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ORIGINAL ARTICLE

Biomarker potential of *hsa-miR-145-5p* in peripheral whole blood of manic bipolar I patients

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Objective: Bipolar I disorder (BD-I) is a type of bipolar spectrum disorder characterized by manic or mixed episodes. Detecting microRNA regulations as epigenetic actors in BD-I is important to elucidate the pathogenesis of the disease and reveal the potential of microRNAs (miRNAs) as biomarkers.
Methods: We evaluated the expression profile of six candidate miRNAs (*hsa-miR-145-5p*, *hsa-miR-376a-3p*, *hsa-miR-3680-5p*, *hsa-miR-4253-5p*, *hsa-miR-4482-3p*, and *hsa-miR-4725*) in patients with BD-I and in healthy controls (aged 11-50 years). We also determined the potential target genes of these miRNAs through *in silico* analysis. The diagnostic values of the miRNAs were calculated through receiver operating characteristic curve analysis.
Results: Four miRNAs were upregulated (*hsa-miR-376a-3p*, *hsa-miR-3680-5p*, *hsa-miR-4253-5p*, *hsa-miR-4482-3p*) and *hsa-miR-145-5p* was downregulated in patients ($p < 0.001$). The target gene analyses showed that *hsa-miR-145-5p* specifically targets the dopamine decarboxylase (*DDC*) gene. The area under the curve of *hsa-miR-145-5p* was 0.987.
Conclusion: Differential expression of five miRNAs in peripheral blood may be associated with the pathogenesis of BD-I, and *hsa-miR-145-5p* has potential as a BD-I biomarker. This miRNA can be used in dopamine-serotonin regulation and dose adjustment in drug therapy via the *DDC* gene.

Keywords: Manic; bipolar I disorder; biomarker; miR-145

Introduction

Bipolar disorder (BD) is a pathological and complex genetic disorder that is usually accompanied by mood and behavioral dysfunctions ranging from extreme depression to mania or vice versa, in which delusions and hallucinations occur during affective periods.[1] BD typically includes remission between episodes, and modern operational diagnostic criteria indicate that it is equally common in men and women, with onset at a mean age of 21 years. Although genetic and family studies strongly suggest that neurobiological deterioration underlies the pathophysiology of BD, its etiology remains unclear.[2] Genetic approaches have provided valuable information about the pathophysiology of BD. Differential results from BD-related genome-wide association studies have revealed the multifactorial nature of the disease. In a study of 25,060 patients with BD-I and 449,978 controls, 44 *loci* were found to be associated with BD-I.[3] The diversity of results from studies investigating the genetic basis of BP may be due to small individual contributions, insufficient sample sizes, and failure to identify genes that may be directly responsible for BD due to its heterogeneity.[4]

Monoamines affect the neurobiology of the disorder, and their potential has emerged with the discovery of effective pharmacological treatments for depression and manic episodes.[5] Many psychotropics (older medications, such as lithium and valproate, and newer ones, such as olanzapine and quetiapine) provide effective treatment by affecting one or more stages of the synthesis, storage, or degradation of monoamine group neurotransmitters. In particular, antidopaminergic drugs, such as olanzapine and quetiapine (through dopamine 2/dopamine 3 receptors), prevent relapse by controlling acute manic episodes through long-term effects.[6] Therefore, monoamine group neurotransmitters, such as dopamine decarboxylase (*DDC*), stand out as attractive candidates for elucidating the pathophysiology of BP.

Many studies have shown that microRNAs (miRNAs) play a role in the pathology of a number of neurological diseases, including BD.[7] Peripheral miRNAs are readily available biological resources and potential biomarkers for brain-based diseases due to their association with the neuroendocrine and neuroimmune systems. miRNAs are potential candidates for these diseases, and plasma miR-134 has been proposed as a potential biomarker for

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mania episodes in BD.[8] miRNAs are epigenetic tools approximately 18-24 nucleotides in length that regulate post-transcriptional gene expression, generally negatively. Overexpression of a miRNA may downregulate its target gene, resulting in a negative correlation. miRNAs are thought to have a role in the pathophysiology of psychiatric disorders. These small non-coding RNA molecules may regulate the expression of single or multiple genes involved in cellular function. Thus, miRNAs could play a prominent role in complex neurodevelopmental syndromes, such as schizophrenia, BD, and other neuropsychiatric disorders. miRNAs are also known to be involved in brain development and dendritic spine morphology, which is presumed to be associated with BD neuropathology.[9] Mania is a common symptom of BD, causing euphoria, intense mood swings, hyperactivity, and delusions. Euthymia is a normal mood and mental state (not manic or depressed) that can be found in healthy individuals.[10] Because of the delicate boundary between euthymia and well-being, miRNA expression is insufficient to explain euthymia.[11,12]

In this study, we aimed to identify new candidate miRNAs for BD-I and target genes in the serotonin-dopamine pathway *in silico* and to evaluate the diagnostic potential of miRNA expression. Therefore, we analyzed peripheral blood samples from manic patients with BD-I for miRNAs and compared them with control samples. Target genes were then determined through bioinformatic analysis and, finally, the diagnostic potential of miRNA expression was evaluated.

