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Investigating Serum miRNA Levels in Autistic Children with and without Bipolar Disorder: A Case-Control Study

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ABSTRACT

Introduction: The presence of bipolar disorder (BD) in individuals with ASD exacerbates social and cognitive impairments, complicates diagnosis and treatment. The aim of this study was to compare the serum levels of specific miRNAs between children with ASD with and without BD, and to explore their association with BD comorbidity in ASD.

Method: A group of 41 pediatric patients with comorbid ASD + BD and a group of 47 pediatric patients with ASD without BD were included in the study. Serum miRNA levels were measured using quantitative real-time polymerase chain reactions, focusing specifically on miRNAs such as miR-132-5p, miR-134-5p, miR-206, and miR-126-5p.

Results: The ASD + BD group exhibited significantly greater ASD severity than the ASD without BD group. Serum levels of miR-134-5p, miR-206, and miR-126-5p were all higher in the ASD + BD group than in the ASD without BD group (p=0.038, p=0.023, and p=0.026, respectively), while miR-132-5p levels were not significantly different (p=0.105). Furthermore, miR-134-5p levels were correlated with the frequency of manic episodes in the ASD + BD group (p=0.007).

Conclusion: The study findings suggest that miRNAs may be involved in the pathophysiology of comorbid BD in children with ASD and may potentially represent candidate biomarkers for early diagnosis and intervention, thus contributing to improved clinical outcomes in this population. Further research is now needed to validate these findings and to understand the underlying mechanisms.

Keywords: Autism, bipolar disorders, miRNA, BDNF, VEGF.

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INTRODUCTION

Bipolar disorder (BD) is a clinically important psychiatric comorbidity in children and adolescents with autism spectrum disorder (ASD), as it may complicate diagnostic assessment, clinical course, and treatment planning (1)**.** The prevalence of comorbid BD in children with ASD has been estimated at 5–7% in recent publications (1–3). Since the prevalence of BD in the neurotypical pediatric population is estimated at 1.8–3.8%, it appears that BD is more common in patients with autism spectrum disorders than in the general population (4). Genetic heritability plays a significant role in both disorders, although specific genetic factors have only been identified in a small number of cases (5,6). Epigenetics has recently emerged as a potential mechanism by which the accumulation of these gene variants, in combination with specific environmental stimuli, may lead to the onset of the disorder (7). MicroRNAs (miRNAs) have recently been investigated as one of the epigenetic mechanisms in psychiatric disorders.

Highlights

- **Serum miR-126-5p, miR-134-5p, and miR-206 levels were elevated in ASD+ BD group.**
- **miR-134-5p correlated with manic episodes, suggesting link to BD severity in ASD.**
- **Co-occurrence of BD and ASD was linked to more severe ASD symptoms.**

miRNAs are short non-coding RNAs of approximately 18–24 nucleotides involved in various cellular processes, such as proliferation, differentiation, growth control, homeostasis, and apoptosis (8). Research has shown that certain miRNAs in peripheral blood correlate with miRNA levels in cerebrospinal fluid (CSF), suggesting that peripheral miRNAs may provide valuable insights into central nervous

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system (CNS) processes (9). This relationship may be explained by the ability of miRNAs to cross the blood-brain barrier, enabling dynamic communication between peripheral and central systems. These correlations suggest that peripheral miRNAs could serve as a window into the molecular changes occurring within the CNS, providing insights into both disease mechanisms and progression. This relationship is critical given the challenges associated with directly sampling CSF, which is an invasive and resource-intensive process. From this perspective, peripheral miRNA studies have gained increasing interest in major psychiatric conditions.

In BD, altered miRNA expression has been reported in both postmortem brain tissue and peripheral blood, suggesting that miRNA dysregulation may be relevant to disease mechanisms and biomarker research (10). Genetic studies have further indicated that several miRNA-related genes are located within BD susceptibility loci (11). Although a wide range of candidate miRNAs has been investigated, only a small number—most notably miR-34a and miR-137—have shown relatively consistent replication across independent studies. Moreover, mood-stabilizing treatments such as lithium and valproate have been reported to modulate miRNA profiles, with some changes paralleling clinical improvement (12). In ASD, the evidence base is comparatively smaller. A recent review highlighted significant alterations in several miRNAs (e.g., miR-28, miR-151, miR-148, and miR-125) in ASD compared with healthy controls (13). Experimental data also suggest links between miRNAs and ASD-relevant pathways: suppression of miR-132 has been associated with increased MECP2 expression and repetitive behaviors, and inhibition of miR-137 has been linked to increased SHANK2 expression in animal models (14,15). Further investigation of the role of miRNAs in psychiatric disorders may improve our understanding of biological correlates and treatment-related changes in psychiatric disorders (11).

Although BD and ASD are known to share a number of genetic links and to co-occur at higher rates than in the general population, few studies have investigated the biological mechanisms underlying BD comorbidity in individuals with ASD (16,17). Even fewer have examined the genetic mechanisms in children with ASD and BD comorbidity. While miRNA research is more established for ASD and BD as separate conditions, less is known about miRNA profiles in patients with comorbid ASD and BD (11,13). This study was intended to fill this gap by investigating serum miRNA levels in children with comorbid ASD and BD and comparing these with children with ASD without BD, and to evaluate their correlations with clinical manifestations of BD.

