	13(%18,6)	
	high school	33(%47,1)	30(%42,9)	
	önlisans	3(%4,3)	1(%0,7)	
	university student	5(%7,1)	6(%8,6)	
	university	8(%11,4)	8(%11,4)	
Marital Status	single	55(%78,6)	32(%45,7)	0.000
	married	15(%21,4)	38(%54,3)	
Smoking		44(%62,9)	37(%52,9)	>0,05
Alcohol Use Disorder		2(%2,9)	0(%0)	>0,05
Substance Use		18(%25,7)	0(%0)	0.000
Comorbid Disease(+)		17(%24,3)	18(%25,7)	>0,05
Diagnosis (for patients)	Schizophrenia	42(%60)		
	Schizophreniform Disorder	4(%5,7)		
	Acute Brief Psychosis	4(%5,7)		
	Psychotic Depression	4(%5,7)		
	Psychotic Mania	9(%12,9)		
	Schizoaffective Disorder	3(%4,3)		
	Delusional Disorder	4(%5,7)		
Scale points (mean±SD)		**Psychosis group**	**Healthy group**	**p**
PANSS points	PANSS positive	22±6,5		
	PANSS negative	21,5±6,8		
	PANSS general	36,7±6,7		
	PANSS total	80,4±14		
Total		13,4±4,6	24±3	0,000
S scale		4,8±1,8	8,4±1	0,000
O scale		3,9±1,3	5±1,4	0,000
D scale		1,2±0,8	2,4±0,5	0,000
M scale		3,5±1,7	8,1±1	0,000
MCQ-30		72,8±14,3	70,3+12,2	>0,05
RMET		18,3±4,8	21,8+3,4	0,000
FEIT		10,5±3,4	12,3+2,8	0,001

FEDT	23,6±4,2	25,2+2,8	0,009
BCIS	0,7±6,4	1,7+4,2	>0,05
GAF	21,9±9,4	86,7±6,6	0,000

Categorical variables are given as number (percent) (n(%)), numerical variables are given as mean±standard deviation (mean±SD). S: Self-Reflectivity; O: Awareness of the Other's Mind; D: Decentration; M: Mastery; Total: Total Score; PANSS: Positive and Negative Syndrome Scale

Reliability and validity analysis':

According to intraclass correlation analyzes (ICC) for three observers, ICC values of the MAS-A total and subscale scores ranged from 0.924 to 0.987 (p<0.001 for each)(Table 2.).

Table 2. Intraclass Correlation Coefficient for MAS-A

		95% Confidence Interval					
MAS-A	ICC	Lower Bound	Upper Bound	mean	min	max	Sig
S Scale	0,981	0,975	0,986	6,306	6,15	6,607	0,000
O Scale	0,944	0,925	0,958	4,432	4,407	4,461	0,000
D Scale	0,924	0,9	0,944	1,827	1,757	1,9	0,000
M Scale	0,985	0,98	0,989	5,575	5,396	5,85	0,000
Total	0,987	0,983	0,991	18,14	17,8	18,675	0,000

S: Self-Reflectivity; O: Awareness of the Other's Mind; D: Decentration; M: Mastery; Total: Total Score

The Cronbach Alpha reliability coefficient is 0.85. Item-item correlations were between r=0.52-0.87 (p<0.05), and item-total correlations were between r=0.73-0.95 (p<0.05).

While there was no significant correlation between MAS-A and MCQ-30 scores in validity analyzes (p>0.05); there was positive weak correlation between decenration subscale and BCIS total score (r=0.198, p<0.05) and self-reflection subscale (r=0.190, p<0.05).Each subscale of MAS-A and MAS-A total score were positively stronge correlated with RMET (r=0.472, p<0.000), FEIT (r=0.275, p<0.01), FEDT (r=0.292, p<0.001) and GAS (r=0.840, p<0.001)(Table 3.).

Table 3. Corelations Between MAS-A and Clinical Parameters

MAS-A		FEIT	FEDT	RMET	BCIS-SR	BCIS-SC	BCIS-T	MCQ-30	GAS
S Scale	r	,217*	,288**	,449***					,802***
O Scale	r	,204*	,194*	,244**					,436***
D Scale	r	,246**	,213*	,441***	,190*		,198*		,706***
M Scale	r	,289**	,285**	,481***					,878***
Total	r	,275**	,292***	,472***					,840***

*:p<0,05; **:p<0,01; ***:p<0,001; S: Self-Reflectivity; O: Awareness of the Other's Mind; D: Decentration; M: Mastery; Total: Total Score; BCIS-SR: BCIS Self-Reflectiveness; BCIS-SC: BCIS Self-Reflectiveness; BCIS-T: BCIS Total score

According to linear regression analysis, there was a positive significant relationship between decentration subscale and RMET (p<0.001), a positive significant relationship between mastery subscale and GAS (p<0.001), a positive significant relationship between MAS-A total score and GAS (p<0.001) and a positive significant relationship between MAS-A total score and RMET (p<0.05).

DISCUSSION

This findings are in line with studies that have high inter-rater consistency for MAS-A and support that a second evaluator isn't needed for accurate metacognitive assessment. This may suggest that the Turkish translation of MAS-A Codebook and IPII interview questions is understandable.

In addition, the limitation of the sample reflecting a more homogeneous psychopathological condition in previous studies was eliminated in this study. In this study, there were not only more stable patients but also patients with more severe psychopathology. Thus, it can be said that MAS-A was reliable and inter-rater validity was good in sample with more heterogeneous psychopathology.

In internal consistency measurement, it was observed that the scores of each MAS-A subscales were well correlated with each other and the subscale scores were well correlated with MAS-A total score. This is in line with other studies showing good internal consistency. It also suggests that MAS-A total score can be used in the evaluation of metacognition.

Considering the converjent validity, no significant correlation was found between MCQ-30 and MAS-A. This is consistent with studies in which clinical interview-scored scales were compared with self-report scales. The reason for this may be that metacognitive reporting required in self-report scales actually requires metacognitive skills. In this case, it may not be possible for participants with metacognitive deficiencies to correctly answer the questions in a self-report scale. Also the lack of a significant relationship with MCQ-30, is related to the fact that MAS-A has a different structure from the MCQ-30, which examines the features of metacognition in anxiety and obsessive compulsive disorder. The MAS-A couldn't be compared with any other clinical scale in this study, since there is no other clinical scale whose validity and reliability studies have been completed in Turkish.

In this study, there was a positive correlation between decentration ability and BCIS total score and the cognitive self-consciousness subscale, which is a component of cognitive insight and an indicator of enhanced cognitive insight. It is in agreement with studies that found that people with enhanced cognitive insight also had better metacognitive abilities.

In this study, all MAS-A subscales and total score was found to be positively correlated with RMET, which measures theory of mind and emotion recognition skills, and FEIT and FEDT, which measure emotion recognition skills. This is consistent with studies showing that metacognition is associated with mentalization, social cognition, and theory of mind.

The positive correlation of all MAS-A subscales and total score with GAS indicates that MAS-A scores are associated with clinical functionality.

The linear regression analysis supports the fact that, mastery ability, which is a reflection of the ability to cope with life's problems, has close connections with social functionality.

The correlation between decentration ability and RMET in linear regression analysis is consistent with studies showing that emotion recognition and theory of mind are related with metacognitive capacity. Better metacognitive abilities also make it easier to understand the feelings and perspective of others. From the point of decentration, it can be thought that in order to see others' perspectives different from one's own, it is necessary to interpret others' perspectives and feelings correctly, and to know what others feel and think in the same situation.

As a result, it can be said that, in this study, the MAS-A was found to be valid and reliable in Turkish as well.

Keywords: MAS-A, Metacognition, Schizophrenia

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[Abstract:0207]

Comparison of Melanocortin-4 Receptör and α-Melanocyte Stimületed Hormone Levels of Healthy Volunteers With Patients Who Developed and Did Not Develop Impaired Sexual Function Due To The Use of Selective Serotonin Reuptake Inhibitor

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ABSTRACT

Objective: Selective Serotonin Reuptake Inhibitors (SSRIs) are the most prescribed drugs worldwide. Studies have shown that SSRIs cause sexual dysfunction (SD). It has been reported that between 10% and 30% of patients using SSGI experience sexual dysfunction secondary to drug use [1/6]. Discontinuation rates due to sexual side effects are very high. Cases have been reported in which it persists after discontinuation of the drug, as it can also occur after the first dose use [2/8]. The biological mechanisms underlying SD due to SSRIs are not yet sufficiently understood and investigated. Views on how SSRIs cause SD are mostly related to level of dopamine, serotonin, and noradrenaline. Recent studies have shown that there is a more complex structure than we thought. In recent studies in animals, Melanocyte Stimulating Hormone (α-MSH) and Melanocortin-4 receptor (MCR4), which are members of the melanocortin family that are involved in an extraordinary variety of physiological functions, pigmentation, energy homeostasis and sexual function, have been found to be related to serotonin(5-HT) and SD.

The melanocortin system consists of the melanocortin peptides α, β, and γ-melanocyte-stimulating hormone, adrenocorticotropic hormone (ACTH), and a family of five seven-transmembrane G protein-coupled melanocortin receptors. The melanocortins are posttranslational products of the proopiomelanocortin (POMC) prohormone. Recent studies have shown that 5-HT released from the raphe nucleus binds to 5-HT_{2C} on POMC, causing the release of α-MSH. α-MSH also stimulates the receptor by binding to MCR4. (heissler) The aim of this study is to investigate the relationship between α-MSH and MCR4 levels in three grups which is healthy volunteers, used SSRI and developed sexual dsyfunction (SSRI-SD (+)) and did not develop (SSRI-SD (-)).

Methods: This study was conducted between January 2022-January 2023 at Ankara Dışkapı Training And Research Hospital, Department of Psychiatry Outpatient Clinic. Sociodemographic data form, sexual history form, Structured Clinical Interview for DSM 5 (SCID 5-CV), Psychotropic Related Sexual Dysfunction (PICIB), Arizona Sexual Experiences Scale, Beck Depression and Anxiety Inventory (BDI,BAI) were used in the evaluation interview with the referred patients. Patient groups were formed according to whether there was SSRI-related SD according to the sexual history and PICIB scale. All participant were women. The study included 52 patients who developed SD due to SSRI use, 40 patients who did not, and 49 healthy volunteers who diagnosed with major depressive disorder and anxiety disorder. Inclusion criteria for patient groups were determined as follows: being over the age of 18 and not having gone through menopause, using antidepressants for at least 1 month, not having a physical illness, marital and relationship problems and not using drugs, alcohol that can lead to impairment of sexual functions. Exclusion criteria for healthy group were determined as follows: having a sexual dsyfunction, using any drugs that can cause impairment of sexual functions, using SSRIs, having gone through menopause, having a physical illness that can lead to impairment of sexual functions and mental and active psychiatric conditions that prevent interviews. Biochemical analyses; after starving for 12 hours, blood samples taken from the participants at 08:00 in the morning by venipuncture method into a tube containing BD Vacutainer SSTII Advance serum separator gel were centrifuged at 1500g for 10 minutes. They were placed in capped ependorf tubes and stored at -20°C. On the day of the ELISA study, the frozen serum were removed from -20°C, allowed to reach room temperature, vortexed and studied in accordance with the kit package insert. Assey range of α-MSH is 15-4200ng/L, assey range of MCR4 is 8-2000ng/L. There is no normal range value for this variables. The data were evaluated with SPSS 25.0 for Windows. Shapiro-wilk test used for assessing normal distribution of data. Chi-square analysis was used for comparison categorical variables. In the comparison of normally distributed data, T test was used between two groups and Anova test was used in the comparison of more than two groups. In the comparison of non-normally distributed data Mann-Whitney U test between two groups, and Mann-Whitney U test between more than two groups Kruskal-Walls test was used. In analyses, $p<0.05$ was considered statistically significant.