Methods

Study groups and procedures

In this case-control study of unrelated individuals, we examined 56 patients (female, n=25; male, n=31) with BD-I and 52 healthy controls (female, n=26; male, n=26) aged 11-50 years. BD-I can begin at any age and may herald a greater burden of depressive symptoms. Early diagnosis may alleviate this burden. The purpose of setting a minimum age that included children was to identify the biomarker potential of miRNAs by catching them at the beginning of the disorder. The research protocol was approved by Mersin University Clinical Research ethics committee (2018/97). Patients and controls were evaluated at the Harran University Faculty of Medicine, Department of Psychiatry. Patients with BD-I were diagnosed according to the DSM-IV-TR and Revising Diagnostic and Statistical Manual of Mental Disorders[13] inclusion criteria: a diagnosis of manic BD-I (individuals who have had at least one manic episode longer than a week) without any other DSM-IV-TR diagnosis, such as substance use disorder, other mental illnesses, or neurological disorders in their family history and no continuous drug use during the sampling process. The exclusion criteria were: anxiety, depression, mania, hypomania, eating disorders, a clinical learning disability, a history of head trauma, loss of consciousness, and a blood transfusion in the last month. The inclusion criteria for controls were: no personal or family history of BD. The psychiatric status of all controls was confirmed with the Schedule for Affective Disorders and Schizophrenia – Lifetime Version.[14] All volunteers provided written informed consent prior to participation in the study.

MicroRNA selection

miRNA expression profiling datasets were selected using the DisGeNET (5.0) database[15] for disease-responsible genes. Genes with a high gene-disease association score, no biomarker studies, defined genetic variation, and varying expression were preferred. The screening steps used to select BD miRNA datasets were: search by Disease menu → Manic-Depressive Illness → Summary of Gene-Disease Associations → Similar Diseases → Bipolar Disorder. Detected genes were screened for expression profiles in the National Center for Biotechnology Information Gene Expression Omnibus (annotation, organism, reporter, DataSet Type). Next, genes related to bipolar etiopathogenesis that have been targeted by antipsychotic drugs were selected for effective target miRNA expression. Using *DDC* algorithms (Target Scan, mirDB), catechol-O-methyltransferase (*COMT*), solute carrier family 6 member 3 (*SLC6A3*), monoamine oxidase A (*MAOA*), and solute carrier family 6 member 4 (*SLC6A4*) – genes involved in dopamine-serotonin metabolism – were analyzed, and six specific mature miRNAs were predicted for manic patients with BD-I whose associations have not been previously investigated in the literature: *hsa-miR-145-5p*, *hsa-miR-376a-3p*, *hsa-miR-3680-5p*, *hsa-miR-4253-5p*, *hsa-miR-4482-3p*, and *hsa-miR-4725*.

RNA extraction and complementary DNA analysis

Total RNAs were obtained using the acid guanidinium-phenol chloroform method[16] by drawing 10 mL of venous blood into 15 mL centrifuge tubes containing 2% ethylene-dimethyl tetraacetic acid. The six miRNA-specific primers and probes (Metabion International AG, Martinsried, Germany), whose nucleotide sequences were obtained using miRbase (release 22.1) and National Center for Biotechnology Information algorithms, were designed according to the stem-loop structure in Primer Express 3.0 (Applied Biosystems, Waltham, MA, USA) (Table 1). Each probe has a fluorescent dye labeled with FAM at its 5′ end, a ''quencher'' (absorber) called Black Hole Quencher™ at the 3′ end, and is bound by a covalent bond that prevents light emission at the wavelength at which radiation occurs. In addition, for some miRNAs, the cytosine analog 5-(1-Propynyl)-dC (pdC) was added instead of cytosine nucleotide and, during probe design, the melting temperature of the fluorescent dye-labeled oligonucleotide was increased to maintain target specificity. Compressed nucleic acid technology (Zip nucleic acids) was used to specifically obtain the miRNA of interest and measure miRNA expression when designing the probe sequence. cDNA was obtained from the total RNA of each participant in the patient and control groups using the standard polymerase chain reaction (PCR)

Table 1 Primer-probe sequences for reverse-transcription PCR and real-time PCR

miRNAs	miRNA ID†	Nucleotide sequence‡	Primer-probe sequences
hsa-miR-26b-5p	407017	NR_029500.1	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTA TTCGCACTGCATACGACACCTAT-3′ F-5′-GCCGCTTCAAGTAATTCAGG-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GA(pdC)A(pdC)CTATCC-ZNA4-BHQ-1-3′
hsa-miR-145-5p	406937	NR_029686	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGC ACTGCATACGACAGGGAT-3′ F-5′-GCCGCGTCCAGTTTTCC-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GA(pdC) AGGGAT-ZNA4-BHQ-1-3′
hsa-miR-4725-3p	100616449	NR_039878	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCAC TGCATACGACCCCGAC-3′ F-5′-GCCGCTGGGGAAGGC-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GAC CCCGAC-ZNA4-BHQ-1-3′
hsa-miR-376a-5p	494325	NR_029868	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGC ACTGCATACGACTACTCA-3′ F-5′-GCCGCGTAGATTCTCCTTCTA-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GA(pdC) TA(pdC)T(pdC)A-ZNA4-BHQ-1-3′
hsa-miR-4482-3p	100616323	NR_039702	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCA CTGCATACGACGAGCCC-3′ F-5′-GCCGCTTTCTATTTCTCAGTGG-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GACGAGCCC-ZNA4-BHQ-1-3′
hsa-miR-4253	100422914	NR_036214	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGCACT GCATACGACACCCCC-3′ F-5-GCCGCAGGGCATGTCCA-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GACACCCCCT-ZNA4-BHQ-1-3′
hsa-miR-3680-3p	100500917	NR_037451	RT-5′-GTCGTATGCAGTGCAGGGTCCGAGGTATTCGC ACTGCATACGACCCTACT-3′ F-5′-GCCGCTTTTGCATGACCC-3′ PR-FAM-5′-TG(pdC)ATA(pdC)GA(pdC) C(pdC)TA(pdC)T-ZNA4-BHQ-1-3′
Universal R primer			R-5′-GTGCAGGGTCCGAGGTAT-3′

BHQ = Black Hole Quencher™; F = forward; miRNA = microRNA; PR = probe; R = reverse; PCR = polymerase chain reaction.
† www.ncbi.nlm.nih.gov/gene.
‡ http://www.ncbi.nlm.nih.gov.

method. For each sample, 10 μL of reaction mixture was prepared with 1.5 μL deoxynucleotide triphosphates (10 mM), 0.25 μL reverse transcriptase (200 U/μL), and 5 μL ddH_2O, 0.2 μL reverse transcriptase primer. The reactions were distributed into plate wells, to which 5 μL of total RNA were added. The plate was incubated for 1 cycle at 16 °C for 30 min, 42 °C for 30 min, and 85 °C for 5 min in an Applied Biosystems 7200 Real-Time PCR System. At the end of the cycle, the plate was immediately placed on ice and stored at -20 °C until expression analysis.

