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 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 BD.

Monoamines affect the neurobiology of the disorder, and their potential has emerged with the discovery of effective pharmacological treatments for depression and manic episodes.2 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 BD.

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 BD.
Mania episodes in BD. 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. 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. Because of the delicate boundary between euthymia and well-being, miRNA expression is insufficient to explain euthymia. 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 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. All volunteers provided written informed consent prior to participation in the study.

miRNA selection

miRNA expression profiling datasets were selected using the DisGeNET (5.0) database 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 by drawing 10 mL of venous blood into 15 mL centrifuge tubes containing 2% ethylenedimethyl 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).
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 ddH2O. 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 Biosytem 7200 Real-Time PCR system. The 25 μL PCR product was prepared using 5 μL cDNA, 12.5 μL of 2X 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.

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. 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^-ΔΔCT method.17

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'-GTCGTATGCAGTGCGGAGGGTGCCAGGGTTTCCGACGAGGTAACGCTGACGCAGACCTAT-3' |
| hsa-miR-145-5p | 406937  | NR_029686           | RT-5'-GTCGTATGCAGTGCAGGGTGCCAGGATTCGCACCTGACAGCCGCAGGGCCGAGG-3' |
| hsa-miR-4725-3p | 10061649 | NR_039878           | RT-5'-GTCGTATGCAGTGCGGAGGGTGCCAGGATTCGCACTGACAGCCGCAGGGCCGAGG-3' |
| hsa-miR-376a-5p | 494325  | NR_029868           | RT-5'-GTCGTATGCAGTGCGGAGGGTGCCAGGATTCGCACCTGACAGCCGCAGGGCCGAGG-3' |
| hsa-miR-4482-3p | 100616323 | NR_039702          | RT-5'-GTCGTATGCAGTGCGGAGGGTGCCAGGATTCGCACTGACAGCCGCAGGGCCGAGG-3' |
| hsa-miR-4253 | 100422914 | NR_036214         | RT-5'-GTCGTATGCAGTGCGGAGGGTGCCAGGATTCGCACTGACAGCCGCAGGGCCGAGG-3' |
| hsa-miR-3680-3p | 100500917 | NR_037451         | RT-5'-GTCGTATGCAGTGCGGAGGGTGCCAGGATTCGCACTGACAGCCGCAGGGCCGAGG-3' |
| Universal R primer | | | R-5'-GTCGTATGCAGTGCGGAGGGTGCCAGGATTCGCACTGACAGCCGCAGGGCCGAGG-3' |

BHQ = Black Hole Quencher; F = forward; miRNA = microRNA; PR = probe; R = reverse; PCR = polymerase chain reaction.

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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 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.

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 |
|-----------------|---------|---------|---------|---------------|----------------|----------------|---------------|----------------|----------------|
| Age, mean ± SD  | > 0.05  | 0.546   | 0.995   | 0.852         | 0.779          | 0.598          |               |               |               |
| Sex, n (%)      |         |         |         |               |               |               |               |               |               |
| Female          | 26 (25) | 25 (22.4)| 0.413   | 0.952         | 0.856          | 0.789          | 0.855         | 0.698          | 0.764          |
| Male            | 26 (25) | 31 (27.6)|         |               |               |               |               |               |               |
| Age, mean ± SD  |         |         |         |               |               |               |               |               |               |
| 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^ΔΔCT

| miRNA            | Control (n=52) | Patient (n=56) | p-value |
|------------------|----------------|----------------|---------|
|                  | Median ± SD    | SE             | [25th-75th] |
| 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.
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. Hsa-miR-145-5p and hsa-miR-4725-3p 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.

**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$^\Delta$AC$^T$ 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.

**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          | ≤ 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.