Results: Although there was no statistically significant difference between the groups in terms of mean age ($p>0.05$), a significant difference was found for mean duration of education ($p<0,001$). 65.2% of the patient group had MDD, 27.2% had generalized anxiety disorder, 2.2% had social phobia, and 5.4% had panic disorder. When the descriptive data related to the measurements in the study were analyzed, the minimum value related to α-MSH measurement in the SSRI-SD (+) group was 110.71ng/L and the maximum value was 4000 ng/L; the mean value was 369.53±787.31 ng/L. In the SSRI-SD (-) group, the minimum value of α-MSH measurement was 110.86 ng/L and the maximum value was 3354.49 ng/L; the mean value was 563.73± 848.83 ng/L, in the control group, the minimum value of α-MSH measurement was 111.38 ng/L and the maximum value was 4000 ng/L; the mean was 978.0± 1050.5 ng/L. In the SSRI-SD (+) group, the minimum value for MCR4 measurement was 16.08 ng/L and the maximum value was 2000 ng/L; the mean value was 259.96±414.38 ng/L, in the SSRI-SD (-) group, the minimum value of MCR4 measurement was 16.10 ng/L and the maximum value was 2000 ng/L; the mean value was 390.69±585.63 ng/L, in the control group the minimum

value for MCR4 measurement was 16.34 ng/L and the maximum value was 2000 ng/L; mean value was 721.71±601.09 ng/L. Among these three groups, the mean α-MSH value of the control group was significantly higher than the mean α-MSH value of the SSRI-SD (+) patient group (p<0,001), the mean MCR4 value of the control group was significantly higher than the mean MCR4 value of the SSRI-SD (+) and SSRI-SD (-) patient groups (p<0,001). The mean total score of the ASEX in the SSRI-SD (+) patient group was 20.08±2.82; in SSRI-SD (-) had a mean total score of 14.30±2.72; in control group had a mean total score of 11.96±2.82. The mean total score of the ASEX in the SSGI-SD (+) patient group was significantly higher than the mean total score of the ASEX in SSGI-SD (-) patient group, and the mean total score of the ASEX in SSGI-SD(-) patient group was significantly higher than the mean total score of the ASEX in the control group. According to the correlation analysis, no correlation was found between α-MSH and MCR4 and age,total score of BDI and BAI. There was a significant difference in α-MSH and MCR4 levels between the groups that used fluoxetine developed sexual dsyfunction and did not develop (p=0.049, p=0,045).

Discussion: The relationship between serum MCR4 and α-MSH levels in the SSRI-SD (+) group was examined and a negative relationship was found as we hypothesized, that is, lower MCR4 and α-MSH levels were found in participants with SD. The relationship between MCR4 and α-MSH levels between the control group, SSGI-SD (+) and SSGI-SD (-) groups was examined and SSGI-SD (+) group had lower MCR4 and α-MSH levels than the other two groups as we hypothesized. These results support that SSRIs cause SD through α-MSH and MCR4. Besides the fact that the ASEX total score of the SSRI-SD(-) group was higher than that of the control group suggests that SSRIs may cause impairment in functions, although not at a level to diagnose sexual dysfunction. .Among SSRIs, the fact that only fluoxetine causes significant differences between the groups with SSRI-SD (+) and SSRI-SD (-) in terms of α-MSH and MCR4 levels supports our hypothesized that SD can be made by a mechanism not found in other SSRIs, such as 5-HT2C antagonism.

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Table 1. Comparison of α-MSH and MCR4 Deceleration Levels between Groups

Variables	Gruplar			Test Result
	SSRI-SD (+) (n=52)	SSRI-SD (-) (n=40)	Control (n=49)	
	Average±Std. deviation	Average±Std. deviation	Average±Std. Deviation	
MSH (ng/L)	369,53±787,30 (a)	563,72±848,83 (b)	978,00±1050,49 (c)	χ^2=18,02 p<0,001* c>a[m]
MCR4 (ng/L)	259,96±414,38 (a)	390,69±585,62 (b)	721,71±601,09 (c)	χ^2=25,65 p<0,001* c>a, c>b[m]
ASEX Total Score	20,08±2,82 (a)	14,30±2,72 (b)	11,96±2,82 (c)	F=112,19 p<0,001** a>b>cn

*Kruskal Walls Testi p<α=0,05, **Anova Testi p<α=0,05

Table 2. Comparison of MSH and MCR4 Levels According to the Causative Decit between Patient Groups

	SSRI-SD(+)	SSRI-SD(-)	Test Result
Fluoxetine			
MSH	615,54±1064,14	1598,04±1288,23	4,212/0,049*
MCR4	424,76±526,83	1037,43±892,60	7,670/0,045*

[Abstract:0211]

The Evaluation of Orexin A, Adiponectin and Apelin-13 Serum Levels in Children with Attention Deficit Hyperactivity Disorder

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Objective: Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder which has not been elucidated in the etiology of neuropathological, genetic and environmental factors. Biological mechanisms associated with the development of ADHD have not been clarified, but few biomarkers have been found [1]. Adiponectin levels, one of these biomarkers, have been shown to decrease in ADHD patients [2]. High Molecular Weight Adiponectin (HMWA) was measured in our study because it was suggested that HMWA is more sensitive in psychiatric disorders [3]. The orexin system seems to be related to dopamine, noreadrenaline and acetylcholine, which are the main neurotransmitter systems related to attention [4] Although studies have been conducted on the role of orexin, in areas such as schizophrenia, bipolar disorder, depression, anxiety disorders, eating disorders and substance abuse, no study has been found examining the role of orexin in pediatric ADHD patients [5]. Since social communication and behavioral deficits ~~have~~ seen in ADHD may be related to the vasopressinergic system, we also examined apelin, which is expressed by the hypotalamoneurohypophysial system, similar to vasopressin [6]. In our study, it was aimed to clarify the place of Orexin A, adiponectin and apelin-13 serum levels in the etiology of ADHD, and to obtain data on the deterioration in cognitive functions seen in ADHD.
Methods: The research was carried out in Pamukkale University Faculty of Medicine, Department of Child and Adolescent Psychiatry, between October 2017 and April 2018. Children and adolescents who accepted to participate in the study from patients who applied to Pamukkale University Faculty of Medicine, Department of Child and Adolescent Psychiatry Outpatient Clinic with ADHD symptoms, were invited to Pamukkale University Faculty of Medicine, Department of Child and Adolescent Psychiatry, for clinical interview with their parents. Cases aged 6-18 years who were evaluated with Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 based clinical interview, Conners Parent and Teacher Rating Scales, Wisconsin Card Sorting Test and diagnosed with ADHD were included in the study group. Case who have been diagnosed with any other psychiatric disorder according to DSM 5 diagnostic criteria other than ADHD, who have received treatment for ADHD in the last 6 months and who were clinically suggestive of mental retardation were not included in the study. In the control group, cases without any psychiatric disorder and mental retardation according to DSM-5 diagnostic criteria based clinical interviews were included. Cases and their parents/legal guardians have given written consent for the use of the case data before participating in the study. According to the researcher's clinical evaluation based on DSM-5, only 37 cases diagnosed with ADHD were defined as the case group, and 35 children and adolescents without any diagnosis were determined as the control group.
From the children and adolescents included in the case and control groups, after 12 hours of fasting, 10 cc venous blood was taken from the antecubital vein into a biochemistry tube, the samples were kept at room temperature for about 15 minutes in Pamukkale University Faculty of Medicine Medical Biochemistry Laboratories, then centrifuged at 3000 rpm for 5 minutes and obtained. Samples were stored at -80 °C until evaluation for orexin A, apelin 13, HMWA levels.
Statistical evaluations were made in the “SPSS (Statistical Package for Social Sciences) for Windows 18.0” package program.
Results: A total of 72 people, 37 of whom were in the study group 35 of whom were in the control group included in the study. When we evaluate the participants according to WCST; The children in the case group were able to complete fewer categories, give fewer correct answers, give more incorrect answers, make more non-perseverative mistakes, make more unique mistakes, try more categories, and have higher set up continuation scores. more perseverative answers, and higher conceptual number and conceptual percentage points. No statistically significant difference was found between the case and control groups in terms of Orexin A, Adiponectin and Apelin-13 levels. Adiponectin levels decreased with increasing age. In addition, there is a moderate negative correlation between adiponectin level and age, weight and height. Therefore, by controlling these factors, it was planned to compare adiponectin values in ADHD and control groups. ANCOVA was planned for the pre-adolescent 6-11 age group and the post-adolescent 12-18 age group. When adiponectin levels in the 6-11 age group were compared between the case-control group by controlling age, height, weight and body mass index, the adiponectin level of the case group (0.52±0.22) and the control group (0.77±0.28) were found to be significant at the F(1.50)=12.455, p=0.001 level. Participants in the serum Orexin A levels, participants; 6-9, 10-15, 16-18 when divided into 3 age groups were found to be statistically significant difference between 6-9 and 10-15 age group.
Discussion: In our study, although Orexin A values were low in the ADHD group, we did not find a statistically significant difference in terms of case and control groups. To the best of our knowledge, there is no study comparing Orexin A levels in children and adolescents with ADHD. However, in their unpublished thesis, Sungur et al. [7] compared 42 adults with ADHD and 46 healthy control groups, and as a result, no difference was found between the ADHD group

and the control group in terms of orexin A levels. Similarly, in our study, no significant difference was found between the ADHD and control groups in terms of Orexin A.
A study evaluating the Orexin levels of normal children found that Orexin levels were higher in newborns and 10-15 years old than in infants, 2-9 years old children and 16-18 years old children [8] . When we grouped our cases according to this study, we found Orexin A plasma levels in pubertal children in the 10-15 age group to be significantly higher than in the 6-9 age group. The reason why we could not find a relationship between Orexin A level and age may be due to the values that increase during adolescence and partially decrease afterward.
Age, weight, height and BMI values were controlled and HMWA values were compared in the case group and control group. According to this comparison, there is a significant difference in HMWA levels between the 6-11 age group in the case group (0.52±0.22) compared to the control group (0.77±0.28) (p=0.001). In the literature, there are contradictions in studies examining the relationship between ADHD-adiponectin/HMWA. In a study conducted with adults with ADHD, serum total adiponectin, HMWA levels and HMWA/Total adiponectin ratio were found to be low in the patient group. It has also been reported that the HMWA/Total adiponectin ratio is strongly correlated with ADHD symptoms [3]. In another study conducted with a total of 76 children, 36 of whom were in the 6-13 age group diagnosed with ADHD but did not receive treatment, and 40 were in the control group, plasma total adiponectin levels were found to be lower in the case group compared to the control group, and low adiponectin levels were found in ADHD cases[9].
In order to understand the role of these and other parameters related to fat metabolism in the etiopathogenesis of ADHD, studies are needed to evaluate genetic and neurochemical factors together with ADHD subtypes. Also evaluation of the cases in terms of the mentioned parameters before and after the treatment; it will contribute to future studies in this field in terms of both seeing whether there is any change in serum levels after treatment and evaluating whether these parameters are related to response to treatment.