Quantitative real-time polymerase chain reaction analysis for microRNA expression

miRNA expression analyses were performed using the TaqMan probe system. Expression profiles for each miRNA from the cDNA library were obtained using the Applied Biosystem 7200 Real-Time PCR system. The 25 μL PCR product was prepared using 5 μL cDNA, 12.5 μL of 2 X Master Mix (Solis Biodyne, Tartu, Estonia), 2.5 μL of universal primer, 2.5 μL of forward primer, 0.2 μL of the probe, and 5 μL ddH2O. The reactions were incubated at 50 °C for 2 min (1 cycle), 95 °C for 10 min (1 cycle), 95 °C for 15 sec (50 cycles) and 60 °C for 1.5 min (1 cycle). miRNAs expression levels were normalized by using the RNA pool of each healthy control and the *hsa-miR-26b* endogenous control. The analyses were read in triplicate and the means were calculated. The real-time growth curves were analyzed in the logarithmic chart in SDS 2.0.6 and the expression levels were determined using the $2^{-\Delta\Delta CT}$ method.[17]

Receiver operating characteristic analysis

We investigated whether these miRNAs could serve as novel and potential biomarkers in manic patients with BD-I by performing receiver operating characteristic (ROC) analysis in two cohorts, determining their diagnostic power using values for all miRNAs. The diagnostic power of each miRNA was evaluated according to a rating system developed by Hosmer et al.[18] In these analyses, the AUC, efficiency, sensitivity, specificity, and positive/negative cutoff value were determined. ROC curves of miRNA expression were calculated with for each miRNA. A p-value < 0.05 was considered statistically significant.

We used MedCalc software (Ostend, Belgium) to perform the ROC analysis.

Statistical analysis

The Mann-Whitney *U* test was used to compare two independent groups. Age and sex differences for the patient and control groups were tested with Pearson's chi-square and likelihood ratio chi-square tests. Non-normally distributed miRNA expression was summarized as medians (25th percentile-75th percentile). False finding rates in multiple gene analyses were controlled with Benjamini-Hochberg corrections to the p-values. The levels of significance were $p < 0.05$ and $p < 0.001$. The analyses were performed in STATISTICA version 13.3.1 (TIBCO Software Inc., Palo Alto, CA, USA). We analyzed the ROC curves and the area under the curve (AUC) to assess the specificity and sensitivity of individual miRNAs when distinguishing manic patients with BD-I from controls. The threshold values of the optimal diagnostic points of the ROC curve were determined at the largest Youden index. Multiple logistic regression analysis was used to calculate AUCs for gene combinations.

Ethics statement

This study was conducted according to the World Medical Association (Declaration of Helsinki) Code of Ethics for experiments involving humans and the Uniform Requirements for manuscripts submitted to biomedical journals. This study was also approved by the Mersin University Faculty of Clinical Research ethics committee. All participants provided written informed consent prior to inclusion.

Results

Differentially expressed microRNAs

We profiled whole peripheral blood miRNAs as biomarker candidates in patients with BD-I and evaluated their association with the disorder. The patient group consisted of 56 individuals (25 [44.64%] females and 31 [55.36%] males) and the control group consisted of 52 individuals (26 [50%] females and 26 [50%] males) (total groups, n=108, aged 11-50 years). The mean ages of the patient and control groups were 30.6±12.6 and 32±6.13 years, respectively. The age and sex differences between the groups were not significant ($p > 0.05$). miRNA expression for covariates was not significant ($p > 0.05$) (Table 2). Calculation of fold changes in miRNA expression values were based on . Benjamini Hochberg's correction was used for multiple comparisons. Consequently, expression levels of *hsa-miR-376a-3p*, *hsa-miR-3680-3p*, *hsa-miR4253*, and *hsa-miR-4482-3p* differed significantly between the control and patient groups ($p < 0.05$). These miRNAs were upregulated and *hsa-miR-145-5p* was downregulated in the patient group. *hsa-miR-4725* expression did not differ significantly between the groups ($p > 0.05$) (Table 3) (Figure 1).

Diagnostic value of the microRNAs

Since miRNA levels differed significantly between groups, the diagnostic performance of expression values in patients with BD-I were evaluated (Table 4). The results indicate that, except for *hsa-miR-4725-3p* ($p = 0.207$), all of the miRNAs have significant sensitivity and specificity ($p < 0.05$). The AUC values were 0.987 ($p < 0.001$) for *hsa-miR-145-5p*, 0.730 for *hsa-miR-376a-5p* ($p < 0.001$),

Table 2 Demographic characteristics and miRNA expressions of the control and patient groups

Characteristics	Control	Patient	p-value	*hsa-miR-145-5p*	*hsa-miR-376a-3p*	*hsa-miR-3680-3p*	*hsa-miR-4253*	*hsa-miR-4482-3p*	*hsa-miR-4725-3p*
Sex, n (%)			0.413	0.952	0.856	0.789	0.855	0.698	0.764
Female	26 (25)	25 (22.4)							
Male	26 (25)	31 (27.6)							
Age, mean ± SD			> 0.05	0.546	0.995	0.498	0.852	0.779	0.598
Female	29.4±14.4	31.3±10.4							
Male	31.5±11.2	30.2±18.3							

SD = standard deviation.