Recent studies involving similar patient groups have shown reduced levels of brain-derived neurotrophic factor (BDNF) and increased levels of vascular endothelial growth factor (VEGF) in ASD patients with BD compared to those without BD (16,17). Experimental and clinical data indicate that neurotrophins can modulate miRNA expression through intracellular signaling molecules, suggesting the presence of integrated positive and negative regulatory loops between miRNAs and neurotrophic factors (18). In the present study, we took these previously identified parameters a step further by selecting miRNAs associated with VEGF and BDNF to investigate whether they would exhibit similar differences. A literature search was therefore performed on PubMed using the terms 'micro-RNA,' 'Brain Derived Neurotrophic Factor,' and 'Vascular Endothelial Growth Factor,' along with their common abbreviations. In this context, miR-132 has been associated with adult neural stem cell neurogenesis and synaptic plasticity, with evidence that it can bind to CREB and increase BDNF levels, while BDNF-related MAPK/ERK1/2 activation may also upregulate miR-132 expression (18). miR-134 is a brain-enriched miRNA implicated in dendritic spine maturation and synaptic development; it can suppress Limk1 and negatively regulate CREB/BDNF gene expression, whereas exposure to BDNF appears to attenuate the inhibitory effect of miR-134 on Limk1 (19). miR-206 has also been shown to repress BDNF expression; moreover, a large clinical study reported an interaction between miR-206 and BDNF polymorphisms associated with BD susceptibility and treatment response (20). miR-126-5p, an endothelial miRNA repeatedly linked to VEGF-related pathways and angiogenic/neurovascular mechanisms, was selected (21). The aim of the present research was to compare serum levels of specific miRNAs between children with ASD with and without BD and to explore their association with BD comorbidity in ASD. The study hypothesis was that serum miRNA levels would be higher in children with ASD+BD compared to those with ASD without BD.

METHODS

Study design and participants

This study was conducted at the Child and Adolescent Psychiatry Outpatient Clinic of Istanbul University between November 2023 and April 2024. Participants were recruited via voluntary response sampling among patients undergoing regular follow-ups in the outpatient clinic. All participants and their parents were informed about the study. Written informed consent was obtained from the parents.

The study was designed with two groups: an ASD+BD group consisting of pediatric patients with ASD comorbid BD, and an ASD without BD group consisting of patients with ASD without BD comorbidity. The following exclusion criteria were applied for both groups, since these might affect serum miRNA levels: the presence of specific metabolic, systemic, genetic, and/or progressive neurologic conditions, a history of infection in the previous month, or a history of nutritional supplement/vitamin use in the preceding month. Patients in the ASD+BD group who were not euthymic at the time of blood collection were excluded. Euthymia was determined using the mood modules of the K-SADS-PL and consensus clinical evaluation by child and adolescent psychiatrists. Blood samples were obtained at least one month after the last manic/hypomanic or depressive episode and only when no syndromal mood symptoms were present.

Sample size estimation was informed by the limited bipolar miRNA literature. Among the four candidate miRNAs, miR-134-5p was selected as the reference target for sample size planning because it has relatively strong support in the bipolar miRNA literature and has been repeatedly investigated in peripheral blood samples in independent cohorts (22,23). The study by Tekdemir et al., which evaluated serum miR-134-5p expression in patients with bipolar disorder and healthy controls, was used as the primary empirical data source for sample size estimation. Specifically, the reported serum miR-134-5p values in that study were used to inform the expected magnitude and direction of group differences in the present study (23). A power analysis was conducted using G*Power (version 3.1) for a two-tailed Mann-Whitney U test, assuming α=0.05, power (1–β)=0.80, an allocation ratio of 1:1, and Cohen's d=0.65 as an assumed moderate planning value (24,25). Under these assumptions, the minimum required sample size was 80 participants (40 per group). Forty-one patients with comorbid ASD and BD and 47 with ASD without BD were enrolled in the study. Figure 1 shows the flowchart.

The study was approved by the Istanbul University Clinical Research Ethics Committee on November 3, 2023, at meeting number 22. The project was supported by a grant from the Istanbul University under project code TTU-2024-40621.

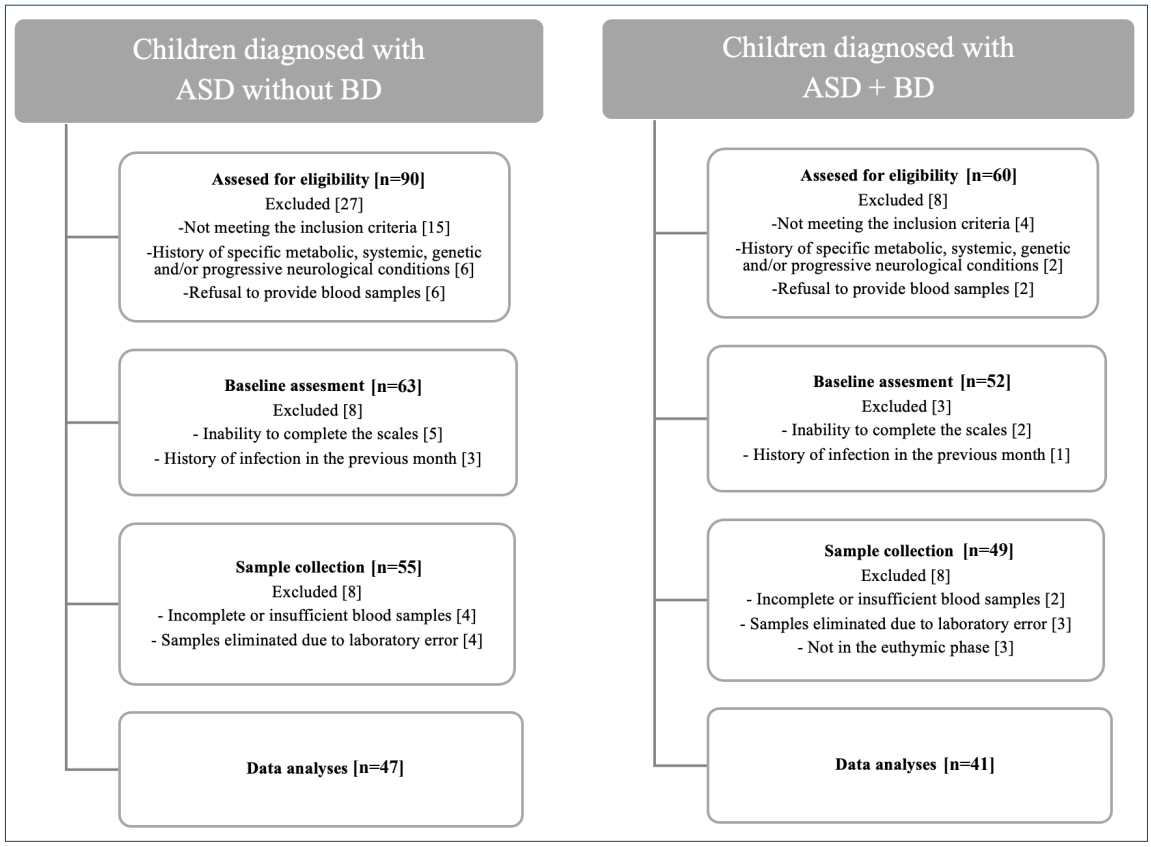

Figure 1. Flowchart diagram for sample selection.