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[Abstract:0306]

Evaluation of Bdnf, Fam19a5, Tas, Tos, Osi And Cytokines Level In Drug-Naive Adolescents with First Episode Major Depressive Disorder

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INTRODUCTION

Although there are multiple theories in the pathogenesis, such as monoamine dysregulation, dysfunction of the HPA (hypothalamic-pituitary-adrenal) axis, cytokine hypothesis, and neurotrophic hypothesis, the underlying biological mechanisms have not been fully illuminated in Major Depressive Disorder (MDD). (1) Current clinical and experimental studies confirm that internal or external stress leads to the emergence of depressive symptoms through various neuroinflammatory pathways mediated by cytokines. It has been reported that stress-triggered proinflammatory

cytokines affect kynurenine pathway enzymes, leading to both a decrease in serotonin synthesis and neuronal excitotoxicity. (2) Another factor with a role in neurodegeneration in the pathogenesis of MDD is oxidative stress, defined as an imbalance in the oxidant-antioxidant parameters that can lead to tissue damage. (3) Despite neurodegeneration, one of the most investigated factors in relation to neurogenesis in MDD is brain-derived neurotrophic factor (BDNF), which is mostly shown to be decreased in individuals with MDD in the literature. In recent years, there is evidence suggesting that the FAM19A5 protein, a member of the TAFA protein family primarily expressed in the brain, which has been found to be associated with neuropsychiatric disorders, may be related to neurodegeneration. (4) In a preclinical study, it was shown that proinflammatory cytokines increase the expression of the FAM19A5 protein and that FAM19A5 leads to hypothalamic inflammation and neurodegeneration. (5) In the literature, no research has been found on serum levels of FAM19A5 in individuals under 18 years of age.

In this study, we aim to contribute to the elucidation of the neuroinflammatory pathophysiology of depression by evaluating the serum levels of FAM19A5 as a novel biomarker of inflammatory activation, along with proinflammatory cytokines, BDNF, and oxidative stress parameters (TAS, TOS, and OSI), using the data we obtained.

METHODS

This study was designed as a case-control investigation and was conducted at our hospital's Child and Adolescent Psychiatry outpatient clinic between May 2020 and March 2021. Ethical approval was obtained from the Manisa Celal Bayar University Faculty of Medicine Health Sciences Ethics Committee on May 6, 2020, with protocol number 20.478.486. Individuals between the ages of 12 and 18 diagnosed with a first episode of unipolar MDD who presented to the outpatient clinic were included in the study after confirming the MDD diagnosis through a semi-structured psychiatric interview. Exclusion criteria were the presence of any psychiatric or medical condition accompanying MDD, any medication use or history of infection in the last 1 month, receiving immunosuppressive treatment in the last 6 months, or substance use in the last 3 months. A total of 119 patients meeting the criteria for MDD diagnosis were interviewed. Among these, 71 individuals were excluded due to exclusion criteria, and 8 individuals declined to participate in the study. For the healthy control group, structured interviews were conducted with individuals aged 12-18 who presented to our Pediatric Clinic for various reasons, had no diagnosed illnesses based on laboratory tests and clinical examination, and showed no signs of psychiatric disorders upon evaluation. Volunteers without psychiatric disorders were included in the study after reviewing the exclusion criteria. Our sample consisted of 40 cases within the 12-18 age range diagnosed with MDD, and a matched control group of 39 healthy volunteers in terms of age and gender. All participants underwent a semi-structured diagnostic interview (*Schedule for Affective-Disorders and Schizophrenia for School-Aged Children Lifetime Version DSM-5 November 2016 - Turkish Adaptation (K-SADS-PL):*) and were administered the DSM-5 Depression, Irritability, Somatic Symptoms, and Sleep Disorders Scales. A sociodemographic data form was created by the authors to determine the sociodemographic characteristics and body mass indexes (BMIs) of the children and their parents included in the study. This form aimed to collect information about various demographic factors and BMI to assess the participants' characteristics. Biochemical Analyses: Venous blood samples were collected from the patients after a 12-hour fasting period. In serum samples, proinflammatory cytokines, FAM19A5, and BDNF concentrations were analyzed using the Enzyme-Linked Immunoassay (ELISA) method. Four types of cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) were analyzed. TNF-α and IFN-γ data were excluded from the evaluation as more than half of the analysis results were below the detection range. BDNF, IL-1β, and IL-6 were analyzed using the ELISA Kit (Elabscience, USA) following the procedures specified in the kit's prospectus. FAM19A5 was analyzed using the TAFA5 ELISA Kit (MyBioSource, USA) according to the procedures stated in the kit's prospectus. Serum TAS and TOS levels were analyzed using the total antioxidant status assay kit and total oxidant status assay kit (Reel Assay Diagnostics, Gaziantep, Turkey) on the Beckman Coulter AU5800 device using an enzymatic spectrophotometric method. The Oxidative Stress Index (OSI), indicated as a marker of oxidative stress, is expressed as the percentage ratio of TOS levels to TAS levels.

Statistical analysis in our study was performed using IBM SPSS (The Statistical Package for Social Sciences) version 23.0. Descriptive statistics included percentages, means (M), and standard deviations (SD). A significance level of $p<0.05$ was considered for assessing the statistical significance of results. Independent Samples t-test was utilized for comparing means between two groups showing a normal distribution for numerical variables. For groups not adhering to normal distribution, the Mann-Whitney U test was employed for comparisons. The relationship between categorical variables was investigated using the Chi-Square test. To explore the correlation between cytokine levels and other numerical variables, Pearson correlation analysis was used for normally distributed values, and Spearman correlation analysis was used for values not following a normal distribution.

RESULTS

The study included 40(50.6%) patients diagnosed with MDD and 39(49.4%) healthy controls who had no history of psychiatric illness and psychotropic drug use. The mean age in the patient group was 14.7±1.57 years, and in the control group, it was 14.41±1.72 years. Among the adolescents in the patient group, 35 (87.5%) were female, while in the

control group, 33 (84.6%) were female. No significant differences were observed between the patient and control groups in terms of age, gender, and body mass index (BMI). There were no significant differences between the groups in terms of the presence of psychiatric or non-psychiatric diseases in the family. (Table 1)

When comparing FAM19A5, IL-1β, IL-6, TAS, TOS levels, and OSI, no significant differences were observed. However, a significant difference was detected in BDNF levels between the two groups ($p<0.001$). Detailed data for all variables are presented in the table. (Table 2)

The potential correlations between the values of FAM19A5, IL-6, IL-1β, BDNF, TAS, TOS, OSI, and the total scores of Level-2 Depression Scale, Level-2 Irritability Scale, Level-2 Sleep Disorder Scale, and Level-2 Somatic Symptom Scale were investigated using correlation analyses. It was found that serum BDNF levels exhibited a positive correlation with the total scores of all scales. ($r=0.413$, $p<0.001$; $r=0.436$, $p<0.001$; $r=0.380$, $p<0.001$; $r=0.459$, $p<0.001$; $r=0.409$, $p<0.001$)

A positive correlation was observed between IL-6 levels and the scores of the Depression Severity Scale, Level-2 Irritability Scale, and Level-2 Somatic Symptom Scale. (Respectively; $r=0.249$, $p=0.030$; $r=0.290$, $p=0.011$; $r=0.312$, $p=0.006$). IL-1β levels exhibited a negative correlation with the scores of the Level-2 Sleep Disorder Scale. ($r=0.230$, $p=0.044$)

When assessing the interrelationship of biochemical parameters, a positive correlation was found between IL-6 with FAM19A5. (Respectively $r=0.485$, $p=0.002$; $r=0.332$, $p=0.042$) No significant correlation were found among the other biochemical parameters.

DISCUSSION

We observed a significant increase in BDNF levels among the patient group compared to the control group in terms of biochemical parameters. However, different outcomes have been found in studies involving children. In several studies examining the relationship between pediatric depressive disorder and serum BDNF levels, it has been suggested that BDNF levels are significantly reduced in adolescents diagnosed with MDD compared to healthy control groups. Animal studies have also demonstrated that BDNF expression can vary with age and gender. BDNF levels exhibited a positive correlation with all scale scores. It has been suggested that BDNF could potentially act as a protective or compensatory mechanism in individuals at risk of depression. This suggests that, unlike in adults, BDNF might serve a protective pre-neurodegenerative last call function in adolescent depression. The finding in our study that IL-6 correlates positively with irritability, somatic symptoms, and depression severity is consistent with the literature. We observed that only IL-1β levels showed a significant negative correlation with participants' sleep disturbance scores. This suggests that REM sleep might have an inhibitory effect on IL-1β production, or there could be a shared underlying circadian factor influencing both IL-1β production and other sleep parameters. When assessing the relationship between serum FAM19A levels and other biochemical data, we found a positive correlation with IL-6. In a recent clinical investigation, higher levels of FAM19A5 were found in adult patients with MDD compared to a healthy population. In contrast to these findings, our study did not find a significant difference in adolescent populations. The youth of our patients, along with their first episode and short disease durations, might be associated with the absence of significant neurodegeneration. When evaluating oxidative stress parameters including TAS, TOS, OSI no significant difference was found between the two groups. This discrepancy could be explained by various factors that influence oxidative stress, such as diet and exposure to smoking, as well as the short duration of illness in our patient group.

Our findings indicate that inflammatory markers and oxidative stress indicators in MDD don't increase in peripheral blood in the adolescent population as they do in adults, while BDNF, which is expected to decrease in neurodegenerative processes, shows an increase. The question of whether elevated BDNF levels in depressed adolescents are pathological or a compensatory mechanism requires longitudinal studies for determination. Considering our findings, designing new studies considering BDNF as both a prognostic factor and a therapeutic target in adolescents with MDD would contribute to the literature.

[Abstract:0308]

Cognitive Functioning of Adolescents Using Methamphetamine: The Impact of Inflammatory and Oxidative Processes

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Objective:

Methamphetamine is substance that causes neurotoxicity and its use is increasing. In a large meta-analysis, results supported that methamphetamine use was associated with cognitive dysfunction(medium effect size). In addition, simple reaction time, attention/working memory, executive functions, learning, memory, motor skills, language, information processing speed and visual structuring were impaired(1). Although the pathogenesis of methamphetamine-induced neurotoxicity has not yet been fully clarified, there are studies suggesting that oxidative stress(2), and inflammatory changes(3) may cause this neurotoxicity. Methamphetamine may trigger neuroinflammatory response with increased production of proinflammatory cytokines and oxidative markers such as TNF-a, IL-1b and IL-6; TAS-TOS. We aim to evaluate the relationship between cognitive functions and biological processes. In this study, it was evaluated the relationship between cognitive processes and biological processes (inflammation, oxidative stress and cytokines) on methamphetamine users.

Methods:

Our sample included 37 adolescents aged 15-19 years diagnosed with Methamphetamine Use Disorder and 32 healthy volunteers without any psychiatric disorder. The exclusion criteria were as follows:(a)any severe level of impairment of the adolescent that disrupts the application of cognitive tests or diseases that disrupt the assessment,(b)having mental retardation in the assessment,(c)having used any anti-inflammatory drug for the last 4 weeks,(d)having received immunosuppressive treatment for the last 6 months,(e)being under the acute effect of a recently used substance. The exclusion criterion for healthy volunteers was to have used any substance even once in their lifetime. The group assesment intrumentals was The Turkish Adaptation of the Schedule for Affective Disorders and Schizophrenia Interview Schedule for School-Age Children Now and Lifetime Version-DSM-5(KD-SADS) The Children's Depression Inventory(CDI) The Childhood Trauma Questionnaire-33(CTQ-33) and Central Nervous System Vital Signs(CNSVS). Those who continued to meet the eligibility criteria were invited back for a second study day, approximately 1 day later, that featured neuropsychological testing. Blood samples were taken for biochemical analysis before the neuropsychological test. The planned parameters were also analyzed from these blood samples. Afterwards, a computer-based test lasting approximately 40 minutes was administered to assess cognitive functions. IL1-Beta,FAM19A5,S100-B,IL-6,TNF-Alpha,BDNF concentrations in serum samples were analyzed by Enzyme Linked Immunoassay(ELISA) method. Oxidative Stress Index(OSI), which is shown as an indicator of oxidative stress, was expressed as a percentage of the ratio of Total Oxidative Stress(TOS) levels to Total Antioxidative Stress(TAS) levels. We used SPSS (version 25.0) to complete all inferential analyses and α value of 0.05 was calculated for decisions regarding statistical significance. The chi-square test was applied to analyze categorical data. In the analysis of continuous variables, it was first checked whether the distribution was normal, and the Independent sample t test was used to compare normally distributed data. For non-normally distributed data, the nonparametric test Mann Whitney U was used. The relationship between the numerical variables in the study was examined by correlation analysis and evaluated by Pearson Correlation Analysis. Multiple Linear Regression Analysis was performed to evaluate the relationship between variables that depend on more than one variable and whose dependent variable shows a linear increase. A model was planned with NCI as the dependent variable and BDNF, duration of METH use and Childhood Trauma Severity Score as independent variables. Model components were included in multiple linear regression analysis.

