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Can miRNA Expression Levels Predict Treatment Resistance to Serotonin Reuptake Inhibitors in Patients with Obsessive-Compulsive Disorder?

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ABSTRACT

Background: Obsessive-compulsive disorder is a psychiatric disorder with different clinical manifestations caused by the interaction of genetic and environmental factors. Recently, it has been shown that microRNAs play a role in the pathogenesis of some psychiatric diseases. We aimed to compare the expression levels of microRNAs between obsessive-compulsive disorder patients and healthy controls and investigate the association between miRNA expression levels and treatment resistance.

Methods: Twelve miRNA expression levels in venous blood of 100 obsessive-compulsive disorder patients and 50 healthy controls were detected by real-time polymerase chain reaction. Patients were assessed using the Hamilton Depression Rating Scale, Yale-Brown Obsessive-Compulsive Scale, and Yale-Brown Obsessive-Compulsive Symptom Checklist. Each patient was scheduled for a monthly follow-up for a minimum 6-month-period after serotonin receptor inhibitor treatments were initiated.

Results: We found that miR-26a-5p ($P < .001$), miR-21-3p ($P < .001$), miR-219a-1-3p ($P = .016$), miR-106b-5p ($P = .039$), miR-6740-5p ($P = .020$), miR-320a ($P = .001$), miR-22-3p, and miR-16b-5p ($P = .010$) expression levels were statistically higher in obsessive-compulsive disorder patients than healthy controls; miR-135a-5p ($P < .001$) and miR-129-6b-5p ($P < .001$) expression levels were statistically lower. Also, it was determined that increased miR-106b-5p levels were associated with treatment-resistance ($P = .020$) and there was a negative correlation between miR-374b-3p and disease severity ($P = .042$).

Conclusion: In obsessive-compulsive disorder, there may be a potential value in the relationship between various miRNA expression levels and treatment resistance and disease severity, and future studies may be beneficial.

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INTRODUCTION

Obsessive-compulsive disorder (OCD) is a psychiatric disorder characterized by the presence of obsessions and/or compulsions, significantly affecting social and daily functionality,[1] and its prevalence is approximately 2%-3%.[2] Although serotonin reuptake inhibitors (SRIs) are known to be the most effective agents in OCD treatment, treatment response varies among patients[3] and it was reported that 40%-60% of patients do not respond completely.[4] Obsessive-compulsive disorder is heterogeneous with different clinical manifestations. These phenotypic differences were suggested to be caused by the interaction of genetic, environmental, and biopsychosocial factors.[5,6] In twin studies, it was reported that genetic factors explain the occurrence of obsessive-compulsive behavior with a 40% variance and the non-shared environment with 51% variance. Thus, it was suggested that in the occurrence of OCD, besides genetic factors, the non-shared environment has an essential role through possible epigenetic factors.[7]

MicroRNAs (miRNAs) are 21-24 nucleotides long, non-coding RNA molecules, recognized as one of the epigenetic modulators.[8] They generally decrease target gene expression at the post-transcriptional level and rarely increase it by binding to the 3' region of mRNA.[9] MicroRNAs are found widely in the central nervous system (CNS) and play an essential role in developing brain and neuronal plasticity. It was reported that alterations in miRNA expression levels

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in different brain regions could lead to changes in neuron structure, circuitry, plasticity, and cognitive functions leading to neurodevelopmental or neurodegenerative disorders.[10] Recently, it has been shown that miRNAs play a role in the pathogenesis of some psychiatric diseases such as bipolar disorder,[11,12] schizophrenia,[13-17] and depression.[18-21]

There are still only a few studies regarding miRNAs in OCD in the literature. These studies have reported that miR-22-3p, miR-24-3p, miR-132, miR-134, miR-106b-5p, miR-125b-5p, miR-155-5p, and miR 485-3p expression levels differed in OCD patients compared to controls.[22-24] However, there are no studies to evaluate the association between miRNA expressions and treatment resistance in OCD.

Revealing the functions of miRNAs, which act as a bridge between the gene and the environment, can help understand the etiology of OCD and the development of effective treatments. In this sense, our study is the first to examine these above relationships. Our study aimed to compare the expression levels of 12 miRNAs that regulate serotonin and glutamate gene regions known to play a role in the etiology of OCD, in untreated OCD patients with a healthy control group. Secondarily, our study determined if baseline miRNAs expression levels may predict treatment resistance in OCD patients.