Assessments

Both ASD and BD were diagnosed based on Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) criteria. The participants were given a survey form consisting of questions intended to elicit sociodemographic and clinical data. Psychiatric diagnoses for all participants were assessed using the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL). Assessments confirmed that the ASD without BD group did not meet the diagnostic criteria for any mood disorder throughout life. The Childhood Autism Rating Scale (CARS) was used to evaluate the severity of ASD symptoms in both groups. Behavioral problems were assessed by means of the parent-reported Aberrant Behavior Checklist (ABC).

Sociodemographic Data Form

The researchers designed a data form to collect information concerning the sociodemographic characteristics of all the patients in the study. This form included individual characteristics such as sociodemographic background, developmental history, the patients' medical records, and the families' demographic characteristics. Phenomenological features of BD such as age at onset and diagnosis, duration of illness, and frequency of episodes were also examined in the ASD+BD group.

Schedule for Affective Disorders and Schizophrenia Interview Chart for School-Age Children Now and Lifetime Style (K-SADS-PL)

K-SADS-PL is a semi-structured interview form widely used to screen for lifetime and current psychopathology in children and adolescents based on DSM-5 criteria (26). The presence and severity of symptoms are assessed by integrating the perspectives of the child/adolescent, parents and clinician. The Turkish version validity and reliability of the scale were investigated by Unal et al. (27).

The Childhood Autism Rating Scale (CARS)

Developed by Schopler et al. (28), CARS is a behavioral rating scale used to differentiate children with ASD from those with other developmental disorders. It is based on clinician observations of the child and information provided by the family. Childhood autism rating scale assesses 15 areas in which impairments associated with autism may be expected. This scale serves as a diagnostic assessment tool, rating individuals on a spectrum from normal to severe, and providing a composite score ranging from non-autistic to mildly, moderately, or severely autistic. The Turkish-language adaptation of the scale was conducted by Incekas et al. (29).

Aberrant Behavior Checklist (ABC)

The ABC, validated in ASD by Kaat et al. (30), was designed to measure psychiatric symptoms and behavioral problems in school-aged children. It consists of five domains: hyperactivity, inertia, repetitive behaviors, damaging behaviors, and other behaviors. The original English version of the scale consists of 58 items, and the Turkish version of 46. The ABC is scored by parents or teachers using a four-point Likert-type scale. The validity and reliability study of the Turkish-language version was conducted by Karabekiroglu et al. in 2009 (31).

Sampling and Evaluation of miRNA expressions

Venous blood samples were collected from patients at the Department of Child and Adolescent Mental Health and Diseases Clinic between December 2023 and January 2024. Samples were transferred to the Department of Biochemistry within fifteen minutes of collection; centrifuged, aliquoted and stored at -80°C until further processed. Total RNA was purified from samples using miRNeasy Serum/Plasma Advanced Kit (Qiagen, USA, Cat no 217204) following manufacturer's instruction. cDNA was synthesized using miRCURY TM LNA RT Kit; (Qiagen, USA, Cat no 339346). miR-132-5p, miR-134-5p, miR-126-5p and miR-206 expressions were quantified on a Qiagen Rotor-Gene Q quantitative real time polymerase chain reaction (qPCR) device. Primers used in the study

were obtained from Qiagen Technologies (USA). The primer sequences were as follows: miRCURY LNA™ miRNA PCR Assay U6 (Qiagen, USA, Cat no YP02119464); miRCURY LNA™ miRNA PCR Assay miR-132-5p (Qiagen, USA, Cat no YP00204552]; miRCURY LNA™ miRNA PCR Assay miR-134-5p, (Qiagen, USA, Cat no YP00205989); miRCURY LNA™ miRNA PCR Assay miR-126-5p, (Qiagen, USA, Cat no YP00206010); miRCURY LNA™ miRNA PCR Assay miR-206, (Qiagen, USA, Cat no YP00206073). Expression levels were calculated using 2−ΔΔCT method and the relative expression of each gene was normalized to that of U6. The selection of U6 as an endogenous control was based on the published literature (32).

Statistical analysis

All analyses were performed on IBM Statistical Package for Social Sciences (SPSS) software program version 26.0. Descriptive statistics were reported as mean ± standard deviation for continuous variables and median with 25 th-75th percentiles and frequency distributions for categorical variables. Pearson's Chi-square test (χ^2) and Fisher's Exact test were applied to assess relationships between categorical variables. The Shapiro-Wilk test was used to assess the normality of distribution of miRNA levels. Since all miRNA levels were non-normally distributed, the Mann-Whitney U test was applied. p values <0.05 were considered statistically significant.

RESULTS

Forty-one pediatric patients with comorbid ASD and BD (ASD+BD group, mean age=13.5±2.9 years) and 47 with ASD without BD comorbidity (ASD without BD group, mean age=13.2±3.2 years) were enrolled in the study. Both groups contained four girls, the remaining participants being boys. A higher prevalence of BD and schizophrenia was determined in the relatives of the ASD+BD group, but there was no difference in terms of anxiety and depressive disorders. A maternal history of depression was more common in the ASD+BD group (p=0.012). The CARS and ABC scores of the ASD+BD group were higher than those of the ASD without BD group (p=0.004 and p=0.008, respectively). According to the KSADS assessments, all patients except one in the ASD without BD group had at least one comorbidity accompanying ASD. Attention deficit hyperactivity disorder was the most common comorbidity in both groups (p=0.276). Oppositional defiant disorder and tic disorder were more common in the ASD+BD group than in the ASD without BD group (p=0.018 and p=0.001, respectively). While all patients in the ASD+BD group received psychiatric treatment (73% were using more than one psychopharmacological agent), 15% of those in the ASD without BD group were followed-up without treatment. In the ASD+BD group, all patients were receiving antipsychotic treatment, whereas 56.5% of the ASD without BD group were on antipsychotic medication. Antidepressants and mood stabilizers (predominantly valproate and, less frequently, lithium) were more common in the ASD+BD group, whereas only a small number of patients in the ASD without BD group received a mood stabilizer. Table 1 and Table 2 show the participants' sociodemographic and clinical characteristics.