Results:

Sample Characteristics:The study included 37 patients diagnosed with METH use disorder and 32 healthy controls without any psychiatric illness or past psychotropic drug use. There were no significant differences between the patient and control groups in terms of age, gender, body-mass index.

Cognitive Performance and Scale Scores: Main test scores in the CNS-VS test were compared for both groups. The mean Neurocognitive Index score was 65.13±16.81 in the group with METH use disorder and 97.15 ± 8.74 in the healthy control group. Statistical differences were found between all cognitive subtest groups between both groups.

Scale scores of adolescents with METH use disorder and healthy controls were compared. The Childhood Depression Scale and Childhood Trauma Scale were given to the participants and analyzed. Both scale scores were found to be significantly higher in METH users.

Biochemical Measurements: In the patient group, IL-6 and TNF-a levels were statistically significantly lower while BDNF levels were significantly higher. CDI showed a significant negative correlation with the year of METH onset(r=-0.325; p=0.05). CDI showed a significant positive correlation with frequency of METH use(r=0.349; p=0.034). CTQ-33 showed a significant negative correlation with age of METH onset and age at first substance use(r=-0.569; p=0.001 and r=-0.398; p=0.015, respectively). CTQ-33 showed a significant positive correlation with duration of METH use(r=0.486;p=0.002).BDNF was significantly positively correlated with duration of METH use(r=0.383;p=0.019).There was a significant positive correlation between TOS and the amount of METH use(r=0.470; p=0.003). There was a significant positive correlation between TAS and METH use(r=0.465; p=0.004).

Predictors of Cognitive Impairment (NCI) in Patients with METH Use Disorder: The correlation between NCI and BDNF, duration of METH use and Childhood Trauma Severity Score was examined. Significant negative correlations were found as r= -0.523,r= -0.701,r=-0.545, respectively. A model was planned with NCI as the dependent variable and BDNF, duration of METH use and Childhood Trauma Severity Score as independent variables. Model components were included in multiple linear regression analysis. BDNF and duration of METH use were found to be variables that showed significant differences among the factors affecting cognitive impairment.R^2=0.557

Trends in Biochemical Parameters According to METH Expiration Date: The changes in biochemical parameters in the blood samples of adolescents with METH use disorder were analyzed. A significant difference was found between those who used METH 1 week ago and those who used it 2 weeks ago.(p=0.002)

Discussion:
Essentially, our study found that(I)cognitive tests and scale scores of the patient and control groups varied significantly;(II)IL-6, TNF-a and BDNF showed significant differences when cytokines were compared in the patient and control groups;(III)the mean NCI score in cognitive tests of adolescents with METH use showed a strong negative correlation with the duration of METH use, BDNF values and childhood trauma severity score.
NCI is a parameter that shows the average of all cognitive test measurements. A significant decrease in this value was found in adolescents with METH use. A meta-analysis in this field showed that adolescents with METH use had impaired general cognitive functions, attention, executive functions, language/verbal fluency, verbal learning and memory, visual memory and working memory compared to healthy controls.
Among the cytokines analyzed, the mean values of IL-6 and TNF-a were significantly lower in the METH group. METH may trigger neuroinflammatory response with increased production of proinflammatory cytokines such as TNF-a and IL-6. However, the levels of the parameters (IL-6, TNF-a) analyzed in our study were found to be lower than healthy controls. In a study on rats, IL-6, TNF-a, IL-1beta were found to be lower in the prefrontal cortex and striatum as a result of METH use, supporting our findings. Although acute METH use caused increases in inflammatory markers, the METH users who participated in the study had last used METH on different dates(Figure-2), which is consistent with the study that found that cytokines may vary according to the duration of last METH use.
In our study, BDNF levels were significantly higher in the METH user group. Studies in this field in the literature have also found high BDNF levels in individuals with METH use disorder.METH has also been shown to cause neurotoxicity, resulting in increased BDNF levels or expression. The correlation of duration of METH use with BDNF may be related to the activation of neuron protective mechanisms as a result of METH exposure in childhood.
No difference was found in TAS-TOS levels of all adolescents participating in the study. In one study, antioxidant capacity and free radicals were found to be lower compared to healthy controls. In another short follow-up study, METH users hospitalized on the first day had higher oxidative parameters compared to healthy subjects. A significant difference may not have been detected because our study was a cross-sectional study and TAS-TOS levels fluctuated.
In our study, the age of METH use and the age of first time substance use were significantly negatively correlated with Childhood Trauma Scale scores. It was observed that the earlier the age at which people started using METH, the higher the severity of trauma. It was found that the duration of METH use was significantly positively correlated with the trauma scale.
Variables affecting the cognitive impairment of adolescents with METH use were included in the regression analysis.Among the factors, it was found that the duration of METH use and BDNF values had significant relationship.The decrease in NCI supports that it activates neuroprotective mechanisms. Duration of METH use; it may cause neuroinflammation by triggering proinflammatory processes, moreover, in chronic use, it may disrupt cognitive abilities by activating the neurotoxication cascade.These findings of our study coincide with other relevant studies in the literature.In our study, cognitive functions of adolescents with METH use were found to be severely impaired.Biochemically, IL-6 and TNF-a levels were significantly lower between the groups.Our study is important as it is the first study to evaluate FAM19-A5 in adolescents with METH use.The most important factors affecting cognitive impairment were found to be the duration of METH use and BDNF levels. IL-6 and TNF-a were not associated with NCI.

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[Abstract:0327]

Comparison of serum complement levels of children with autism spectrum disorder and healthy controls

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INTRODUCTION
Autism Spectrum Disorder is a neurodevelopmental disorder whose symptoms begin in early childhood, manifested by difficulties in social relationships and communication, stereotyped and repetitive behaviors, limited interests, and whose effects last a lifetime (1). Today, according to reports from the Center for Disease Control and Prevention

(CDC), ASD is steadily increasing in prevalence and around 1 in 36 children have been reported with ASD (2). Genetic inheritance and environmental factors have been reported to have a key role in the etiopathogenesis of ASD but the cause of ASD and underlying pathological mechanisms are yet to be fully elucidated (3). Early diagnosis of ASD allows for early implementation of behavioral interventions to improve core and associated symptoms of autism (4). For these reason, there has been an increased interest in determining biomarkers for etiology in ASD.
The complement system is a system in the innate immune system and forms the basis of the effector mechanisms of antibody-mediated humoral immunity. It can be activated by three different pathways including classical, alternative and lectin (5). In addition to its role in host defense, the immune system also plays critical roles in neuronal developmental steps in the brain, including brain development, neurogenesis, neuronal migration and synaptic pruning. It also plays an important role in brain homeostasis, protection from infection and inflammation, and in the elimination and regeneration process of damaged cells (6).
Dysfunctions of the complement system have been associated with autoimmune, chronic and neurodegenerative diseases (7). Recently, it has been suggested that the complement system may also be involved in the etiology of psychiatric disorders; studies have reported that it may be associated with schizophrenia, depression and bipolar disorder (8).
C1q is a target recognition protein of the classical complement pathway. C2 and C4 are involved in the classical and lectin pathway. C3 is an important component of the complement system that is central to all three pathways. Mannose Binding Lectin and Ficolin are activate the complement system via the lectin pathway (9). C1q, C3 and C4 have been shown to play an important role in synaptic pruning and neural development in the CNS (10).

The present research aimed to explore whether the concentrations of serum C1q, C2, C3, C4, MBL and L-Ficolin vary between ASD patients and healthy controls. The present research also intended to explore whether there is an association between C1q, C2, C3, C4, MBL and L-Ficolin concentrations and ASD severity. We also aimed to investigate the hsCRP level to strengthen the results.

MATERIALS AND METHODS

Participants and Process

The study group included 44 children (36 boys and 8 girls) aged 2–6 years, who were diagnosed for the first time or followed-up with ASD at the outpatient department of child and adolescent psychiatry at the Ankara University School of Medicine between February 2022 and May 2022. The control group consisted of 44 children (36 boys and 8 girls) matched for age and gender, who attended the child and adolescent psychiatry outpatient clinic of our institution in the same period with the study group and discharged without any psychopathologic diagnosis or treatment.
Exclusion criteria for both groups were: a concomitant neurological, genetic, or other medical diseases; the use of any medication or immunomodulatory support; and having had any infectious disease in the last 1 month.
A semi-structured form was completed for the ASD and control groups to assess various sociodemographic and clinical variables and the medical records of all participants were also reviewed. ASD was diagnosed after a detailed clinical interview based on the Diagnostic and Statistical Manual of Mental Disorders, 5th edition [APA, 2013]. A developmentally appropriate IQ test was administered for both groups. The children in both groups were taken to the observation room for diagnostic confirmation. Turkish versions of the Autism Behavior Checklist (ABC) and Repetitive Behavior Scale-Revised (RBS-R-TV) were completed by the parents of ASD group. Childhood Autism Rating Scale (CARS), was completed by the psychiatrist in the observation room.
The ABC is a scale used for assessing the autistic symptoms with 57 items.The CARS (15 items) was used to generate a single total score defining the severity of autism, in which a score between 30 and 36.5 indicates “mild-to-moderate autism,” while the scores from 37 to 60 denotes “severe autism.” The Repetitive Behavior Scale-Revised (43 items) indicates the severity of repetitive behaviors. In this study, these scales were used to investigate the symptomatic correlation with plasma parameters.

Blood Samples

Venous blood samples were collected from an antecubital vein of all participants after an overnight fasting for12 hours and 10 ml of venous blood from each patient were sampled in biochemistry tubes. The biochemistry tubes were centrifuged at 3000 xg for 10 min and the serum was separated and stored in eppendorfs at -80°C refrigerated until biochemical analysis. C1q, C2, C3, C4, MBL, L-Ficolin and hsCRP levels were measured by enzyme-linked immunosorbent assay (ELISA) method.

Statistical analysis

Statistical analyses for the evaluation of tests and scales were performed using IBM SPSS Statistics for Windows 28.0 software. Chi-Square or Fisher's Exact Test will be used in the analysis of categorical data, and Student t test/Mann Whitney U test will be used in the analysis of continuous data according to the number of groups and the fulfillment of test assumptions. Pearson/Spearman Correlation Coefficient was used to examine the co-variance of continuous variables. Pearson/Spearman Correlation Coefficient was used to examine the co-variation of continuous variables.

RESULTS:

The mean age of the children with ASD included in the study was 42.6±14.10 months; the mean age of the healthy control group was 43.95±15.07 months. There was no statistically significant difference between the ages of the children according to the groups (p= 0.485) (Table 1).