Table 3 MicroRNA expression analyses for $2^{-\Delta\Delta CT}$

	Control (n=52)			Patient (n=56)			
miRNA	Median ± SD	SE	[25th-75th]	Median ± SD	SE	[25th-75th]	p-value
hsa-miR-145-5p	527.291±9,893.657	1,428.026	[51.596-3,774.886]	0.138±0.821	0.107	[0.043-0.329]	< 0.001*
hsa-miR-376a-3p	1.466±29.662	4.471	[0.061-5.819]	17.208±8.659	1.167	[1.379-77.273]	< 0.001*
hsa-miR-3680-3p	1.403±1.908	275.535	[0.002-52.811]	205.746±3.285	4.390	[29.829-1811.22]	< 0.001*
hsa-miR-4253	13.019±7,976.664	1,151.332	[0.192-510.354]	248.247±2.454	3.251	[23.356-4150.996]	0.001*
hsa-miR-4482-3p	13.640±3,044.813	422.239	[0.357-129.498]	227.821±1.166	1.519	[61.313-14038.632]	< 0.001*
hsa-miR-4725-3p	46.642±5,249.445	765.710	[0.997-799.596]	117.586±3.506	4.603	[9.694-863.455]	0.209

miRNA = microRNA; SD =standard deviation; SE = standard error.
* $p < 0.05$ = statistically significant.

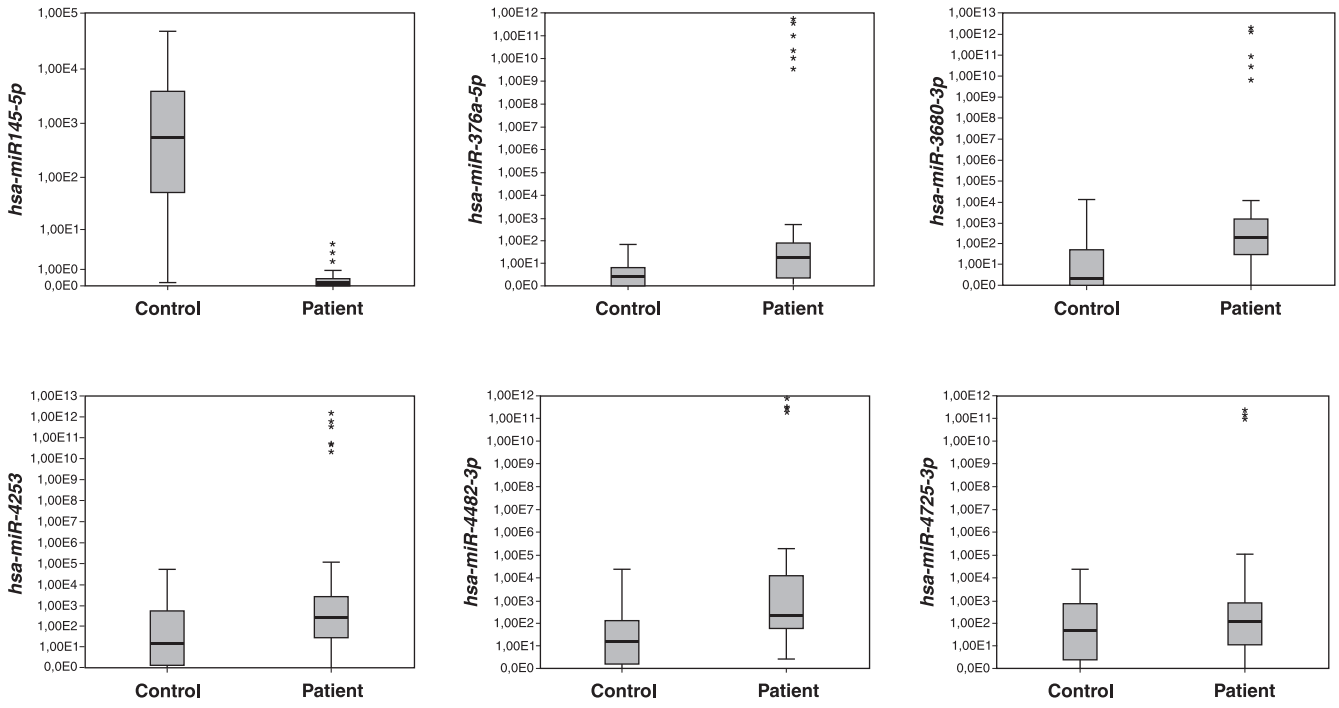

Figure 1 Boxplot graphic of microRNA (miRNA) expressions in whole peripheral blood of patients with BD-I. Data are reported as log transformation and 95% confidence interval (95%CI) median for $2^{-\Delta\Delta CT}$ values. Expression rates in the patient and control groups were compared and normalized using *hsa-miR-26b* endogenous control. *hsa-miR-376a-5p*, *hsa-miR-3680-3p*, *hsa-miR-4253*, and *hsa-miR-4482-3p* appear to be upregulated in patients compared to controls, while *hsa-miR145-5p* and *hsa-miR-4725-3p* appear to be downregulated. Stars represent high variance of expressions between individuals. There was a statistically significant difference between the groups, except for *hsa-miR-4725-3p*.