Bipolar I disorder was diagnosed in 63.4% of the ASD+BD group and bipolar II in 34.1%. Mean age at onset was 10.58±2.62 years. The mean time since the last mood episode was 5.64±7.17 months. Sixty-one percent of the patients in the ASD+BD group exhibited a stable course between attacks, the others manifesting subsyndromal symptoms in the inter-attack period. Distractibility, increased activity, and a decreased need for sleep were the most common symptoms and signs other than mood change and increased energy (92.7%, 87.8% and 85.4%,

Table 1. The study groups' demographic and clinical characteristics

Variable	ASD+BD [n=41]	ASD w/o BD [n=47]	p value	t / z value
Age, years, mean ± SD	13.5±2.9	13.2±3.2	0.646[a]	-0.461
BMI [kg/m²], mean ± SD	25.05±5.0	23.05±6.8	**0.026[b]**	-2.22
Family history of BD [n, %]	36.6% (15)	2.1% (1)	**<0.001[c]**	17.478
Family history of schizophrenia [n, %]	29.3% (12)	8.5% (4)	**0.004[c]**	8.111
Perinatal maternal depression [n, %]	29.3% (12)	8.5% (4)	**0.012[c]**	6.343
CARS-total score, mean ± SD	42.37±6.36	38.04±7.13	**0.004[a]**	-2.990
ABC-total score, mean ± SD	74.63±31.0	59.70±27.1	**0.008[b]**	-2.639
Multiple psychiatric drug use [n, %]	73.2% (30)	27.7% (13)	**<0.001[a]**	14.438

SD: standard deviation; BMI: body mass index; ASD: autism; BD: bipolar disorder; CARS: childhood autism rating scale; ABC: aberrant behavior checklist; w/o: without; [a]: Student t-test; [b]: Mann Whitney-U test; [c]: Pearson Chi-square test; [d]: Fisher's exact test; p <0.05 shown in bold.

Table 2. Distributions of psychiatric diagnoses in the study groups

Psychiatric diagnoses	ASD+BD [n=41]	ASD w/o BD [n=47]	p value	z/t value
Psychosis	2.4% (1)	0%	0.466[b]	
Anxiety	43.9% (18)	27.7% (13)	0.112[a]	2.532
OCD	31.7% (13)	27.7% (13)	0.678[a]	0.172
ADHD	95.1% (39)	87.2% (41)	0.276[b]	
ODD	24.4% (10)	6.4% (3)	**0.018[a]**	5.640
Enuresis	24.4% (10)	8.5% (4)	0.042[a]	4.127
Encopresis	17.1% (7)	8.5% (4)	0.226[a]	1.468
Tic disorder	29.3% (12)	4.3% (2)	**0.001[a]**	10.241
PTSD	2.4% (1)	0%	0.466[b]	

[a]: chi-square test; [b]: Fisher's exact test; ASD: autism spectrum disorder; BD: bipolar disorder; OCD: obsessive compulsive disorder; ADHD: attention deficit hyperactivity disorder; ODD: oppositional defiant disorder; PTSD: post traumatic stress disorder; w/o: without; p <0.05 shown in bold.

Table 3. Clinical characteristics of the ASD+BD group

Variable	Values
BD-1[n, %]	63.4%
BD-2[n, %]	34.1%
Age at onset [years] mean ± SD	10.58±2.62
Attack frequency, episodes/year, mean±SD	1.55±0.87
Total number of manic/hypomanic episodes, mean ± SD	2.60±1.80
Total number of depressive episodes, mean ± SD	2.20±2.16
No full recovery between episodes (n, %)	37.5%

SD: standard deviation; ASD: autism spectrum disorder; BD: bipolar disorder.

Table 4. The relative expression levels of miR-132-5p, miR-134-5p, miR-126-5p, and miR-206 in study groups

Variable	ASD+BD [n=41]	ASD w/o BD [n=47]	Mann-Whitney U-Test	
			p value	z value
miR-126-5p	49.31±24.5	37.39±12.31	**0.026**	-2.223
miR-132-5p	42.03±13.28	33.86±4.54	0.105	-1.622
miR-134-5p	45.44±9.63	34.70±4.72	**0.038**	-2.079
miR-206	48.41±9.09	36.32±4.54	**0.023**	-2.281

p <0.05 shown in bold; ASD: Autism spectrum disorder; BD: Bipolar disorder; w/o: without.

Table 5. Correlation analysis between clinical characteristics and miRNA Levels in ASD+BD group

Clinical characteristics	miR-132-5p		miR-134-5p		miR-206		miR-126-5p	
	p value	r	p value	r	p value	r	p value	r
Age at onset	0.787	-0.046	0.798	0.043	0.658	-0.046	0.970	0.006
Total Number of depressive episodes	0.788	-0.046	0.521	0.107	0.768	0.049	0.733	0.057
Total number of hypomanic/manic episodes	0.079	-0.292	**0.007**	-0.432	0.186	-0.219	0.572	-0.093
CARS total score	0.124	0.179	0.372	0.102	0.521	0.072	0.863	0.019
ABC total score	0.233	0.139	0.168	0.157	0.498	0.075	0.639	0.052

p <0.05 shown in bold; r indicates Spearman's rho; ASD: autism spectrum disorder; BD: bipolar disorder; CARS: childhood autism rating scale; ABC: aberrant behavior checklist; r indicates Spearman's rho.

respectively). The least common symptoms and signs were exhibiting activities with negative consequences and flight of thought (31.7% and 29.3%, respectively). Episodes triggered by seasonal cycles were detected in 63.4% of the patients. While 19.5% of the patients described a psychosocial stress factor before the first episode, 51.2% had a history of episode exacerbation with psychopharmacological agents. The clinical features of the ASD+BD group are presented in Table 3.