METHODS

Subjects

One hundred patients between the ages of 18 and 65 (no previous treatment or medication free for at least 1 month) among patients whose primary diagnosis was OCD according to the Diagnostic and Statistical Manual of Mental Disorders- Fifth Edition (DSM-5) diagnostic criteria were included in our study. The sociodemographic data form, 17-item Hamilton Depression Rating Scale (HDRS),[25] Yale-Brown Obsessive-Compulsive Scale (YBOCS),[26] and Yale-Brown Obsessive-Compulsive Symptom Checklist were used. Exclusion criteria of the study were determined as (i) dementia, psychotic disorder, and bipolar disorder; (ii) substance/alcohol abuse in the last 6 months; (iii) active suicide risk; (iv) major physical illness (such as neurological diseases, cancer, cardiovascular diseases, and diabetes) and pregnancy. Insight level was determined according to the 11th item of the YBOCS, and accordingly, it was divided into "good insight" or "poor insight." Symptom dimensions were divided into 5 sub-dimensions: (i) contamination/cleaning; (ii) aggression/sexual/religious obsessions, that is, taboo thoughts; (iii) doubt/checking obsessions; (iv) symmetry/ordering obsessions; and (v) hoarding.[4] About 50 age- and sex-matched healthy volunteers without any psychiatric or physical illness were included in the study. Body mass indexes (BMIs) were recorded before taking blood samples due to being potential confounder for miRNAs. Participants were informed about the study's purpose and design before their informed consent was obtained. The study was approved by the Ethics Committee of the Health Sciences University Şişli Hamidiye Etfal Teaching and Research Hospital (number of commission agreement: 2083/2018).

MAIN POINTS

- miR-26a-5p, miR-21-3p, miR-219a-1-3p, miR-106b-5p, miR-6740-5p, miR-320a, miR-22-3p, and miR-16b-5p expression levels were statistically higher in obsessive-compulsive disorder (OCD) patients than healthy controls.
- miR-135a-5p and miR-129-6b-5p expression levels were statistically lower in OCD patients than in healthy controls.
- Increased miR-106b-5p levels were associated with treatment resistance in OCD.
- There was a significant negative correlation between Yale-Brown Obsessive-Compulsive Scale and miR-374b-3p.

Follow-up

The medication of patients was selected among the SRIs (clomipramine, fluoxetine, sertraline, escitalopram, citalopram, fluvoxamine, and paroxetine) which are first-line treatments for OCD. This preference was made considering the patient's complaints, clinical characteristics, and side-effect profiles of the drugs. Each patient was scheduled for a monthly follow-up for a minimum of a 6-month-period after SRI treatments were initiated. The average follow-up was 6 months to 2 years. Patients were evaluated for treatment resistance at their sixth month visit and separated into treatment-response (n=43) and treatment-resistant (n=35) groups. Twenty-two patients could not be assigned to treatment-resistant or -response groups in the study for the reason of non-compliance (n=18; did not accept drugs or dropped out), and partial compliance (did not receive effective dose) to treatment protocol (n=3) and hypomanic shift (n=1). The severity of the disease was evaluated with the YBOCS during the monthly visits. Treatment resistance was determined as failure to respond to at least 2 SRIs at an effective dose and sufficient time,[27] and treatment response was determined as a 35% or more decrease in YBOCS score. The sufficient time was determined by waiting until the end of the twelfth week after the SRIs were increased to the effective dose in the eighth week.[28] The effective dose was determined as clomipramine 250 mg, fluvoxamine 300 mg, fluoxetine 80 mg, sertraline 200 mg, escitalopram 40 mg, citalopram 40 mg, and paroxetine 60 mg.[27] The maximum dose was determined as clomipramine 300 mg, fluvoxamine 300 mg, fluoxetine 80 mg, sertraline 200 mg, escitalopram 40 mg, citalopram 60 mg, and paroxetine 60 mg in this study.

MicroRNAs Selection Process

Twelve miRNAs (miR 26a-5p, miR 374b-3p, miR 21-3p, miR 6740-5p, 219a-1-3p, miR 320a, miR 106b-5p, miR 129-6b-5p, miR 16b-5p, miR 135a-5p, miR 22-3p, and miR 96b-5p) were selected. During this selection, http:/mirtarbase.cuhk.edu.cn and http://mirdb.org/ sites were also used.