Table 4 The ROC curve analyses of the significant five microRNAs

miRNAs	AUC	95%CI	SE	p-value	Specificity (%)	Sensitivity (%)	Criterion
hsa-miR-145-5p	0.987	0.943-0.999	0.011	< 0.001	97.92	96.55	$\leqslant 1.937$
hsa-miR-376a-5p	0.730	0.632-0.815	0.050	< 0.001	77.27	67.27	> 140.396
hsa-miR-3680-3p	0.834	0.748-0.900	0.039	< 0.001	93.75	48.21	> 13.424
hsa-miR-4253	0.695	0.597-0.781	0.051	< 0.001	52.08	84.21	> 14.048
hsa-miR-4482-3p	0.811	0.726-0.879	0.038	< 0.001	65.38	79.66	> 49.013

Compared groups: patients with manic episode BP-I vs. controls. The state variable for *miR-145-5p* is the control group.
95%CI = 95% confidence interval; AUC = area under the curve; miRNA = microRNA; ROC = receiver operating characteristic; SE = standard error.

and 0.834 ($p < 0.001$) for *hsa-miR-3680-3p*, 0.695 ($p < 0.001$) for *hsa-miR-4253*, 0.811 ($p < 0.001$) for *hsa-miR-4482-3p*, and 0.572 ($p = 0.207$) for *hsa-miR-4725-3p* (Figure 2). Strikingly, *hsa-miR-145-5p* has a high diagnostic value for distinguishing healthy individuals from patients.

Combined comparisons of the miRNAs showed that the AUC values of some miRNA combinations have potential for diagnosing BD-I (Table 5). We analyzed the miRNAs together and investigated their diagnostic power with the ROC curve. The highest values were obtained for miRNAs modeled with *hsa-miR-145-5p* (95% confidence interval [95%CI] 0.99065-0.9985) (Table 5). The diagnostic value (as percentages) for the miRNAs were: 98.7% for *hsa-miR-145-5p*, 73% for *hsa-miR-376a-5p*, 83.4% for *hsa-miR-3680-3p*, 69.5% for *hsa-miR-4253*, and 81.1% for *hsa-miR-4482-3p*. These results suggest that a co-signature of miRNAs, particularly hsa-miR-145-5p, have strong diagnostic value in differentiating healthy individuals from patients with BD-I.

Discussion

In BD, the patient's symptoms at admission, the physical and mental examination, and the data obtained from interviews are all descriptive, with symptoms differing between individuals. This psychiatric disorder is complex due to the transition between its stages, which causes clinical heterogeneity,[19] and diagnostic biomarkers are sorely needed. Insufficient understanding of the disease's pathogenesis harms the diagnosis and treatment process. Family studies of patients may facilitate the diagnostic process, but this information is insufficient to explain the underlying mechanisms of this complex

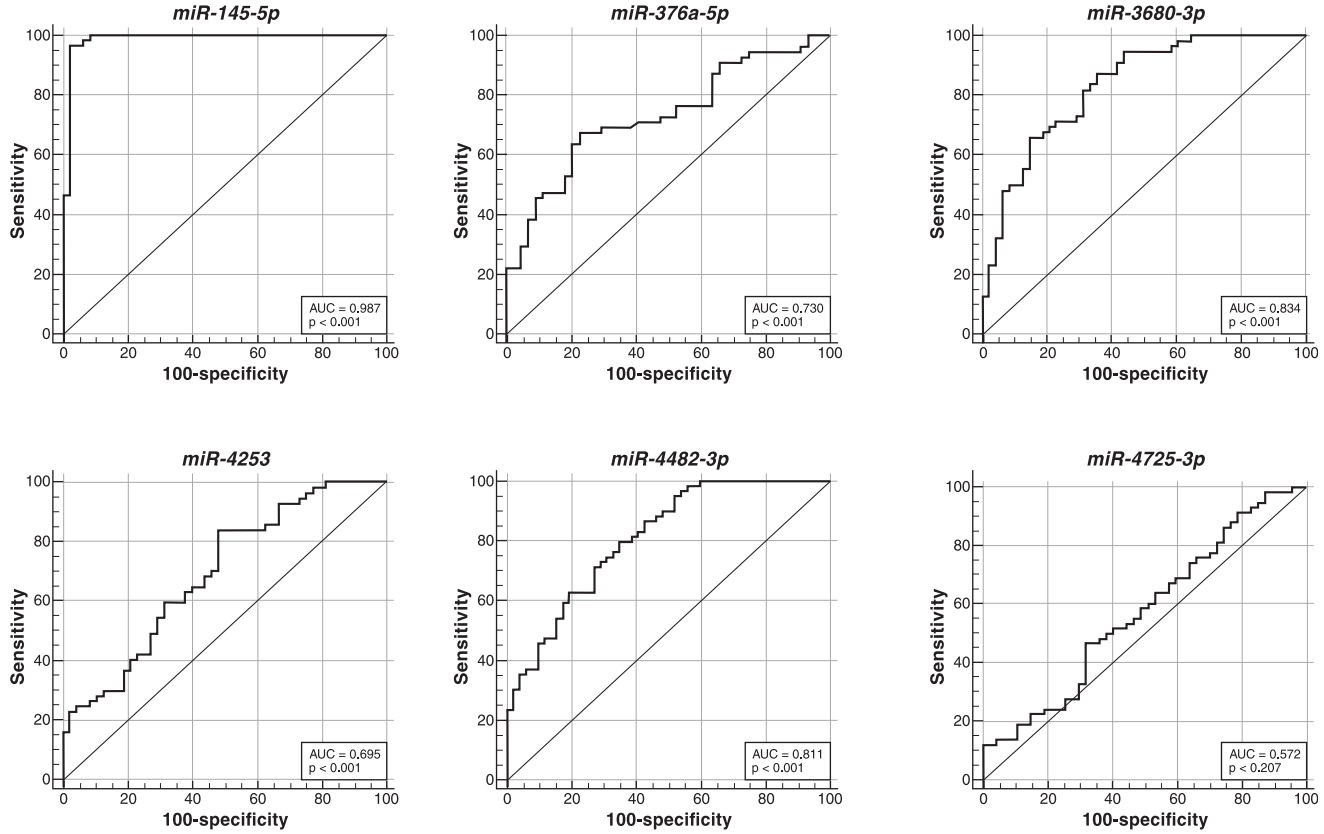

Figure 2 Receiver operating characteristic curve analysis for the expression of six microRNAs. The area under the curve (AUC) values indicate $2^{-\Delta\Delta CT}$. According to the results, *hsa-miR-145-5p* has excellent diagnostic values for identifying controls, *hsa-miR-3680-3p* and *hsa-miR-4482-3p* have good values, *hsa-miR-376a-5p* has fair values, and *hsa-miR-4253* has poor values.