Levels of miR-132-5p, miR-134-5p, miR-206, and miR-126-5p were compared between the study groups. This selection was based on previous studies highlighting variations in BDNF and VEGF levels in individuals with ASD+BD comorbidity compared to those with ASD alone. By selecting these miRNAs — known for their association with the BDNF and VEGF pathways — we aimed to further investigate these specific molecular differences. miR-134-5p, miR-206, and miR-126-5p levels were all significantly higher in the group than in the ASD without BD group (p=0.038 z=-2.07, p=0.023 z=-2.281, and p=0.026 z=-2.223, respectively), while miR-132-5p levels were similar between the groups. The examined miRNAs showed high correlations with each other (miR-126-5p – miR-132-5p p<0.001; miR-126-5p – miR-134-5p p<0.001; miR-126-5p – miR-206 p<0.001; miR-132-5p – miR-134-5p p=0.006; miR-132-5p – miR-206 p=0.004; miR-134-5p - miR-206 p=0.002) (Table 4).

No significant correlations were observed for any of the miRNAs with age (miR-126-5p p=0.378; miR-132-5p p=0.134; miR-134-5p p=0.422; miR-206 p=0.310), suggesting that age did not have a measurable impact on their expression levels in this study population. Comparisons between miRNA levels and BD types in the ASD+BD group revealed lower miR-132-5p and miR-134-5p levels in patients with BD type 1 compared to those with bipolar type 2 (p=0.014, z=-2.452 and p=0.005, z=-2.795, respectively). However, no similar differences were observed between serum miR-206 and miR-126-5p levels and bipolar types (p=0.476, p=0.355, and p=0.824, respectively). An analysis of clinical features showed that miR-134-5p levels were negatively correlated with the total number of manic episodes (p=0.007, r=-0.432). No other significant correlations were identified between miRNA levels and clinical parameters. The relationships between clinical features and miRNA levels are shown in Table 5.

DISCUSSION

Recent years have witnessed a growing understanding that changes in miRNA expression may contribute to the pathogenesis of psychiatric disorders and be capable of acting as peripheral biomarkers of the disease and markers of therapeutic responses (10,11). While numerous studies have separately investigated the role of miRNAs in ASD and BD, there has been a notable absence of research into miRNA levels in patients with ASD and comorbid BD (11,13). In order to investigate whether altered miRNA expression is associated with the presence of ASD with comorbid BD, the present research focused on miRNAs related to BDNF and VEGF, since previous studies have reported significant differences in these neurotrophic factors in patients with ASD with comorbid BD (16,17). Specifically, miR-132-5p, miR-134-5p, miR-206, and miR-126-5p were examined due to their previously reported associations with BDNF and VEGF. The results suggested that miR-134-5p, miR-206, and miR-126-5p levels were associated with BD comorbidity in pediatric patients with ASD.

miR-134-5p, known for its important role in synaptic development and plasticity, is often investigated in studies of BD and schizophrenia (11). miR-134-5p regulates dendritic structure by inhibiting LIM domain kinase (Limk1) synthesis, which adversely affects dendritic spine growth. The suppressive effect of miR-134-5p on Limk1 is reduced in neurons exposed to BDNF, resulting in increased dendritic spine growth (19). miR-134-5p also transcriptionally down-regulates CREB expression, thereby inhibiting BDNF gene expression (33). Brain-derived neurotrophic factor (BDNF) is crucial for neurotransmitter modulation, synaptogenesis, and neuroplasticity, and abnormalities in BDNF expression, release, and activity have been implicated in neurological and psychiatric disorders (34). Numerous studies have reported that low BDNF levels correlate with response to treatment, age at onset, and severity of episodes

(35,36). Despite decreased BDNF levels observed in BD, a recent meta-analysis reported increased BDNF levels in children with ASD compared to neurotypical controls (37). Consistent with that finding, Yıldız Bayındır et al. (17), also reported decreased BDNF levels in patients with both ASD and BD compared to those without BD. In the present study miR-134-5p levels were higher in the BD group with ASD than in the ASD without BD group, consistent with previous findings associating low BDNF levels with high miR-134-5p levels and BD. In contrast, studies involving the general population have reported that miR-134-5p levels decrease during manic episodes of BD and unipolar depression and increase after treatment (22). The relationship between miR-134-5p and BDNF appears to be complex and inconsistent across studies, potentially varying by clinical state, developmental stage, and sample characteristics. The observed increase in serum miR-134-5p levels is thought to be due to decreased levels of BDNF, which exerts opposite effects to those of miR-134-5p in dendritic development. The results of the present study suggest that different mechanisms may be involved in BD comorbidity in patients with ASD compared to the neurotypical population.

miR-206, another key element in this complex network, is highly expressed in muscle tissue and plays a critical role in muscle development and remodeling (38). It regulates the synthesis of BDNF and several other proteins, including epidermal growth factor receptor (EGFR), histone deacetylase 4 (HDAC4), and cyclin D1. In their study of 288 patients with BD, Wang et al. (20) identified an interaction between miR-206 and BDNF polymorphism associated with susceptibility to BD and response to treatment. In line with these findings, elevated levels of miR-206 were also observed in the BD comorbidity group in the present study. Considered together, the data for miR-134-5p and miR-206 highlight the complex interactions between these microRNAs and BDNF in the pathogenesis of BD, particularly in patients with ASD. These interactions highlight the need for further research in order to understand the different mechanisms involved in BD comorbidity in patients with ASD compared to the neurotypical population. Understanding these mechanisms may provide further insights into targeted therapeutic strategies for managing BD in the context of ASD.