Sample Collection and MicroRNA Expression Method

On the first visit, fasting venous blood samples (5 cc) was collected from the participants between 9.00 AM and 11.00 AM. The samples were stored in DNA/RNA Shield tubes. As required by the tubes' storage condition (+4°C, maximum 1 month), samples were kept in the refrigerator at +4°C, and the samples collected before the end of the period were transferred to the laboratory. Serum was separated (5 minutes at 4500 rpm) from the blood by centrifugation (Allegra® X-30 Series Benchtop Centrifuges, Beckman Coulter, California, USA). Total RNA was obtained from this separated serum using the RNeasy mini kit (Qiagen, Hilden, Germany). The RNA amount was measured with a spectrophotometer (NanoDrop 2000/2000c Spectrophotometer, Waltham, MA, USA). The cDNA was synthesized with miRNA-specific primers, and then miRNA expression levels were evaluated by real-time polymerase chain reaction using SYBR-Green as a dye. RNU6 was used as the reference gene for accurate measurement in the real-time phase. The cDNA samples' expression levels were amplified with primers specific to the miRNAs whose expression levels were desired to be determined, and the expression levels were determined by the standard delta Ct calculation method.

Statistical Analysis

Statistical Package for the Social Sciences version 20 (IBM Corp.; Armonk, NY, USA) was used for statistical analysis. According to power analysis, the sample size of the study was determined to be at least 50 individuals for each group in order to reach 80% power. Descriptive statistics were given as number and percentage for categorical variables, mean, standard deviation, median, and minimum-maximum values. The Pearson's chi-square test was used for differences between categorical variables in independent groups. Comparing numerical variables between 2 independent groups was made using Student's *t*-test under normal distribution conditions and Mann-Whitney *U* test if not met. Analysis of covariance (ANCOVA) was applied by fixing age, gender, and BMI to compare the patient and control groups' miRNA expression levels. Analysis of covariance compared miRNA levels between the sex groups (gender, age, YBOCS score, treatment response, symptom dimensions, insight, and onset) as covariates. Logistic regression analysis was performed to determine the predictors of treatment resistance. Correlation analysis was performed using Pearson's or Spearman's correlation coefficients. Statistical alpha significance level was considered as $P < .05$.

RESULTS

Subjects

Sociodemographic data and clinical characteristics of the patient and control groups are presented in Table 1. There was no difference between the patient and control groups in terms of sociodemographic data (age, gender, BMI, and education, respectively) (P = .105, .806, .702, .378). Other clinical characteristics can be accessed through Supplementary materials. In ANCOVA analysis, miRNA-26a-5p ($\eta p2 = 0.142$, $P < .001$), miRNA-21-3p ($\eta p2 = 0.172$, $P < .001$), miR-6740-5p ($\eta p2 = 0.036$, $P = .020$), miRNA-219a-1-3p ($\eta p2 = 0.038$, $P = .016$), miRNA-22-3p ($\eta p2 = 0.046$, $P = .008$), miRNA-16b-5p ($\eta p2 = 0.044$, $P = .010$), miRNA-106b-5p ($\eta p2 = 0.028$, $P = .039$), and miRNA-320a ($\eta p2 = 0.066$, $P = .001$) expression levels of the OCD patients were statistically significantly higher than the control group; miRNA-129-6b-5p ($\eta p2 = 0.458$, $P < .001$) and miRNA-135a-5p ($\eta p2 = 0.177$, $P < .001$) expression levels were statistically significantly lower, miRNA-96b-5p ($\eta p2 = 0.012$, $P = .181$) and miRNA-374b-3p ($\eta p2 = 0.002$, $P = .609$) expression levels did not differ between OCD and control groups (Figure 1).

Table 1. Sociodemographic Data of the Participants and Clinical Characteristics of the Patients

	OCD (n = 100)	Healthy Controls (n = 50)	*P*
Age, median (min-max)	24 (18-62)	28(18-45)	.105[a]
Gender (female), n (%)	66 (66)	34 (68)	.806[b]
BMI, median (min-max)	22.8 (15.8-36.5)	24 (17.9-36)	.702[a]
Education, median (min-max)	12 (1-16)	11 (5-18)	.378[a]
Age of onset, (years)	18.4 ± 9	-	
Duration of disorder	9.4 ± 8.8	-	
YBOCS-total	26.6 ± 4.9	-	
HDRS	8.4 ± 4.4	-	

[a]Mann–Whitney *U* test.
[b]Chi-squared test.
BMI, body mass index; YBOCS, Yale-Brown Obsessive-Compulsive Scale; HDRS, 17-item Hamilton Depression Rating Scale.