disorder. Discovery of the genetic mechanisms responsible for the disorder's pathogenesis will positively affect its diagnosis and treatment process. Having additional psychiatric disorders, such as attention-deficit/hyperactivity disorder, anxiety, personality disorder, eating disorder, and substance use disorders, can affect the diagnosis, treatment, general morbidity, and mortality of BD.[20] When accompanied by neuropsychiatric syndromes, the survival rate of BD decreases considerably. Current pharmacological treatments for BD may cause side effects that increase cognitive impairment. Genetic, neuroimaging, histological, and biochemical studies are the most important way out of this vicious circle. The results of these studies will allow BD to be examined as a biologically different disease category, providing a different perspective. Several drug treatment modalities have been developed specifically for manic and depressive episodes (the anticonvulsants valproate, lamotrigine, carbamazepine for symptoms and recurrence; the antipsychotics olanzapine, quetiapine, aripiprazole, ziprasidone; and the antidepressants fluoxetine, paroxetine, sertraline, bupropion). Although lithium is the most conventional pharmacological treatment for BD, several anticonvulsants have recently added to reduce the recurrence of acute depression and mania episodes.[21] However, these current treatments have many side effects and unfortunately, they are neither completely effective nor a definitive solution.

Candidate gene approaches suggest that dysfunctions in certain genes, such as *COMT*, brain-derived neurotrophic factor, and neuregulin-1, may be strong risk factors for BD.[22] *COMT*'s function in the brain is to mediate the degradation of certain chemical messengers called neurotransmitters. *COMT* gene dysfunction may be important in the pathogenesis of neuropsychiatric disorders. Studies have shown that miRNA expression involves epigenetic changes that play a role in different stages of BD.[23] miRNAs are descriptive epigenetic markers. They are transcriptional regulators of gene expression and, due to their involvement in disease pathology, may serve as potential therapeutic targets. A BD-II study found upregulation of *miR-7-5p*, *miR-23b-3p*, *miR-142-3p*, *miR-221-5p*, and *miR-370-3p*.[24] Although many studies have been conducted at the level of single nucleotide polymorphisms associated with the disease, it has been suggested that the relationship between the disease and the gene is clearer in studies investigating miRNAs that can control *COMT* gene expression. It is believed that polymorphism studies cannot provide clear results regarding diagnosis of the disorder. In our study, *hsa-376a-5p* and *hsa-miR-4482-3p*, which were detected *in silico* as targeting the *COMT* gene, had higher

Table 5 Multiple logistic regression analyses of miRNA predictor variables to determine associations with manic bipolar I disorder