Studies have reported increased levels of miR-132-5p in unmedicated relatives of BD patients, in postmortem brain tissue from patients with BD and schizophrenia, and in peripheral blood from patients with schizophrenia (39). Li et al. (40) investigated the relationship between BDNF and miR-132-5p levels in patients with depression and found that BDNF levels decreased while miR-132-5p and miR-182 levels increased in patients compared to healthy controls. They also reported that administration of miR-132-5p resulted in a decrease in BDNF levels. Nevertheless, there are inconsistent results in the literature regarding the relationship between BDNF and miR-132-5p. Some experimental studies have reported that miR-132-5p increases BDNF levels via CREB, while others have shown a decrease in BDNF levels with miR-132-5p administration (12,18). Some studies have also suggested that BDNF may be related to a decrease in miR-132-5p levels through feedback mechanisms involving MAP/kinase and CREB (18). The discrepant findings in the literature regarding miR-132-5p may be due to the complex interactions between miRNAs and various biochemical parameters, as well as to their interactions with one another (41). No significant difference in miR-132-5p levels was observed between the study groups in the present study. Some other studies investigating miR-132-5p in patients with BD have also reported either a significant decrease or no difference compared to healthy controls (11). We attribute the unclear correlations between miR-132-5p and BDNF to compensatory mechanisms that develop as a result of psychopathology.

Studies have shown that miR-126-5p, which is specifically expressed in endothelial cells and hematopoietic progenitor cells, is associated with angiogenesis and VEGF expression (42). VEGF is known for its role in neuronal plasticity and neurogenesis, potentially affecting synaptic transmission (43). Elevated VEGF levels have been reported in patients with BD, particularly during depressive episodes (44). A study published in 2017 reported increased miR-126-5p and VEGF levels in patients with psychotic depression compared to healthy controls, and that these decreased to levels similar to those of healthy controls following electroconvulsive therapy (45). In the light of previous studies reporting high VEGF levels in BD patient groups and the correlation between miR-126-5p and VEGF, our study hypothesis was that higher miR-126-5p levels would be in the ASD+BD group compared to the ASD without BD group. Consistent with both our hypothesis and the previous literature, serum miR-126-5p levels were higher in the group with ASD and comorbid BD. Guldiken et al. (16) also reported higher levels of VEGF in cases with comorbid ASD and BD compared to controls. These results suggest that further studies with larger samples are now needed to clarify the roles of miR-126-5p and VEGF in the BD in patients with comorbid ASD, including their potential relevance to therapeutic interventions.

This study also investigated whether these miRNAs would correlate with the clinical features of BD. Analysis revealed that serum miRNA levels were not associated with clinical features such as age at first diagnosis, number of symptoms, duration of illness, mixed/psychotic features, and seasonal cycling in patients with BD. miR-134-5p levels, by contrast, were associated with the total number of manic/hypomanic episodes. Similarly, miR-132-5p and miR-134-5p levels were higher in patients diagnosed with BD II compared to those with BD I in our study sample. Our observation of higher miR-134-5p levels in BD II is incompatible with Rong et al. (22), who suggested that a decrease in miR-134-5p levels may be associated with the severity of mania. The finding of an increase in miR-134-5p levels both in line with the total number of manic/hypomanic episodes and in BD II is compatible with the results of studies reporting a higher total number of episodes in BD II compared to BD I (46). Further studies with larger samples and examining a greater number of miRNAs are now needed to verify the correlation between miRNA levels and clinical features of BD.

This study addresses the changing clinical features associated with the presence of BD in individuals with ASD. Previous research has suggested that the co-occurrence of BD and ASD may result not only in impairments characterized by mood disturbance, but also in impairments in core ASD symptoms (2,47). In agreement with previous research, the present study shows that children with comorbid BD are more likely to exhibit more severe symptoms of ASD (47). Our findings also indicated an increased prevalence of a history of self-injurious behavior and sleep difficulties within the comorbid BD group. The symptomatology of BD typically includes increased aggression, irritability, and persistent sleep disturbance even during euthymic periods. Individuals with early-onset BD and neurodevelopmental problems often exhibit subsyndromal symptoms between episodes (48). This may contribute to the increased frequency of these clinical features in the comorbid BD group. In addition, patients with comorbid BD have a greater overall burden of psychiatric disorders. Previous research has also shown a higher prevalence of comorbid psychiatric diagnoses in cases of ASD and BD, a reflection of the clinical complexity of this comorbidity (48,49). These clinical findings highlight the critical importance of accurate diagnosis of and early intervention in BD in the ASD population.

A number of limitations to this study need to be considered when interpreting the findings. First, the cross-sectional design of the study limits our ability to infer causality from the observed associations; therefore, all relationships between serum miRNA levels and clinical characteristics should be interpreted as correlations rather than evidence of direct causal effects. The members of the ASD+BD group were euthymic at the time of data collection, thus limiting insight into fluctuations in miRNA levels during manic and depressive episodes. Longitudinal studies

are now needed to elucidate the temporal relationships between miRNA expression patterns and the onset and progression of comorbid ASD and BD. Second, comorbidity is common in ASD, and individuals with ASD often present with multiple coexisting conditions. Although excluding participants with significant comorbidities might have enhanced the interpretability of our results, we cannot exclude these cases due to the nature of ASD, where such comorbidities are frequently observed. This inclusion is a limitation of our study, as it may introduce additional variability; at the same time, it reflects the real-world clinical complexity of ASD with BD comorbidity. Furthermore, differences in psychotropic medication exposure between groups may have influenced serum miRNA levels and should be considered a potential confounder. These treatment differences partly reflect the clinical reality of ASD patients with BD, in whom polypharmacy and long-term pharmacotherapy are often unavoidable; nevertheless, the lack of standardized medication regimens is an important limitation of our study. Third, the relatively small and clinically heterogeneous sample—including both BD I and BD II—may limit the generalizability of our findings to the broader population. Moreover, because effect sizes from BD-versus-control biomarker studies may be larger than those expected for within-ASD stratification (ASD+BD vs ASD without BD), the present study may be underpowered to detect smaller true effects; therefore, further studies with larger sample sizes are needed before clinical translation can be considered. Fourth, the use of peripheral blood samples may not fully capture miRNA expression patterns in the brain, where the pathophysiology of psychiatric disorders is primarily manifested. Future studies using postmortem brain tissue or neuroimaging techniques may provide further insight into the molecular mechanisms underlying comorbid ASD and BD. In addition, the lack of a neurotypical control group complicates broader comparisons of serum miRNA levels and BD-specific clinical features. These limitations highlight the need for more comprehensive studies, including healthy controls, to investigate the alterations in serum miRNA levels in individuals with comorbid ASD and BD.