Table 1. Age Distribution of ASD and Control Groups

	ASD Mean±SD (min.-max.)	CONTROL Mean±SD (min.-max.)	p
Age (month)	42,6± 14,10 (25,00-72,00)	43,95±15,07 (24,00-72,00)	0,485

Mann- Whitney U Test
SD: Standard deviation

In the ASD group, 18.2% (n=8) were female and 81.8% (n=36) were male, while in the control group, 18.2% (n=8) were female and 81.8% (n=36) were male. There was no statistically significant difference between the groups in terms of gender (p=1) (Table 2).

Table 2. Gender Distribution of ASD and Control Groups

	ASD N (%)	CONTROL N (%)	p
Gender			
Girl	8 (18,2)	8 (18,2)	1,000
Boy	36 (81,8)	36 (81,8)	

Chi-square Test

The mean total mean score of the CARS was 40.14±5.50 points, the mean total score of the ABC was 43.65±23.85 points, and the mean total score of the RBS-R-TV was 19.65±16.60 points (Table 3).

Table 3. Scale Assessments of the ASD Group

	Mean±SD
Childhood Autism Rating Scale (CARS)	40,14 ± 5,50
Autism Behavior Checklist (ABC)	43,65 ± 5,03
Repetitive Behavior Scale-Revised (RBS-R-TV)	19,65 ± 16,60

C1 q level was found to be statistically significantly lower in the ASD group compared to the control group (p<0.001). MBL level was statistically significantly higher in the ASD group compared to the control group (p=0.007). There was no statistically significant difference between ASD and control groups in terms of C2, C3, C4, Ficolin and hsCRP levels (p=0.748; p=0.216; p=0.725; p=0.756; p=0.575) (Table 4).

Tablo 4. Mean/Medians of Serum Complement Proteins and hsCRP Levels of Children in ASD and Control Groups

	ASD Mean±SD/Median(IQR)	**KONTROL Mean±SD/Median(IQR)**	**p**
C1q (ng/ml)	4,12 (3,91)	8,67 (11,75)	**<0,001****
C2 (ng/ml)	2,45(4,75)	2,96 (6,27)	0,748**
C3 (ng/ml)	302,53±98,59	327,91±89,88	0,216*
C4 (ng/ml)	155,68 (119, 30)	163,54 (144,85)	0,725**
MBL (ng/ml)	26,13±13,92	19,37 ± 10,02	**0,007***
L-Fikolin (ng/ml)	4,96 (5,02)	4,76 (6,23)	0,908**
hsCRP (ng/ml)	19,20 (38,36)	17,70 (43,98)	0,575**

*Student T Test **Mann- Whitney U Test

There are no statistically significant correlation between the total scores of CARS, ABC and RBS-R-TV and C1q levels (p=0,568; p=0,599; p=0,544). There are no statistically significant correlation between the total scores of CARS, ABC and RBS-R-TV and C2 levels (p=0,157; p=0,331; p=0,543). There are no statistically significant correlation between the total scores of CARS, ABC and RBS-R-TV and C3 levels (p=0,267; p=0,886; p=0,487). There are no statistically significant correlation between the total scores of CARS, ABC and RBS-R-TV and C4 levels (p=0,933; p=0,357; p=0,480). There are no statistically significant correlation between the total scores of CARS, ABC and RBS-R-TV and C4 levels (p=0,505; p=0,915; p=0,487) (Table 5)

Tablo 5. The Relationship Between Complement Levels and Scale Scores of Children in ASD Group

	C1q (ng/ml)**		**C2 (ng/ml)****		**C3 (ng/ml)***		**C4 (ng/ml)****		**MBL (ng/ml)***		**L-Fikolin (ng/ml)****	
	r	p	r	p	r	p	r	p	r	p	r	p
CARS Total Score	0,092	0,568	-0,231	0,157	0,171	0,267	0,013	0,933	0,106	0,505	0,204	0,208
ABC Total Score	0,085	0,599	-0,160	0,331	-0,022	0,886	-0,144	0,357	0,017	0,915	0,229	0,156
RBS-R-TV	0,097	0,544	-0,1	0,543	-0,108	0,487	-0,111	0,480	-0,108	0,487	**0,342**	**0,031**

Total Score						

*Pearson Correlation Analysis** Spearman Correlation Analysis

There is a significant negative correlation between the C2 levels and the scores of the 'taste, smell, touch' and 'general impressions' subscales of the CARS (r=-0.355; p= 0.027; r=-0.359, p=0.025); there is a significant positive correlation between the C3 levels and the scores of the 'verbal communication' subscale of the CARS; a significant positive low-severity correlation was found between picolin levels and the scores of the 'emotional responses' subscale (r=0.326; p=0.040); A significant positive moderate correlation was found between Ficolin levels and the scores of the 'listening response' subscale of the CARS (r=0.482; p=0.002) (Table 6)

Tablo 6. The Relationship Between C2, C3, L-Ficolin Levels and the CARS Scores of Children in the ASD Group

CARS	**C2 (ng/ml)****		**C3 (ng/ml)***		**L-Fikolin (ng/ml)****	
	r	p	r	p	r	p
Total	-0,231	0,157	0,171	0,267	0,204	0,208
Emotional Response	0,057	0,730	0,059	0,702	**0,326**	**0,040**
Listening Response	-0,075	0,648	0,053	0,732	**0,482**	**0,002**
Taste-smell-touch response	**-0,355**	**0,027**	0,098	0,526	-0,075	0,647
Verbal Communication	-0,125	0,448	**0,313**	**0,039**	0,001	0,993
General Impressions	**-0,359**	**0,025**	0,233	0,128	0,131	0,409

*Pearson Correlation Analysis** Spearman Correlation Analysis

There was a significant positive correlation between picolin levels and ODCL 'sensory' and 'relationship building' subscale scores. A significant positive correlation was found between ficolin and TEDÖ-R-TV total score and 'self-injurious behaviors' sub-score (Table 7).

Tablo 7. The Relationship Between L-Ficolin Levels and ABC, RBS-R-TV Scores of Children in ASD Group

SCALE	**L-Fikolin (ng/ml)**	
	r	p
ABC		

Total	0,229	0,156
Sensory	**0,366**	**0,020**
Relating/Social Skills	**0,383**	**0,015**
RBS-R-TV		
Total	**0,342**	**0,031**
Compulsive Behavior	**0,337**	**0,034**

Spearman Correlation Analysis

DISCUSSION

The present study examined whether serum C1q, C2, C3, C4, MBL and L-Ficolin levels are related to ASD in childhood. The results demonstrated that patients with ASD had lower serum C1q and higher serum MBL levels than controls. Although, there was no statistically significant difference between the groups in terms of serum C2, C3, C4 and L-Ficolin levels. And also no significant correlation was found between serum C1q, C2, C3, C4, MBL and L-Ficolin levels levels and total and subscores of the scales used for ASD group.

In our study, serum C1q levels were significantly lower in the ASD group compared to the controls. In contrast to our study, Corbett et al. (11) found that serum C1q levels were higher in patients diagnosed with ASD. The authors suggested that this increase in serum C1q levels may be a compensatory mechanism for the disfunction of C1q. An in vitro study conducted with rodents, it was shown that there was decreased C1q mRNA expression in the middle frontal gyrus of the ASD group compared to controls (12).

In postmortem studies of brain tissues of individuals diagnosed with ASD, it was found that synaptic pruning was slow, resulting in an excess of synapses, increased dendritic spiny protrusion density and decreased spiny protrusion pruning in layer 5 pyramidal neurons (13). Bourgeron (2009) reported that abnormal synaptic growth and excitatory and inhibitory synaptic imbalance may be involved in the etiology of ASD (14). C1q plays an important role in neurogenesis, synaptogenesis and synaptic pruning during early CNS development, and accordingly, it is thought that C1q dysfunction may be involved in the etiology of ASD with evidence such as an increase in excitatory synapses in rodent lacking C1q protein (15).

In the literature, there are a limited number of studies examining the association of MBL with psychiatric disorders, and to our knowledge, there is no study examining its association with ASD. Considering the literature, there are a number of studies on blood MBL levels in schizophrenia, with inconsistent findings (16,17). In our study, serum MBL levels were found to be significantly higher in the ASD group. The results of our study suggested that complement system activation may also play a role in the etiopathogenesis of ASD, but further studies are needed.

In our study, no significant difference was found between the groups in serum C3 and C4 levels. Similar to our study, Ashaat EA et al compared serum C3 and C4 levels between ASD and control groups and found no significant difference. However, unlike our study, there was a significant difference in C3 and C4 levels between moderate and severe autism groups in the ASD group (18). Another recent study showed a significant increase in serum C3 and C4 levels in children with ASD compared to healthy control group (19). Future studies are needed to explore this issue by evaluating the potential role of C3 and C4 in the etiology of ASD.

In of our study, no significant difference was found between the C2 and L-Ficolin levels of the two groups.

As a result, our findings suggest that disorders of C1q and MBL may be involved in the etiopathogenesis of ASD. No significant difference was found between the groups in C2, C3, C4 and L-Ficolin levels. Although C1q and MBL proteins may affect the brain by causing dysregulation in immune response, neurogenesis, synaptic pruning, or with other unknown mechanisms, no correlation was found between other complement proteins and ASD. Further studies are needed to explain the difference in the complement system proteins and the role of complement system disorders in the etiology of ASD.

In addition to the small sample size, the cross-sectional nature of the study is an important limitation. The fact that our study was not a follow-up study and the presence of active infection was not excluded by any biochemical test are other limitations.
Despite these limitations, our study is the first in the literature to examine serum C2, MBL, and L-Ficolin levels in individuals with ASD. It is also the first study in the literature to examine all three pathways of the complement system simultaneously. Another strength of the study is that the ASD and control groups were matched in terms of gender

Keywords: autism spectrum disorder, complement system, immune system, C1q, MBL

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[Abstract:0340]

Vortioxetine improved autistic-like behaviors in the prenatal valproic acid model of autism in rats

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Introduction

Autism spectrum disorder is a neurodevelopmental disorder characterized by impairments in social interaction and communication, stereotyped or repetitive behaviors. According to data published by the Center for Disease Control and Prevention in 2020, it is seen that 1.85% of 8-year-old children living in the United States have autism. According to the data published by the WHO in 2022, it is seen that approximately one out of a hundred children worldwide have autism. According to the diagnostic and statistical manual of mental disorders, autism can be diagnosed by two main symptoms. These two main symptoms are (i) deterioration in social interaction and communication and (ii) repetitive behaviors. In neurological disorders such as autism, it has been shown in the literature that various physiopathological changes occur during fetal development, such as irregular inflammatory cascades, neuronal degeneration, microglial cell activation, and oxidative stress through reactive oxygen production in the brain. There are two drugs approved by the FDA for use in individuals with autism. These drugs are aripiprazole and risperidone. The atypical antipsychotics aripiprazole and risperidone were not developed specifically to treat autism. In addition, serious side effects such as tardive dyskinesia, hyperprolactinemia, metabolic syndrome and agranulocytosis may occur during treatment with these drugs. Therefore, research has focused on new therapeutic approaches to better treat autism [1].

Valproic acid is an anticonvulsant drug that acts by increasing the concentrations of GABA, an inhibitory neurotransmitter in the central nervous system, in the brain. In addition, valproic acid can suppress repetitive neuronal excitation by inhibiting voltage-sensitive sodium channels. It can also cause hyperpolarization by opening potassium channels in the neuron membrane [2]. The most widely used model for constructing an experimental autism model in rodents is the prenatal valproic acid-induced model [3]. Autism-related symptoms such as poor cognitive performance, social interaction deficits, and repetitive and stereotypical behaviors are observed in offspring born as a result of a single dose of valproic acid administration in animals during pregnancy [4],[5],[6].