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, 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
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. 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. 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. 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. 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-4482-3p, and miR-370-3p. 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-miR-376a-5p and hsa-miR-4482-3p, which were detected in silico as targeting the COMT gene, had higher...
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       | R²   |
|-------------------------------|-------------------------------------|--------------|---------------|---------------|------|
| 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                               | 0.967-1.000  | 0.010         | < 0.0001*     | 0.685|
| hsa-miR-145-5p + hsa-miR-4253  | 0.989                               | 0.970-1.000  | 0.010         | < 0.0001*     | 0.688|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.988                               | 0.994-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-145-5p + hsa-miR-4725-3p | 0.985                               | 0.763-0.926  | 0.041         | < 0.0001*     | 0.707|
| hsa-miR-376a-5p + hsa-miR-3680-3p | 0.744                               | 0.645-0.844  | 0.051         | < 0.0001*     | 0.637|
| hsa-miR-376a-5p + hsa-miR-4253  | 0.821                               | 0.739-0.903  | 0.042         | < 0.0001*     | 0.698|
| hsa-miR-376a-5p + hsa-miR-4482-3p | 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*     | 0.683|
| hsa-miR-4253 + hsa-miR-4482-3p  | 0.764                               | 0.668-0.860  | 0.049         | < 0.0001*     | 0.685|
| hsa-miR-4482-3p + hsa-miR-4725-3p | 0.699                               | 0.597-0.802  | 0.052         | < 0.0001*     | 0.696|
| hsa-miR-145-5p + hsa-miR-376a-5p | 0.986                               | 0.963-1.000  | 0.012         | < 0.0001*     | 0.682|
| hsa-miR-145-5p + hsa-miR-3680-3p | 0.987                               | 0.965-1.000  | 0.011         | < 0.0001*     | 0.683|
| hsa-miR-145-5p + hsa-miR-4253  | 0.988                               | 0.968-1.000  | 0.010         | < 0.0001*     | 0.689|
| hsa-miR-145-5p + hsa-miR-376a-5p | 0.998                               | 0.994-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.987                               | 0.966-1.000  | 0.011         | < 0.0001*     | 0.683|
| hsa-miR-145-5p + hsa-miR-4725-3p | 0.987                               | 0.993-1.000  | 0.002         | < 0.0001*     | 0.703|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.998                               | 0.968-1.000  | 0.010         | < 0.0001*     | 0.696|
| hsa-miR-376a-5p + hsa-miR-4253  | 0.998                               | 0.993-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-4253 + hsa-miR-4482-3p  | 0.842                               | 0.761-0.923  | 0.041         | < 0.0001*     | 0.667|
| hsa-miR-376a-5p + hsa-miR-3680-3p | 0.704                               | 0.591-0.818  | 0.058         | < 0.0001*     | 0.664|
| hsa-miR-376a-5p + hsa-miR-4253  | 0.735                               | 0.632-0.837  | 0.052         | < 0.0001*     | 0.688|
| hsa-miR-376a-5p + hsa-miR-4482-3p | 0.662                               | 0.549-0.776  | 0.058         | < 0.0001*     | 0.698|
| hsa-miR-3680-3p + hsa-miR-4253  | 0.772                               | 0.678-0.876  | 0.048         | < 0.0001*     | 0.688|
| hsa-miR-3680-3p + hsa-miR-4482-3p | 0.593                               | 0.477-0.709  | 0.059         | 0.119         | 0.092|
| hsa-miR-4253 + hsa-miR-4482-3p  | 0.723                               | 0.621-0.825  | 0.052         | < 0.0001*     | 0.685|
| hsa-miR-145-5p + hsa-miR-376a-5p | 0.986                               | 0.962-1.000  | 0.012         | < 0.0001*     | 0.681|
| hsa-miR-145-5p + hsa-miR-3680-3p | 0.986                               | 0.963-1.000  | 0.012         | < 0.0001*     | 0.682|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.986                               | 0.993-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-145-5p + hsa-miR-4725-3p | 0.987                               | 0.966-1.000  | 0.011         | < 0.0001*     | 0.683|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.987                               | 0.993-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-145-5p + hsa-miR-4725-3p | 0.987                               | 0.966-1.000  | 0.011         | < 0.0001*     | 0.683|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.998                               | 0.993-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-145-5p + hsa-miR-4725-3p | 0.998                               | 0.993-1.000  | 0.002         | < 0.0001*     | 0.705|
| hsa-miR-145-5p + hsa-miR-4482-3p | 0.688                               | 0.581-0.795  | 0.055         | < 0.0001*     | 0.683|

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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. 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. 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. Accordingly, disruptions in this gene’s activity are associated with many neuronal pathologies. Specific miRNAs that target single nucleotide polymorphisms in the SLC6A3 gene have been identified in BD bioinformatics studies. 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 and modulates serotonergic signaling by transporting serotonin molecules from the synaptic cleft to the presynaptic terminal for reuse. One study found that the expression of SLC6A4 was significantly higher in patients with schizophrenia and BD. 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.\textsuperscript{32} Comparative analyses in mouse and human brain tissue have shown that miR-15 and miR-16 regulate the expression of SLC6A4.\textsuperscript{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\textsuperscript{34} and methylation status in the promoter region of the gene seem to be effective in determining the pathology of the disease.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{39} and that transmission is increased in mania.\textsuperscript{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 through 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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