As a result, this study yielded valuable insights into the biological basis of the comorbidity of ASD and BD by investigating the levels of specific miRNAs in affected individuals. The results indicate significant elevation of serum levels of miR-126-5p, miR-134-5p and miR-206 in patients with ASD and BD comorbidity compared to those with ASD alone. These results support the hypothesis that these miRNAs, particularly those associated with BDNF and VEGF, may be involved in the pathophysiology of BD in the context of ASD.

Ethics Committee Approval: The study was approved by the Istanbul University Clinical Research Ethics Committee on November 3, 2023, at meeting number 22.

Informed Consent: All participants and their parents were informed about the study. Written informed consent was obtained from the parents.

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REFERENCES

1. Rosen TE, Mazefsky CA, Vasa RA, Lerner MD. Co-occurring psychiatric conditions in autism spectrum disorder. Int Rev Psychiatry. 2018;30:40–61. [Crossref]
2. Skokauskas N, Frodl T. Overlap between autism spectrum disorder and bipolar affective disorder. Psychopathology. 2015;48:209–216. [Crossref]
3. Hossain MM, Khan N, Sultana A, Ma P, McKyer ELJ, Ahmed HU, et al. Prevalence of comorbid psychiatric disorders among people with autism spectrum disorder: an umbrella review of systematic reviews and meta-analyses. Psychiatry Res. 2020;287:112922. [Crossref]
4. Van Meter A, Moreira ALR, Youngstrom E. Updated meta-analysis of epidemiologic studies of pediatric bipolar disorder. J Clin Psychiatry. 2019;80:18r12180. [Crossref]
5. Ruderfer DM, Ripke S, McQuillin A, Boocock J, Stahl EA, Pavlides JMW, et al. Genomic dissection of bipolar disorder and schizophrenia, including 28 subphenotypes. Cell. 2018;173:1705–1715. [Crossref]
6. Chaste P, Leboyer M. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues Clin Neurosci. 2012;14:281–292. [Crossref]
7. Mahgoub M, Monteggia LM. Epigenetics and psychiatry. Neurotherapeutics. 2013;10:734–741. [Crossref]
8. Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell. 2006;11:441–450. [Crossref]
9. Burgos K, Malenica I, Metpally R, Courtright A, Rakela B, Beach T, et al. Profiles of extracellular miRNA in cerebrospinal fluid and serum from patients with Alzheimer's and Parkinson's diseases correlate with disease status and features of pathology. PLoS One. 2014;9:e94839. [Crossref]
10. Van den Berg MMJ, Krauskopf J, Ramaekers JG, Kleinjans JCS, Prickaerts J, Briedé JJ. Circulating microRNAs as potential biomarkers for psychiatric and neurodegenerative disorders. Prog Neurobiol. 2020;185:101732. [Crossref]
11. Fries GR, Carvalho AF, Quevedo J. The miRNome of bipolar disorder. J Affect Disord. 2018;233:110–116. [Crossref]
12. Zhou R, Yuan P, Wang Y, Hunsberger JG, Elkahloun A, Wei Y, et al. Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. Neuropsychopharmacology. 2009;34:1395–1405. [Crossref]
13. Li J, Xu X, Liu J, Zhang S, Tan X, Li Z, et al. Decoding microRNAs in autism spectrum disorder. Mol Ther Nucleic Acids. 2022;30:535–546. [Crossref]
14. Klein ME, Lioy DT, Ma L, Impey S, Mandel G, Goodman RH. Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat Neurosci. 2007;10:1513–1514. [Crossref]
15. de Sena Cortabitarte A, Berkel S, Cristian FB, Fischer C, Rappold GA. A direct regulatory link between microRNA-137 and SHANK2: implications for neuropsychiatric disorders. J Neurodev Disord. 2018;10:15. [Crossref]
16. Guldiken G, Karayagmurlu A, Kucukgergin C, Coskun M. VEGF, IGF-1 and FGF-2 serum levels in children and adolescents with autism spectrum disorder with and without bipolar disorder. J Autism Dev Disord 2023. [Crossref]
17. Yıldız Bayındır B, Coskun M, Kucukgergin C, Karayagmurlu A. A case-controlled study of serum BDNF, GDNF, and NGF levels in autistic youth with and without bipolar disorder. J Affect Disord. 2026;399:121042. [Crossref]
18. Remenyi J, Hunter CJ, Cole C, Ando H, Impey S, Monk CE, et al. Regulation of the miR-212/132 locus by MSK1 and CREB in response to neurotrophins. Biochem J. 2010;428:281–291. [Crossref]
19. Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, et al. A brain-specific microRNA regulates dendritic spine development. Nature. 2006;439:283–289. [Crossref]
20. Wang Z, Zhang C, Huang J, Yuan C, Hong W, Chen J, et al. MiRNA-206 and BDNF genes interacted in bipolar I disorder. J Affect Disord. 2014;162:116–119. [Crossref]
21. Sabry D, El-Deek SE, Maher M, El-Baz MA, El-Bader HM, Amer E, et al. Role of miRNA-210, miRNA-21 and miRNA-126 as diagnostic biomarkers in colorectal carcinoma: impact of HIF-1α-VEGF signaling pathway. Mol Cell Biochem. 2019;454:177–189. [Crossref]
22. Rong H, Liu TB, Yang KJ, Yang HC, Wu DH, Liao CP, et al. MicroRNA-134 plasma levels before and after treatment for bipolar mania. J Psychiatr Res. 2011;45:92–95. [Crossref]
23. Tekdemir R, Selvi Y, Altınbaş K, Koçak N. Decreased miR-15b-5p/miR-155-5p levels and increased miR-134-5p/miR-652-3p levels among BD patients under lithium treatment. J Affect Disord. 2022;317:6–14. [Crossref]
24. Cohen J. Statistical Power Analysis for the Behavioral Sciences, 2nd ed. New York: Routledge; 2013. [Crossref]
25. Kang H. Sample size determination and power analysis using the G* Power software. J Educ Eval Health Prof. 2021;18. [Crossref]
26. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry. 1997;36:980–988. [Crossref]