One of the main symptoms of autism and the most resistant to treatment is social behavior deficiencies. There is ample evidence that serotonergic system dysfunction plays a role in social behavior deficits in autism. Clinical studies show that the behavioral symptoms of autism worsen with reduced 5-HT availability and improve with selective 5-HT reuptake inhibitor (SSRI) fluoxetine treatment in some patients. Restrictive-repetitive behaviors, one of the main autism symptoms, do not respond to SSRI treatments. These rigid compulsions in autism are most often controlled through use of antipsychotics such as risperidone, which acts as a potent 5-HT2 and dopamine D2 antagonist. Antagonists or partial-agonists of 5-HT1A, HT1B, or 5-HT7 also reduce restrictive-repetitive traits [7]. Vortioxetine; It is a new multimodal antidepressant with 5-HT1A receptor agonist, 5-HT1B receptor partial agonist, 5-HT3, 5-HT7 and 5-HT1D receptor antagonist and inhibitory effect on serotonin reuptake pumps [8]. It has been thought that vortioxetine may have a therapeutic effect on autism due to its multimodal regulatory role in the serotonergic system in the brain. Therefore, in this study, it was aimed to investigate the effects of vortioxetine on autism-like behaviors in a the prenatal valproic acid model of autism in rats.

Material and Methods

Adult female Wistar albino rats were used in our study. Rats were obtained from Erciyes University Experimental Research Application and Research Center. All experiments were conducted in this study were approved by the Erciyes University Animal Research Ethics Committee. Standard care procedures, including constant temperature (22 ± 1°C), 12/12 light and dark cycle, no restriction for feeding and water were applied in our experiments.

Female rats 10-12 weeks old were mated and the next morning vaginal smears of the rats were examined under a light microscope. Successful mating was demonstrated by the presence of more than 10 spermatozoa in the vaginal plug and smear, and that day was designated as embryonic day 0.5 (E0.5). At embryonic day 12.5 (E12.5), pregnant rats were given a single intraperitoneal dose of 500 mg/kg VPA or saline at the same dose. After VPA and saline applications, all pregnant rats were kept in separate cages until delivery. From birth, all lactating rats were allowed to raise their offspring. On postnatal 21st day (PND 21, weaning day), the female pups were separated from their mothers. Offsprings were randomly divided into five groups (n=8 for each group): Saline , VPA, VPA+Vortioxetine 2.5 mg/kg, VPA+Vortioxetine 5 mg/kg, VPA+Vortioxetine 10 mg/kg.

Valproic acid and vortioxetine were dissolved in saline. The injection volume of chemicals was 0.1 mL/100 g. All treatments were administered daily intraperitoneally for twenty eight days (from PND42 to 69). Behavioral experiments were performed from the twenty-first day of treatments. Drugs were administered intraperitoneally 30 minutes before all behavioral tests. Locomotor activity, three-chamber social interaction, novel object recognition and prepulse inhibition tests were performed to evaluate autism-like behaviors in rats. Ketamine (80 mg/kg) and xylazine (10 mg/kg) combination was used as anesthetic. The animals were sacrificed by cervical dislocation when the reflexes disappeared.

Windows operating system based GraphPad Prism 8.0 program was used for all statistical analysis of the data obtained as a result of our studies. Data are presented as mean ± standard error, and our data was considered statistically significant when $p<0.05$. Comparisons between groups were performed with one-way and two-way analysis of variance (one-way ANOVA and two-way ANOVA), and Dunnett's test was used as a post hoc test. In addition, the paired Student-t test was also used in the new object recognition test. In addition, GraphPad Prism 8.0 program was used for all graphics.

Result

Vortioxetine increased the discrimination index in the NOR task

Discrimination indexes were compared to evaluate the ability of rats in different experimental groups to distinguish between novel and familiar objects. Valproic acid administration decreased discrimination index ($p<0.001$) compared to the saline group. It was observed that vortioxetine (5 mg/kg) ($p<0.05$) and vortioxetine (10 mg/kg) ($p<0.001$) groups significantly reversed the deterioration caused by valproic acid (Figure 1).

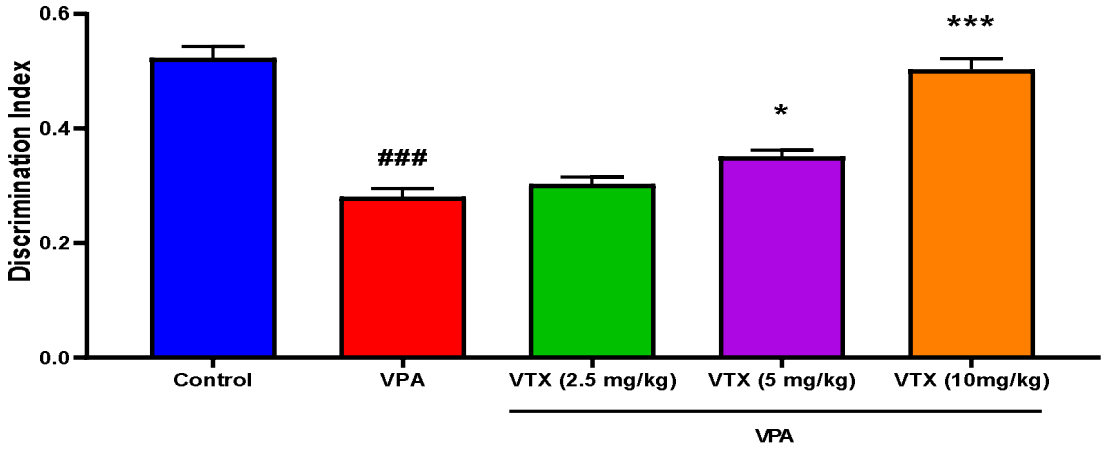

Figure 1. Comparison of the effect of the treatment groups on the discrimination index in the novel object recognition test with the control and VPA groups. Values were expressed as mean ± SEM. Comparisons between groups were made using one-way ANOVA and post hoc Dunnett's test. Compared with the control group, it is ###: $p<0.001$, when compared with the VPA group, ***: $p<0.001$ and *: $p<0.05$.

Vortioxetine decreased total distance traveled, self-grooming and time spent in the centre in locomotor activity test

The effects of different doses of vortioxetine and VPA on locomotor activity parameters were evaluated. When we compare the total distance traveled of the VPA group with the control group, a significant increase is observed in the VPA group ($p<0.05$). Vortioxetine (5 mg/kg) ($p<0.01$) and vortioxetine (10 mg/kg) ($p<0.001$) significantly decreased the increase in total distance traveled caused by valproic acid (Figure 2).

When we compare the self-grooming of the VPA group with the control group, a significant increase is observed in the VPA group ($p<0.001$). Vortioxetine (5 mg/kg) ($p<0.001$) and vortioxetine (10 mg/kg) ($p<0.001$) significantly decreased the increase in self-grooming caused by valproic acid (Figure 2).

When we compare the time spent in the centre of the VPA group with the control group, a significant increase is observed in the VPA group ($p<0.001$). Vortioxetine (5 mg/kg) ($p<0.05$) and vortioxetine (10 mg/kg) ($p<0.001$) significantly decreased the increase in time spent in the centre caused by valproic acid (Figure 2).

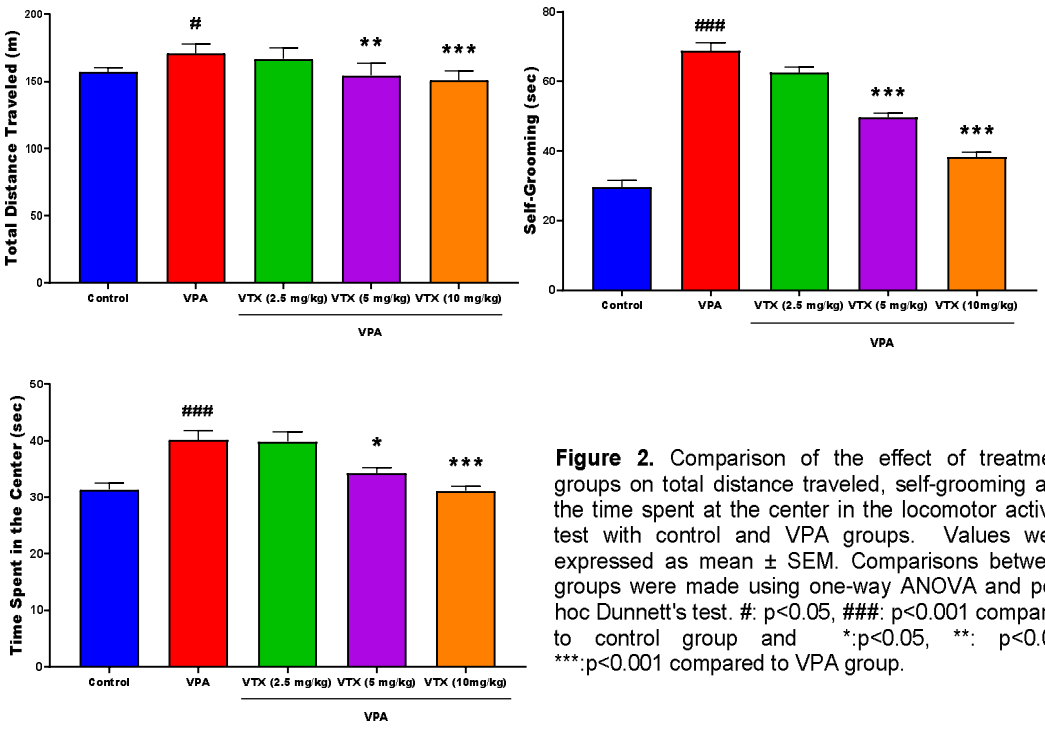

Figure 2. Comparison of the effect of treatment groups on total distance traveled, self-grooming and the time spent at the center in the locomotor activity test with control and VPA groups. Values were expressed as mean ± SEM. Comparisons between groups were made using one-way ANOVA and post hoc Dunnett's test. #: p<0.05, ###: p<0.001 compared to control group and *:p<0.05, **: p<0.01, ***:p<0.001 compared to VPA group.