In ANCOVA analysis (gender, age, YBOCS score, treatment response, symptom dimensions, insight, onset as covariates), miR-6740-5p expression levels ($\eta p2 = 0.063$, $P = .012$) in female OCD patients were significantly increased compared to men. While miR-6740-5p expression levels were nearly significant ($P = .051$) in females in the healthy control group compared to males. There was no statistically significant difference between other miRNA levels and sex groups ($P = .108$-$.496$). There was no significant correlation between miRNA levels and BMI in OCD patients ($P = .056$-$.993$). While there was a statistically significant negative correlation between YBOCS-total score and miR-374b-3p ($r = -0.203$, $P = .042$), there was no significant correlation with other miRNA levels ($r = -0.081$-0.103, $P = .308$-$.957$).

Treatment Response and Treatment Resistance

The mean age ($P < .001$), the age of onset ($P = .029$), the duration of the disease ($P = .008$), the YBOCS total score ($P = .033$), the postpartum onset rate ($P = .006$), the

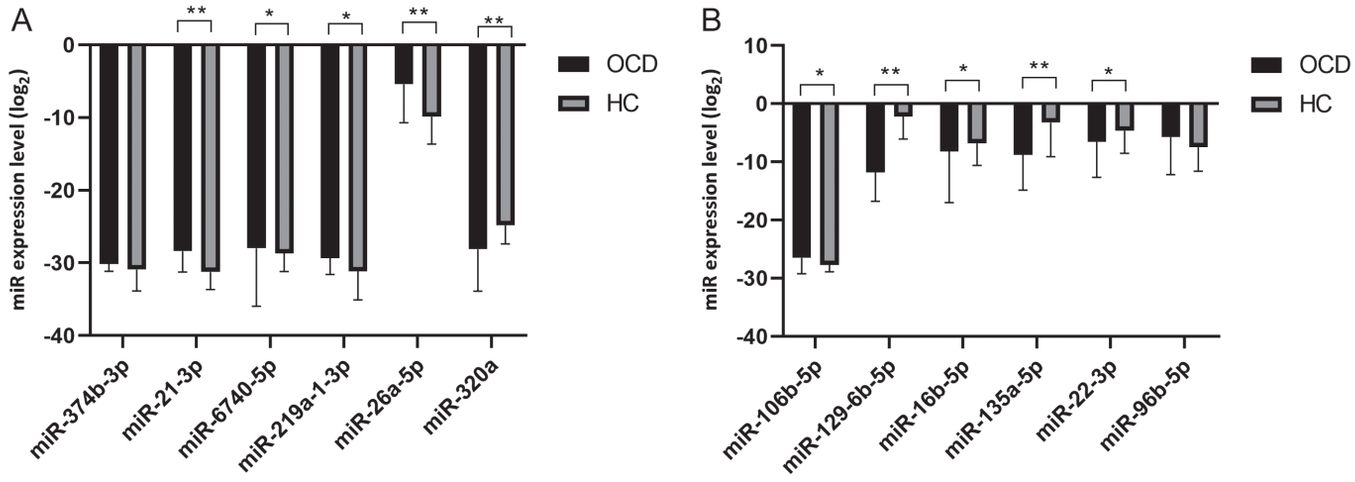

Figure 1. A, B. MicroRNA expression levels of participants. (A) Relative expression levels of miR-374b-3p, miR-26a-5p, miR-21-3p, miR-6740-5p, miR-219a-1-3p, and miR-320a (log_2 transformed) were compared with OCD patients and healthy controls. (B) Relative expression levels of miR-106b-5p, miR-129-6b-5p, miR-22-3p, miR-135a-5p, miR-16b-5p, and miR-96b-5p (log_2 transformed) were compared with OCD patients and healthy controls. $^{*}P < .05$, $^{**}P < .001$. HC, healthy control; OCD, obsessive-compulsive disorder.

depression scores (P = .001), and the previous diagnosis rates (P = .005) of the treatment-resistant patients were higher than the patients who responded to the treatment, while the average years of education were lower (P = .018). However, there was no statistically significant difference in treatment-resistant patients in terms of sex (P = .116), BMI (P = .066), OCD prevalence rates in their family (P = .632), type of insight (P = .239) or symptom dimensions (P = .068-.929), compared to patients who responded to treatment. In ANCOVA analysis, while the expression levels of miRNA-106b-3p were statistically higher in patients who were treatment-resistant compared to those who were not (P = .027), there was no difference in terms of expression levels of other miRNAs (P = .091-.907) (Table 2).