27. Unal F, Oktem F, Cetin Cuhadaroğlu F, Cengel Kültür SE, Akdemir D, Foto Özdemir D, et al. Reliability and validity of the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5. November 2016 -Turkish adaptation (K-SADS-PL-DSM-5-T). Turk J Psychiatry. 2019;30:1–9. [Crossref]
28. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Autism Dev Disord. 1980;10:91–103. [Crossref]
29. Incekas Gassaloglu S, Baykara B, Avcil S, Demiral Y. Validity and reliability analysis of Turkish version of Childhood Autism Rating Scale. Turk J Psychiatry. 2016;27:266–274. [Crossref]
30. Kaat AJ, Lecavalier L, Aman MG. Validity of the Aberrant Behavior Checklist in children with autism spectrum disorder. J Autism Dev Disord. 2014;44:1103–1116. [Crossref]
31. Karabekiroglu K, Aman MG. Validity of the Aberrant Behavior Checklist in a clinical sample of toddlers. Child Psychiatry Hum Dev. 2009;40:99–110. [Crossref]
32. Duan ZY, Cai GY, Li JJ, Bu R, Wang N, Yin P, et al. U6 can be used as a housekeeping gene for urinary sediment miRNA studies of IgA nephropathy. Sci Rep. 2018;8:10875. [Crossref]
33. Gao J, Wang WY, Mao YW, Gräff J, Guan JS, Pan L, et al. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature. 2010;466:1105–1109. [Crossref]
34. Grande I, Fries GR, Kunz M, Kapczinski F. The role of BDNF as a mediator of neuroplasticity in bipolar disorder. Psychiatry Investig. 2010;7:243–250. [Crossref]
35. Fernandes BS, Gama CS, Cereser KM, Yatham LN, Fries GR, Colpo G, et al. Brain-derived neurotrophic factor as a state-marker of mood episodes in bipolar disorders: a systematic review and meta-regression analysis. J Psychiatr Res. 2011;45:995–1004. [Crossref]
36. Brunoni AR, Baeken C, Machado-Vieira R, Gattaz WF, Vanderhasselt MA. BDNF blood levels after electroconvulsive therapy in patients with mood disorders: a systematic review and meta-analysis. World J Biol Psychiatry. 2014;15:411–418. [Crossref]
37. Liu SH, Shi XJ, Fan FC, Cheng Y. Peripheral blood neurotrophic factor levels in children with autism spectrum disorder: a meta-analysis. Sci Rep. 2021;11:15. [Crossref]
38. McCarthy JJ. MicroRNA-206: the skeletal muscle-specific myomiR. Biochim Biophys Acta Gene Regul Mech. 2008;1779:682–691. [Crossref]
39. Kim AH, Reimers M, Maher B, Williamson V, McMichael O, McClay JL, et al. MicroRNA expression profiling in the prefrontal cortex of individuals affected with schizophrenia and bipolar disorders. Schizophr Res. 2010;124:183–191. [Crossref]
40. Li YJ, Xu M, Gao ZH, Wang YQ, Yue Z, Zhang YX, et al. Alterations of serum levels of BDNF-related miRNAs in patients with depression. PLoS One. 2013;8:e63648. [Crossref]
41. Huang YWA, Ruiz CR, Eyler EC, Lin K, Meffert MK. Dual regulation of miRNA biogenesis generates target specificity in neurotrophin-induced protein synthesis. Cell. 2012;148:933–946. [Crossref]
42. Alhasan L. MiR-126 modulates angiogenesis in breast cancer by targeting VEGF-A mRNA. Asian Pac J Cancer Prev. 2019;20:193–199. [Crossref]
43. Ruiz de Almodovar C, Lambrechts D, Mazzone M, Carmeliet P. Role and therapeutic potential of VEGF in the nervous system. Physiol Rev. 2009;89:607–648. [Crossref]
44. Castillo MFR, Cohen A, Edberg D, Hoppensteadt D, Fareed J, Martin B, et al. Vascular endothelial growth factor in bipolar depression: a potential biomarker for diagnosis and treatment outcome prediction. Psychiatry Res. 2020;284:112781. [Crossref]
45. Kolshus E, Ryan KM, Blackshields G, Smyth P, Sheils O, McLoughlin DM. Peripheral blood microRNA and VEGFA mRNA changes following electroconvulsive therapy: implications for psychotic depression. Acta Psychiatr Scand. 2017;136:594–606. [Crossref]
46. Vieta E, Gasto C, Otero A, Nieto E, Vallejo J. Differential features between bipolar I and bipolar II disorder. Compr Psychiatry. 1997;38:98–101. [Crossref]
47. Ghaziuddin M, Ghaziuddin N. Bipolar disorder and psychosis in autism. Child Adolesc Psychiatr Clin N Am. 2020;29:433–441. [Crossref]
48. Sapmaz D, Baykal S, Akbaş S. The clinical features of comorbid pediatric bipolar disorder in children with autism spectrum disorder. J Autism Dev Disord. 2018;48:2800–2808. [Crossref]
49. Gedik CU, Karayagmurlu A, Kucukgergin C, Bingul I, Coskun M. Peripheral markers of nitrosative stress in children with autism spectrum disorder and bipolar disorder comorbidity during euthymic phase. Res Autism Spectr Disord. 2023;109:102259. [Crossref]