Vortioxetine increased the sociability index and social preference index in the three-chamber social interaction

In the three-chamber social interaction test, VPA exposure significantly decreased the sociability index (p<0.001) and social preference index (p<0.001) compared to the control group. Vortioxetine (2.5 mg/kg) (p<0.05), vortioxetine (5 mg/kg) (p<0.01) and vortioxetine (10 mg/kg) (p<0.001) increased the sociability index. Vortioxetine (5 mg/kg) (p<0.01) and vortioxetine (10 mg/kg) (p<0.001) increased the social preference index (Figure

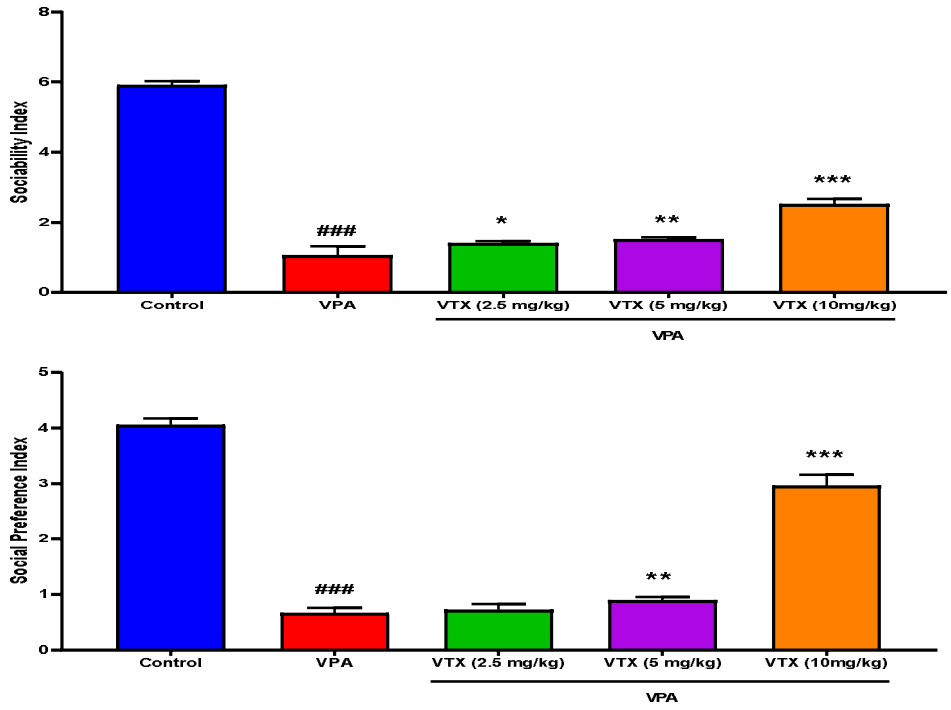

Figure 3. Comparison of the effect of the treatment groups on the in the three-chamber social interaction test with the control and VPA groups. Values were expressed as mean ± SEM. Comparisons between groups were made using one-

way ANOVA and post hoc Dunnett's test. ###: p<0.001 compared to control group and **: p<0.01, ***:p<0.001 compared to VPA group.

Vortioxetine failed to correct VPA-induced patency in the prepulse inhibition test

The results showed that VPA application reduced prepulse inhibition (%) compared to saline at prepulse intensities of +4 dB (p<0.001), +8 dB (p<0.001) and +16 dB (p<0.001) in the PPI test. The decreased prepulse inhibition (%) due to VPA could not be corrected in 3 different doses of vortioxetine (Figure 4).

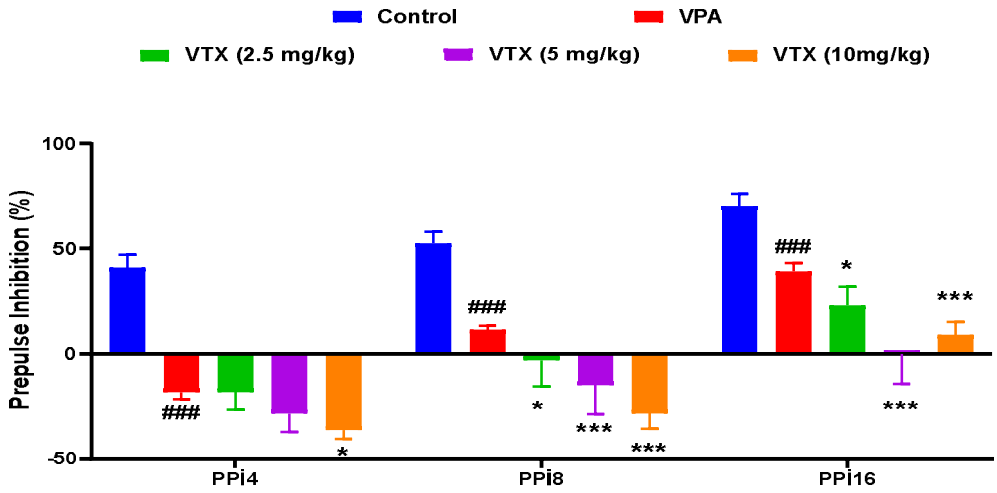

Figure 4. Comparison of the effect of treatment groups on percent inhibition in the PPI test with control and VPA groups. Values were expressed as mean ± SEM. Comparisons between groups were made using one-way ANOVA and post hoc Dunnett's test. ###: p<0.001 compared to control group and *:p<0.05 and ***:p<0.001 compared to VPA group.

Conclusion

In this study, we investigated the effects of vortioxetine on VPA-induced autism-like behaviors in rats. Self-grooming was used to test for repetitive behaviors, sociability and social preference index were used to test for social interaction disorder, discrimination index was used to test learning and memory, and PPI was used to test sensorimotor gating function. The offspring of VPA-treated rats showed social dysfunction, repetitive stereotyping, poor cognitive performance, and impaired sonsorimotor gating function. Vortioxetine improved social performance, learning and memory, while reducing repetitive stereotypes. It exacerbated the deterioration in the sensorimotor gating function caused by VPA. According to the literature review, vortioxetine's reduction in restrictive-repetitive behaviors and its reinforcing effect in social interaction were attributed to its being a 5-HT1B partial agonist and 5-HT7 antagonist. The procognitive effects of vortioxetine have been tried to be explained in the literature by serotonergic, glutamatergic, GABAergic or histaminergic enrichments in the prefrontal cortex [7],[8]. Our results showed that VPA administration mimicked the behavioral of autism-like in rats. Vortioxetine improved the autistic-like behavioral in the VPA model of rats. As a result of the study, we think that vortioxetine will provide a therapeutic effect on autism with its multimodal mechanism of action. We need to validate our study in different models of autism and elucidate possible mechanisms underlying these effects of vortioxetine.

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[Abstract:0061]

Investigating the Role of PI3K/Akt/mTOR Signaling Pathway and Effect of Agmatine in Experimental Autism Model in Rats

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Objective: Autism spectrum disorder (ASD); is a heterogeneous set of neurodevelopmental disorders that occur early in life and are characterized by impaired social interaction and communication, and unusually limited and repetitive stereotypes and interests. Current study aimed to investigate the role of PIK3/Akt/mTOR signaling pathway and possible effect of agmatine (AGM) which is an endogenous polyamine, known to be involved in the regulation of many functions with its widespread distribution in the mammalian brain and the periphery, and accepted to be an atypical neurotransmitter and neuromodulator in valproic acid (VPA) induced ASD in rats.

Methods: Sprague Dawley female rats were grouped according to the smear results, female rats with spermatozoa in their samples were accepted 0.5th day (E0.5) of their pregnancies such as Control, ASD (400 mg/kg VPA; sc) and ASD+AGM (400 mg/kg VPA; sc and 50 mg/kg AGM; ip). In order to generate the model, a single dose of 400 mg/kg VPA injection was applied to pregnant rats on E12.5 AGM was applied for 15 days beginning from P30 [3]. Animals were observed for malformations and decapitated after behavioral tests. Open field test (OFT) for locomotor activity and to evaluate the stereotypical behavior, novel object recognition test (NORT) for recognition index and the discrimination index, social interaction test for social withdrawal and three-chamber test (TCT) for sociability preference index. Prefrontal brain regions were dissected and mRNA expressions were evaluated for PI3K, Akt and mTOR with real time PCR test.

Statistical analysis: Data were analyzed by one-way analysis of variance (ANOVA) for OFT, discrimination and recognition indices in NORT, social interaction test and sociability and social preference indices in TCT which were followed by post-hoc Tukey's multiple comparison test. Two-way ANOVA was used to analyze time spent with old and novel objects in NORT which were followed by post-hoc Post-hoc Šídák test. Data are shown as mean ± SEM and differences with $p < 0.05$ were considered statistically significant.

Results: According to the findings of current study, total activity, and grooming time were significantly higher in ASD group in the OFT compared with control group ($p<0.05$) while AGM treatment supressed this increase in grooming time ($p<0.001$) (Figure 1 A,B).

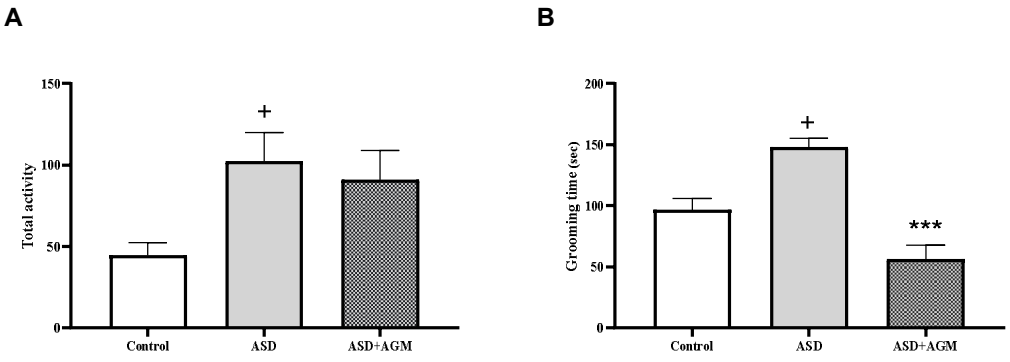


Figure 1. Total activities of groups (A) and grooming time (B) in OFT. Data are shown as mean ± SEM. Statistical analysis was done with one-way ANOVA, Post-hoc Tukey test was applied (n=12/groups). +p<0.05; Compared to the control group, ***p<0.001; compared to the ASD group. Autism Spectrum Disorder; ASD, Agmatine; AGM.

Time spent with the novel object was significantly higher in control group ($p<0.001$) compared to the old object, wheras there was no significant difference in ASD group in the NORT (Figure 2 A). Discrimination and recognition index were

significantly lower in ASD compared to control group ($p<0.001$). It also found that treatment with AGM, significantly increased both indexes ($p<0.001$) (Figure 2 B,C).