As per the logistic regression analysis, the predictive factors for treatment resistance were late-onset (odds ratio OR=3.440; P = .026), miR-106b-5p expression level (OR=1.307; P = .020), and HDRS score (OR=0.873; P = .048) were statistically significant. This model could predict treatment-resistance at 69.2% overall (χ^2=82.951, Nagelkerke R^2 = 0.359, $P < .001$) (Table 3).

While there was a statistically significant negative correlation between YBOCS-total score and miR-374b-3p (r=−0.203, P = .042), there was no significant correlation with other miRNA levels (r=−0.0081-0.103, P = .308-.957).

DISCUSSION

Our study examined expression levels of 12 miRNAs which have been shown to regulate glutamate and serotonin genes in OCD patients and the relationship between these miRNA levels and resistance to treatment. miR-26a-5p, miR-21-3p, miR-219a-1-3p, miR-106b-5p, miR-6740-5p, miR-320a, miR-22-3p, and miR-16b-5p expression levels were statistically higher in OCD patients than healthy controls; miR-135a-5p and miR-129-6b-5p expression levels were statistically lower. Additionally, it was determined that increased miR-106b-5p levels predict the treatment resistance and there was a negative correlation between miR-374b-3p levels and disease severity.

Although few studies have examined the relationship between OCD and miRNA, so far they have not studied the resistance to treatment. In a previous study, it was found that the miR-22-3p, miR-24-3p, miR-106b-5p, miR-125b-5p, and miR-155-5p expression levels increased in children and adolescents diagnosed with OCD compared to a healthy control group, and there was no difference in miR-18a-5p and miR-107 expression levels.[22] Similar to that study, we found that miRNA 22-3p and miR106b-5p expression levels were increased in OCD patients compared to the control group. It was previously shown that miRNA 22-3p regulates the *RGS2* gene associated with *HTR2C*, *MAO-A*, *BDNF*, and G protein, particularly associated with panic disorder.[29] Yue et al[17] found that miRNA-132 and miRNA-134 expression levels were higher in OCD patients than in the healthy control group. In the same study, it was suggested that miRNA-132 and miRNA-134 regulate synaptic plasticity and *BDNF* expression in OCD so that *BDNF* may be associated with OCD through epigenetic mechanisms.

Similar to our study, an increase in miR-106b-5p expression levels has been shown in schizophrenia,[14] depression,[21] bipolar disorder,[30] and OCD.[22] In contrast, miR-106b-5p expression levels were low in autism[31] and attention deficit hyperactivity disorder (ADHD).[32] In studies, target genes of miRNA-106b-5p have been indicated as HTR2A,[14] and the TGF-ß signal pathway.[33] Our study found that the miR-106b-5p expression levels were significantly higher in treatment-resistant patients than in those who respond to treatment. Our study showed that through miRNAs, epigenetic factors may play a role in predicting

Table 2. Comparisons of Clinical Characteristics and miRNA Expression Levels Between Treatment-Resistant and Treatment-Response Groups