miRNA	AUC for multiple logistic regression	95%CI	Standard error	p-value	R^2
hsa-miR-145-5p+ hsa-miR-376a-5p	0.988	0.966-1.000	0.011	< 0.0001*	0.687
hsa-miR-145-5p+ hsa-miR-3680-3p	0.987	0.966-1.000	0.011	< 0.0001*	0.683
hsa-miR-145-5p+ hsa-miR-4253	0.987	0.967-1.000	0.010	< 0.0001*	0.685
hsa-miR-145-5p+ hsa-miR-4482-3p	0.989	0.970-1.000	0.010	< 0.0001*	0.688
hsa-miR-145-5p+ hsa-miR-4725-3p	0.988	0.994-1.000	0.002	< 0.0001*	0.705
hsa-miR-376a-5p+ hsa-miR-3680-3p	0.845	0.763-0.926	0.041	< 0.0001*	0.076
hsa-miR-376a-5p+ hsa-miR-4253	0.744	0.645-0.844	0.051	< 0.0001*	0.097
hsa-miR-376a-5p+ hsa-miR-4482-3p	0.821	0.739-0.903	0.042	< 0.0001*	0.098
hsa-miR-376a-5p+ hsa-miR-4725-3p	0.606	0.491-0.721	0.059	0.081	0.082
hsa-miR-3680-3p+ hsa-miR-4253	0.593	0.480-0.706	0.058	0.111	0.088
hsa-miR-3680-3p+ hsa-miR-4482-3p	0.794	0.709-0.879	0.043	< 0.0001*	0.083
hsa-miR-3680-3p+ hsa-miR-4725-3p	0.764	0.668-0.860	0.049	< 0.0001*	0.085
hsa-miR-4253+ hsa-miR-4482-3p	0.789	0.703-0.875	0.044	< 0.0001*	0.095
hsa-miR-4253 + hsa-miR-4725-3p	0.699	0.597-0.802	0.052	0.001*	0.096
hsa-miR-4482-3p+ hsa-miR-4725-3p	0.735	0.639-0.831	0.049	< 0.0001*	0.093
hsa-miR-145+ hsa-miR-376a-5p+ hsa-miR-3680-3p	0.986	0.963-1.000	0.012	< 0.0001*	0.682
hsa-miR-145+ hsa-miR-376a-5p+ hsa-miR-4253	0.987	0.965-1.000	0.011	< 0.0001*	0.683
hsa-miR-145+ hsa-miR-376a-5p+ hsa-miR-4482-3p	0.988	0.968-1.000	0.010	< 0.0001*	0.689
hsa-miR-145+ hsa-miR-376a-5p+ hsa-miR-4725-3p	0.998	0.994-1.000	0.002	< 0.0001*	0.705
hsa-miR-145+ hsa-miR-3680-3p+ hsa-miR-4253	0.987	0.966-1.000	0.011	< 0.0001*	0.683
hsa-miR-145+ hsa-miR-3680-3p+ hsa-miR-4482-3p	0.987	0.966-1.000	0.011	< 0.0001*	0.683
hsa-miR-145+ hsa-miR-3680-3p+ hsa-miR-4725-3p	0.998	0.993-1.000	0.002	< 0.0001*	0.703
hsa-miR-145-5p+ hsa-miR-4253+ hsa-4482-3p	0.998	0.968-1.000	0.010	< 0.0001*	0.686
hsa-miR-145-5p+ hsa-miR-4253+ hsa-4725-3p	0.998	0.993-1.000	0.002	< 0.0001*	0.705
hsa-miR-145-5p+ hsa-miR-4482-3p+ hsa-4725-3p	0.998	0.994-1.000	0.002	< 0.0001*	0.706
hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4253	0.650	0.533-0.767	0.060	0.017*	0.079
hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4482-3p	0.842	0.761-0.923	0.041	< 0.0001*	0.077
hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4725-3p	0.704	0.591-0.818	0.058	0.001	0.064
hsa-miR-376a-5p+ hsa-miR-4253+ hsa-miR-4482-3p	0.735	0.632-0.837	0.052	< 0.0001*	0.100
hsa-miR-376a-5p+ hsa-miR-4253+ hsa-miR-4725-3p	0.662	0.549-0.776	0.058	0.009*	0.098
hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4482-3p	0.772	0.678-0.876	0.048	< 0.0001*	0.088
hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4725-3p	0.593	0.477-0.709	0.059	0.119	0.092
hsa-miR-3680-3p+ hsa-miR4482-3p+ hsa-miR-4725-3p	0.723	0.621-0.825	0.052	< 0.0001*	0.085
hsa-miR-4253+ hsa-miR-4482-3p+ hsa-miR-4725-3p	0.669	0.563-0.774	0.054	0.004*	0.099
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4253	0.986	0.962-1.000	0.012	< 0.0001*	0.681
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4482-3p	0.986	0.963-1.000	0.012	< 0.0001*	0.682
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4725-3p	0.998	0.993-1.000	0.003	< 0.0001*	0.703
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-4253+ hsa-4482-3p	0.987	0.966-1.000	0.011	< 0.0001*	0.684
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-4253+ hsa-4725-3p	0.998	0.993-1.000	0.002	< 0.0001*	0.705
hsa-miR-145-5p+ hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4482-3p	0.987	0.966-1.000	0.011	< 0.0001*	0.683
hsa-miR-145-5p+ hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4725-3p	0.998	0.993-1.000	0.002	< 0.0001*	0.703
hsa-miR-145-5p+ hsa-miR-4253+ hsa-miR-4482-3p+ hsa-miR-4725-3p	0.998	0.993-1.000	0.002	< 0.0001*	0.706
hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4482-3p+ hsa-miR-4725-3p	0.688	0.581-0.795	0.055	0.002*	0.092
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4253+ miR-4482-3p	0.986	0.962-1.000	0.012	< 0.0001*	0.681

Continued on next page

Table 5 (*continued*)

miRNA	AUC for multiple logistic regression	95%CI	Standard error	p-value	R^2
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4725-3p	0.998	0.992-1.000	0.003	< 0.0001*	0.704
hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4482-3p+ miR-4725-3p	0.694	0.581-0.807	0.058	0.002*	0.080
hsa-miR-145-5p+ hsa-miR-376a-5p+ hsa-miR-3680-3p+ hsa-miR-4253+ hsa-miR-4482-3p+ hsa-miR-4725	0.898	0.811-0.954	0.039	< 0.0001*	0.872

The area under the curve (AUC) values were calculated using the predicted value obtained by logistic regression analysis.
95%CI = 95% confidence interval; miRNA = microRNA.
*p < 0.05 = statistically significant.

expression in patients than controls, which indicates that the gene may have decreased activity due to miRNA control.

The MAOA enzyme, which is encoded by the *MAOA* gene, is responsible for the degradation of biogenic amines, and has been associated for many years with mood disorders. As non-invasive peripheral biomarkers, changes in miRNA expression have been found to correlate with miRNA changes in neuronal tissue in neuropsychiatric disorders.[25] One study found that miR-22, miR-138-2, miR-148a, and miR-488 regulate several genes and pathways related to *MAOA* and anxiety in patients with panic disorder.[26] Although the data only indicate *MAOA*'s potential in BD, the effects of miRNA on the disease can be predicted. However, the true relationship between them can be established by detecting specific miRNAs. It is believed that *MAOA* gene dysfunction leads to psychiatric disorders. At this point, it seems that polymorphism studies are insufficient to reveal the relationship between *MAOA* and disorders. In our study, *hsa-miR-3680-3p* was highly expressed in the patient group. According to the algorithms, this miRNA targets *MAOA*. *hsa-miR-3680-3p* may be associated with low levels of the MAOA enzyme in BD-I.

The *SLC6A3* (dopamine transporter 1-*DAT1*) gene plays a critical role in the regulation of dopaminergic neurotransmission and regulates active reuptake of dopamine from synapses.[27] Accordingly, disruptions in this gene's activity are associated with many neuronal pathologies. Specific miRNAs that target single nucleotide polymorphisms in the *SLCA6A3* gene have been identified in BD bioinformatics studies.[28] Although U.S. SNP-based genome-wide association studies have targeted the *SLC6A3* gene, there is limited knowledge about miRNAs. The results of the present study suggest that *hsa-miR-4725-3p* may not regulate the *SLC6A3* gene. Considering the epigenetic framework, there are other candidate miRNAs. As we demonstrated bioinformatically, miRNAs with gene-specific and tissue-specific targeting may play an important role in BD-I. The specificity of miRNAs for the target gene and tissue strengthens their association with the disease. We detected *in silico* that this miRNA targets the *SLC6A3* gene and found it to be downregulated in the sample. Although this miRNA does not play a role in gene regulation, it may contribute to miRNA-based pathogenesis and biomarker studies.