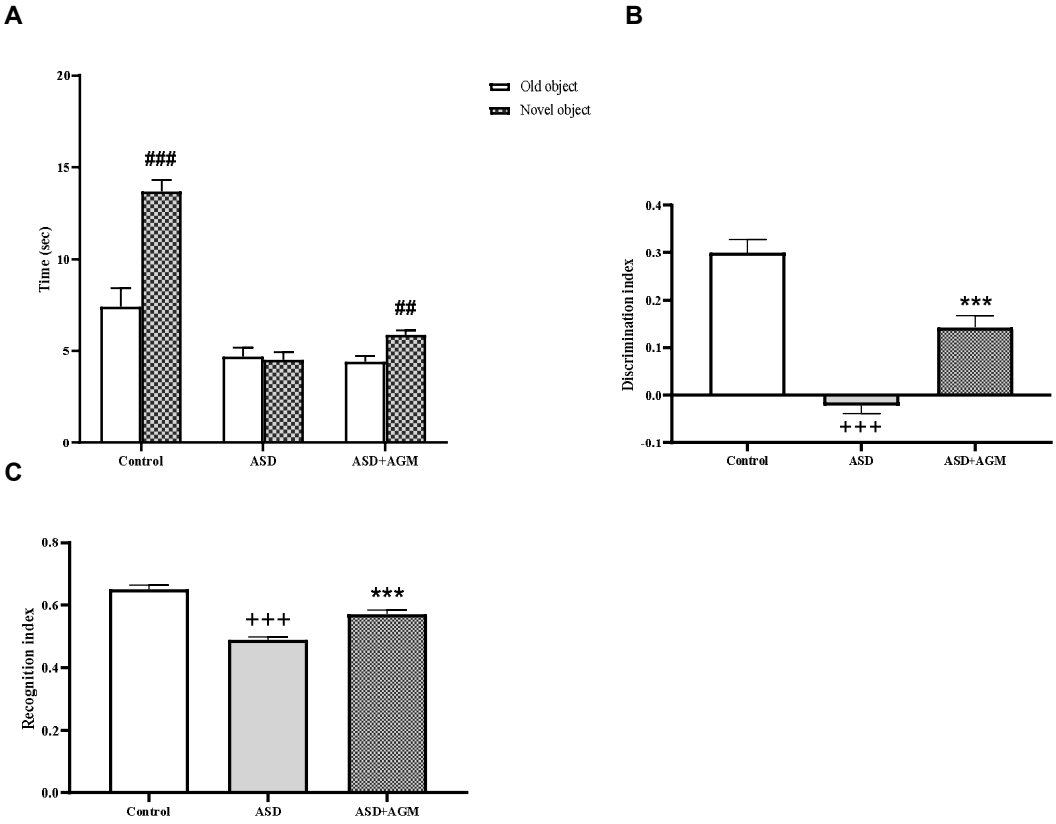
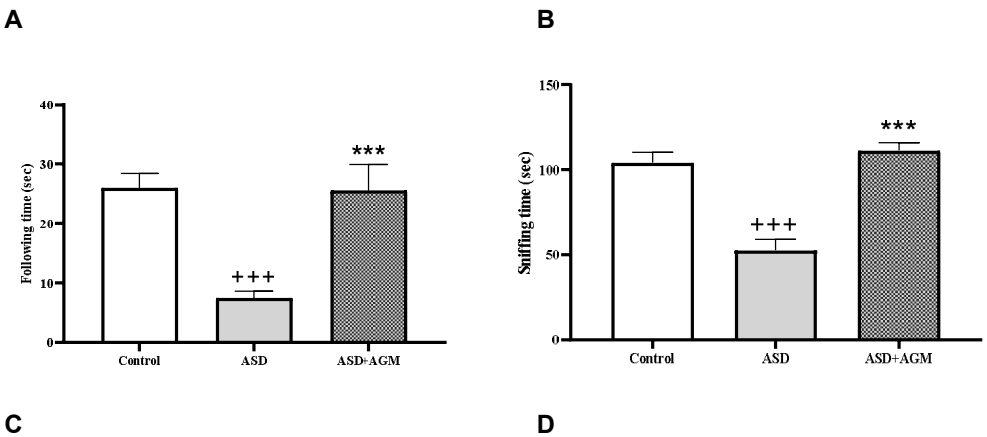


Figure 2. The time spent (A), discrimination index (B) and recognition index (C) in NORT. Data are shown as mean ± SEM. The time spent with old and novel objects was compared within each group. Statistical analysis was performed with two-factor ANOVA (A) and one-way ANOVA (B,C), Post-hoc Šídák test was applied (A) and, Post-hoc Tukey test was applied (B,C) (n=12/groups). ##p<0.01 and ###p<0.001; Compared to the old object, +++p<0.001; compared to the control group, ***p<0.001; compared to the ASD group. Autism Spectrum Disorder; ASD, agmatine; AGM.

In the social interaction test following ($p<0.001$), sniffing ($p<0.001$), and climbing ($p<0.05$) behaviors were significantly lower in ASD group compared to control and AGM treatment were able to significantly reverse these effects ($p<0.001$) while avoidace time was significantly higher in ASD group ($p<0.01$) and treatment with AGM significantly reversed this effect ($p<0.01$)(Figure 3 A,B,C,D).



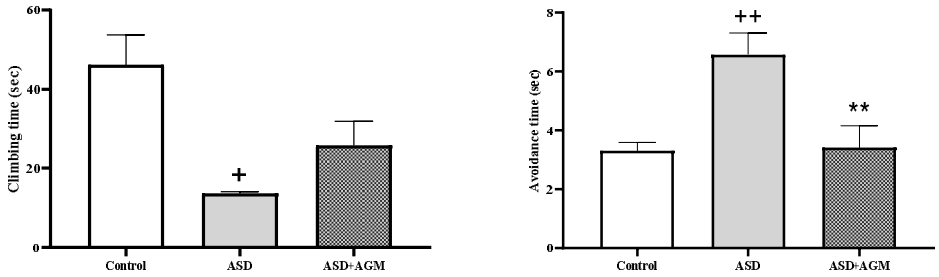


Figure 3. Following time (A), sniffing time (B), climbing time (C) and avoidance time (D) of groups in the social interaction test. Data are shown as mean ± SEM. Statistical analysis was performed by one-way ANOVA, Post-hoc Tukey test was applied (n=12/groups). + p<0.05, ++p<0.01 and +++ p<0.001; Compared to the control group, **p<0.01 and ***p<0.001; compared to ASD group. Autism Spectrum Disorder; ASD, agmatine; AGM.

In comparison with the control group in the ASD group sociability index (p<0.001) and sociability preference (p<0.05) were significantly lower in the TCT. AGM treatment significantly increased sociability but not in preference alleviated (p<0.05) significantly decreased sociability preference index but not in sociability index (Figure 4 A,B).

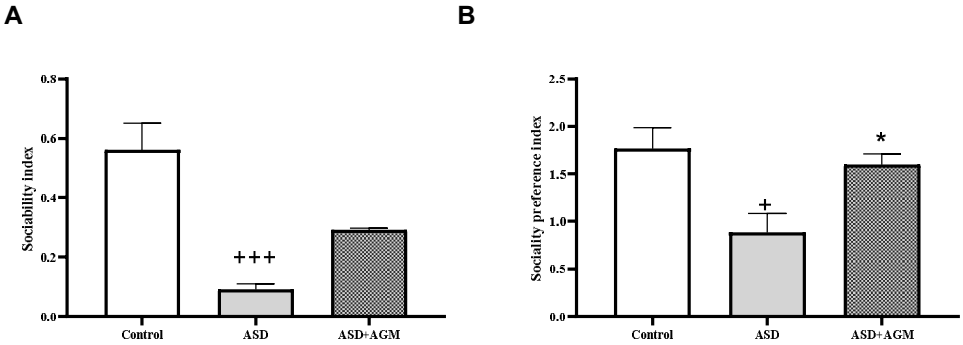
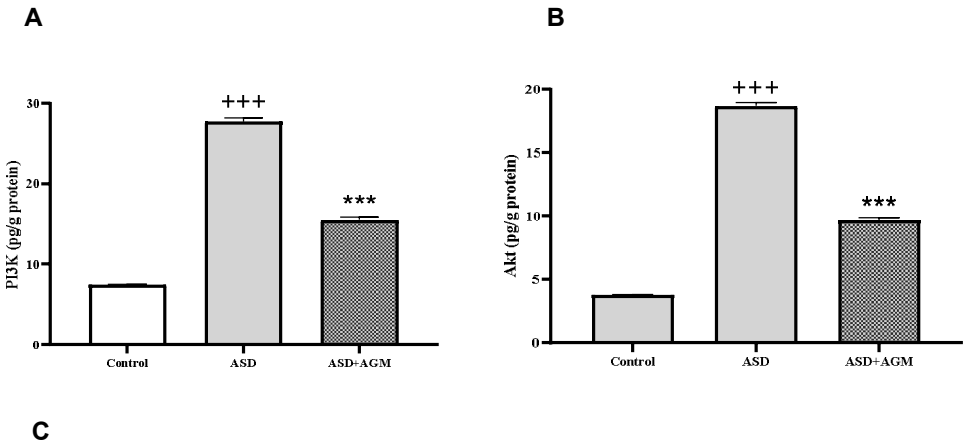


Figure 4. Sociability index (A) and sociality preference index (B) of groups in TCT. Data are shown as mean ± SEM. Statistical analysis was done with one-way ANOVA, Post-hoc Tukey test was applied (n=12/groups). +p<0.05 and +++p<0.001; Compared to the control group, *p<0.05; compared to the ASD group. Autism Spectrum Disorder; ASD, agmatine; AGM.

In prefrontal cortex regions mRNA expressions of PI3K, Akt, mTOR were found significantly increased in ASD group (p<0.001) compared to control group. AGM treatment significantly reduced all three parameters (p<0.001) (Figure 5 A,B,C).



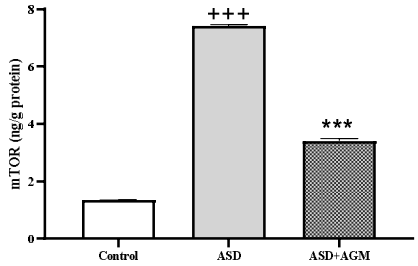


Figure 5. PI3K (A), Akt (B) and mTOR (C) relative mRNA expression. Data are shown as mean ± SEM. Statistical analysis was done with one-way ANOVA, Post-hoc Tukey test was applied (n=12/groups). +++p<0.001; Compared to the control group, ***p<0.001; compared to the ASD group. Autism Spectrum Disorder; ASD, agmatine; AGM.

Discussion:

It has been reported that the incidence of hyperactivity in patients with ASD is higher than in the normal population [4]. In our study, the total locomotor activity of the ASD group was found to be increased compared to the control group, in line with the literature [1]. Repetitive and stereotyped behaviors, which are considered as the main symptoms of ASD, are evaluated by the increase in the self-cleaning behavior called grooming in the animal model and its continuation as a stereotyped session. In our study, similar to the literature [2], it was observed that grooming behaviours increased significantly in the ASD group compared to the control group. AGM treatment reduced stereotypical and repetitive caregiving behaviors to control levels. This study is the first finding of the AGM effect in the experimental ASD model.

Early symptoms of ASD include also atypical visual exploration and cognitive rigidity [2]. Most studies in the literature report that animals in the ASD group tend to spend time with the old object rather than recognizing the novel object, which is an indicator of cognitive rigidity [1]. In our study, similar to the literature, ASD group spent more time with the old object compared to the control group, and the discrimination index and recognition index were found to be significantly lower than the control group. While there is no study in the literature showing the effect of AGM treatment, our study was revealed that animals in the AGM treatment group spent statistically significantly more time with the novel object, and the discrimination index and recognition index decreased significantly in ASD.

Limitation in social interaction, difficulty in understanding and maintaining relationships and emotional limitation are among the most frequently used symptoms in the early diagnosis of ASD [2]. Social interaction behavior in the ASD animal model is evaluated using the social interaction test and TCT. In the social interaction test, sniffing, following, and climbing on the other animal are considered signs of social interaction, while avoidance behavior is considered a sign of social withdrawal. In our study sniffing and following behavior found to be significantly lower than the control group in ASD; AGM treatment significantly improved these behaviors. Also, while avoidance time increased in the ASD compared to the control group, AGM treatment reduced this behavior to a significantly. In this regard, the data obtained in our study is in accordance with a single study in literature [3].

Studies show that in TCT, social interaction is impaired in the ASD model and animals tend to spend time with the familiar animals rather than the unfamiliar ones [1]. In our study, sociability index and sociability preference index were found to be significantly decreased in the ASD group compared to the control group and AGM treatment was increased these indices. Our results consistent with the previous study with AGM were obtained [3].

In a study conducted in patients with idiopathic ASD, increased PI3K/Akt/mTOR intracellular signaling pathway activity was reported compared to the healty controls [5]. Studies show that in the ASD model established in rodents, PI3K/Akt/mTOR pathway activity is increased in the prefrontal cortex of rats with ASD [6]. Our results were shown that PI3K, Akt and mTOR expressions were significantly increased in the ASD model group compared to the control group, and this increase was statistically significantly improved by AGM treatment.

In the light of the findings of the study, it was thought that the PI3K/Akt/mTOR pathway may have an important role in the neurobiology of ASD. It has been evaluated that molecules effective on AGM synthesis and metabolism may provide new treatment approaches in the future, based on the fact that the treatment performed due to the external application of AGM, an endogenous molecule, improves the behavioral and molecular parameters of ASD.

Keywords: Autism, agmatine, PI3K/Akt/mTOR pathway, valproic acid

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