	Treatment Response (n=43)	Treatment Resistant (n=35)	*P*	η_p^2
Age, median (min-max)	22 (18-42)	35 (18-62)	**<.001**[a]	
Gender (female), n (%)	26 (60.5)	27 (77.1)	.116	
BMI, median (min-max)	22.3 (15.8-35.1)	24.7 (16.9-36.5)	.066[a]	
Education, median (min-max)	13 (5-16)	11 (1-16)	**.018**[a]	
Age of onset, median (min-max)	16 (7-41)	20 (3-44)	**.029**[a]	
Duration of disorder	5 (1-22)	10 (1-59)	**.008**[a]	
Insight (good) n, %	33 (76.7)	31 (88.6)	.239	
Postpartum onset	0	6 (17.1)	.006[d]	
First diagnosis	26 (60.5)	10 (28.6)	.005	
OCD in family	22 (51.2)	16 (45.7)	.632	
YBOCS-total	25.67 ± 4.77	28.06 ± 4.87	**.033**[b]	
HDRS	7.09 ± 3.72	10.29 ± 4.59	**.001**[b]	
Symptom dimensions, n (%)				
Contamination/cleaning	24 (55.8)	25 (71.4)	.156	
Doubt/checking	23 (53.5)	18 (51.4)	.856	
Symmetry/ordering	18 (41.8)	15 (42.8)	.929	
Taboo thoughts	19 (52.9)	16 (45.7)	.733	
Hoarding	7 (16.3)	1 (2.8)	.068[d]	
miRNA expression levels (Log_2 transformed)[c] Mean ± SD				
miR26a-5p	−6.1 ± 5.1	−4.2 ± 5.4	.091	0.037
miR-21-3p	−28.4 ± 2.7)	−28.3 ± 1.7	.351	0.011
miR-219a-1-3p	−29.4 ± 2.1	−28.9 ± 2.7	.859	-
miR-106b-5p	−27.3 ± 2.2	−25.9 ± 2.9	**.027**	0.063
miR-320a	−27.1 ± 5.4	−29.2 ± 5.9	.320	0.032
miR-129-6b-5p	−12.3 ± 4.9	−11.2± 5.4	.392	0.01
miR-135a-5p	−8.9 ± 5.9	−8.8 ± 6.4	.854	-
miR-22-3p	−6.2 ± 6.6	−5.9 ± 6.4	.907	-
miR- 96-5p	−6.6 ± 7.1	−4.3 ± 6.5	.236	0.018
miR-16b-5p	−7.1 ± 8.7	−7.3 ± 10.3	.743	0.001
miR-6740-5p	−29.1 ± 11.5	−26.2 ± 3.4	.125	0.031
miR-374-3p	−30.2 ± 1	−29.8± 1.7	.250	0.017

[a]Mann–Whitney *U* test.
[b]Student's *t*-test.
[c]Analysis of covariance (age, gender, age of onset, YBOCS score, insights, and symptom dimensions as covariates).
[d]Fisher's exact test.
BMI, body mass index; YBOCS, Yale-Brown Obsessive-Compulsive Scale; HDRS, 17-item Hamilton Depression Rating Scale; OCD, obsessive-compulsive disorder; miRNA, microRNA.

resistance to treatments with SRIs in OCD. In another study, oxytocin receptor gene hypermethylation was reported to be a significant epigenetic factor in predicting treatment response in OCD patients undergoing cognitive behavioral therapy.[34] In a rat study with OCD modeling, it was reported that a decrease in obsessive-compulsive symptoms was observed after an epigenetic modulating agent (RG108) was given as treatment.[35] Thus, studying epigenetic factors through miRNAs may be promising for future treatment studies.

According to our findings, the miRNA-129-6b-5p expression levels of the patients were significantly lower than those of the control group. A study conducted in cachectic patients with cancer revealed that the target gene of miRNA-129-6b-5p reduces serotonin signaling by *HTR2A* gene regulation and induces myogenesis in this way.[36] Its effects and functions in the CNS are not yet known. Thus, it is early to say anything about the relationship of this miRNA with OCD, and further studies are needed.

Table 3. Predictors of Treatment-Resistant OCD

	P	OR	95% CI	
			Lower	Upper
Early onset	**.026**	3.440	1.160	10.196
miR-106b-5p expression level (log_2)	**.020**	1.307	0.610	0.959
HDRS	**.048**	0.873	0.763	0.999
YBOCS	.090	0.898	0.793	1.017
Constant	.266	0.028		

$\chi^2 = 82.951$, Nagelkerke $R^2 = 0.359$, $P < .001$.
YBOCS, Yale-Brown Obsessive-Compulsive Scale; HDRS, 17-item Hamilton Depression Rating Scale; OR, odds ratio.