The serotonin transporter gene (*SLC6A4*) encodes a membrane protein that transports the neurotransmitter serotonin from the synaptic gap to the presynaptic neurons[29] and modulates serotonergic signaling by transporting serotonin molecules from the synaptic cleft to the presynaptic terminal for reuse.[30] One study found that the expression of *SLC6A4* was significantly higher in patients with schizophrenia and BD.[31] This indicates that studies should explain the relationship between *SLC6A4* and BD. miRNAs play an important role in biomarker studies, which are especially needed for brain-based diseases, such as major depressive disorder. A diagnostic study in the cerebrospinal fluid of patients with major depressive disorder found that downregulation of miR-16,

which targets the *SLC6A4* gene, is associated with the disorder through the serotonin neurotransmitter system.[32] Comparative analyses in mouse and human brain tissue have shown that miR-15 and miR-16 regulate the expression of *SLC6A4*.[33] According to the algorithms in our study, *hsa-miR-4253* was the target of this gene and was upregulated in manic BD-I. This miRNA targets the 3′-UTR of the gene, and its upregulation may result in *SLC6A4* dysfunction in BD-I. *hsa-miR-4253* may be associated with disease, but the analyses showed it has low diagnostic power. The variants[34] and methylation status in the promoter region of the gene seem to be effective in determining the pathology of the disease.[35] The dopamine decarboxylase, aromatic-L-amino acid decarboxylase (*DDC*) enzyme plays an important role in the dopaminergic system and participates in the uptake and decarboxylation of amine precursors from peripheral tissue.[36] In addition to catecholamines, *DDC* catalyzes the biosynthesis of serotonin and trace amines. *DDC* acts as an endogenous modulator of central nerve transmission through its role in the biosynthesis of trace amines.[37] Therefore, *DDC* is a potential susceptibility gene for various neuropsychiatric disorders. Some studies have focused on the relationship between *DDC* and miRNAs. One of these studies found that miRNAs regulate *DDC* expression, which supports our bioinformatics results. According to our results, miR-145 is a candidate for *DDC* regulation. In addition, it has been shown that miR-145 does not affect protein levels and that, by reducing mRNA expression, the *DDC* gene is a potential target of miR-145.[38] Our results indicate that *hsa-miR-145*, which targets the *DDC* gene, has a high diagnostic value in differentiating healthy individuals from patients with BD-I. The *DDC* gene is involved in the production of dopamine and serotonin, which provides signal transmission between neurotransmitters. Thus, it plays an important role in the BD mechanism as the target of *hsa-miR-145-5p*. It has been reported that dopaminergic system activity is high in BD[39] and that transmission is increased in mania.[40] The active metabolic involvement of *DDC* indicates the importance of *hsa-miR-145* control. In addition, this miRNA may be a therapeutic target in both manic BD-I and neurological and psychiatric disorders.

In this study, we investigated the biomarker potential of six miRNAs that target five genes and discussed their roles in the pathogenesis of BD-I. Current research on the genetics of BD-Is is predominantly based on single nucleotide polymorphisms. Although these studies were conducted to clarify the genetic and neurobiological causes of BD, the disorder's emergence through the deterioration of more than one factor, the inconsistency of polymorphism studies, and the inability to conduct candidate gene and linkage studies have led to confusion. On the other hand, these studies were conducted in different populations and cannot give a clear idea about the etiopathogenesis of the disease due to genetic differences between ethnic groups. However, the use of miRNAs as markers for diagnosis can be facilitated through proper sampling, exclusion and inclusion criteria, investigating age and sex factors, determining BD's association with other diseases, and predicting specific miRNAs bioinformatically. Our objective was to determine the diagnostic value of six selected miRNAs in BD and interpret their effects on its pathogenesis. In conclusion, *hsa-miR-145-5p* is a powerful epigenetic diagnostic tool to distinguish patients with BD from healthy individuals, and upregulation of this miRNA may improve the clinical symptoms of BD by controlling *DDC* activity.

In this study, we examined miRNA biomarkers that could be used in BD-I diagnosis, discussed target genes, and interpreted their contribution to pathogenesis. Thus, we provided a new perspective by using epigenetic markers for BD, whose diagnosis and treatment process differs from patient to patient and cannot be fully elucidated though family, polymorphism, or candidate gene studies. *hsa-miR-145b-5p* can be used in the diagnosis of BD-I. By silencing this miRNA, the associated mechanisms may be regulated, and its upregulation as a treatment target may lead to symptom control. The presence of expression of these miRNAs in the peripheral blood of patients with BD-I and healthy controls are both informative and promising for determining drug dose, dopamine, and serotonin levels during the therapeutic process.

Our study involves certain limitations. We sampled whole blood miRNAs rather than specific samples such as plasma, serum, or peripheral blood monocytes. However, plasma miRNA is likely to be mixed with serum in case of coagulation, and exosome-derived serum miRNA may be misleading for disease diagnosis. It would be appropriate to also investigate the six selected miRNAs in other sample types to confirm our findings. In addition, comparisons with hypomania, depression, and BD-II, in addition to manic groups, may enable fine-tuning. Testing these results in larger populations can more clearly demonstrate their potential as biomarkers and provide strong evidence for their diagnostic use. This cross-sectional study has shed light, albeit in a limited fashion, on the causal relationship between miRNA expression and BD-I, providing a basis for functional studies to determine the role of miRNAs in the pathogenesis of BD-I. Analyses of patients during depressive episodes may more clearly reveal the contribution of candidate miRNAs to the pathogenesis of the disease and their therapeutic potential.

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Disclosure

The authors report no conflicts of interest.

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