In our study, miR-16b-5p expression levels increased in OCD patients compared to the control group, while miR-135a-5p expression levels were found to be decreased. It was reported in previous studies that these 2 miRNAs regulate *SLC6A4* gene expression levels.[20,37] Moreover, in rat studies with depression modeling, it has been shown that the therapeutic efficacy of antidepressants is due to the reduction of SERT expression in the raphe nucleus via miR-16.[37-39] Additionally, miR-16 increases bcl-2 expression in rat hippocampus, increasing neurogenesis.[40] In another study, it was reported that miR-16 levels in the CSF in patients with depression were lower than the control group, and miR-16 levels had a significant positive correlation with serotonin level and a negative correlation with the severity of depression.[41] Unfortunately, no significance as mentioned above was found with miR-16 levels measured from blood in the same study. In another study, a relationship was found between low miR-16 and miR-135a levels and treatment response in patients with depression.[42] Also, it was reported that miR-135 regulates *HTR1A* gene expression alongside *SLC6A4* and that anxiety-depress ive-like behavior was observed in miR-135 knockout rats and they did not respond to antidepressants.[20] However, no relation was found between treatment response and miR-135a-5p and miR-16b-5p in our study. For future studies, it might be interesting to examine the pre- and post-treatment miRNA expression levels which is a limitation in our study.

In our study, miR-26-5p expression levels significantly increased in patients with OCD compared to the control group. It is also known that miR-26a is mainly expressed in nerve cells.[42] Also, miR-26a-5p could be a potential marker for IFN-ß treatment response in multiple sclerosis patients and negatively affected the expression level of the target gene, *SLC1A1*.[43] Another study reported that in Wistar rats with memory loss caused by exposure to stress or scopolamine injection, miR-26 expression levels in the corticolimbic pathway decreased, which was negatively associated with BDNF, c-FOS, p-CREB/CREB levels.[44] Although further studies are needed on this point, miRNA may play a role in inflammation in the CNS and may be related to OCD in these respects.

In our study, there was no change in miR-374b-3p expression levels in OCD patients compared to the control group, but there was a negative correlation between miR-374b-3p expression levels and YBOCS severity. The miR-374 family has been associated with reproductive system diseases, various cancers, and epilepsy.[45] An autopsy study showed that miR-374 levels increased in mesial temporal lobe epilepsy patients with hippocampal sclerosis compared to the control group in samples taken from the hippocampal region.[46] Therefore, it is emphasized that the miR-374 family mainly plays a role in cell growth and differentiation and may play a role in neurodegenerative diseases.[47] Although the exact mechanisms are unknown, the relationship of miR-374 expression levels to disease severity may be related to neurodegeneration.

In our findings, it was found that miR-21-3p was expressed more in OCD patients compared to the control group. In a postmortem study conducted with autistic patients, miR-21-3p expression levels were higher than those of the control group. Thus, miR-21-3p is widely expressed in different brain regions during development and up-regulates the neuronal migration (*PAFAH1B1/LIS1*) and *DLGAP1* genes.[46] Unfortunately, there is no such study yet on OCD and its spectrum.

In our study, miR-219a-1-3p expression levels were significantly higher in OCD patients compared to healthy controls. Dizocilpine, a phencyclidine-like N-Methyl D-Aspartic Acid (NMDA) receptor antagonist, has been shown to downregulate miRNA-219 in the mouse prefrontal cortex significantly.[48] Besides the *GRIN2B* gene, miR-219 was reported to regulate CaMKIIgamma, which is part of the NMDA receptor signaling cascade.[49] All these findings show that miR-219 is a crucial component of the NMDA receptor signaling pathway.[50] It was reported that serum miR-219-1 levels are increased in patients with schizophrenia,[51] and another study[52] found that the presence of miR-219-1 rs107822 SNP reduces the risk of developing schizophrenia. However, miR- 219 has not been previously studied in OCD.

According to our findings, miR-320a expression levels were significantly higher in OCD patients than in the healthy control group. It was stated that miR-320 could be used as a biomarker in various neuropsychiatric diseases.[18,53,54] In studies, miR-320a expression levels were found to be decreased in depression[18] and autism,[54] while it was found to be increased in schizophrenia.[53] There are no data in OCD and further studies are needed.

In our study, miR-6740-5p expression levels were significantly higher in OCD patients than in the control group. Furthermore, female OCD patients had significantly higher miR-6740-5p expression levels than men. The target genes of miR-6740-5p contain glutamate receptor subunits (GRIA2 and GRID2) and GABA receptor subunits.[55] In some studies, differences in clinical and genetic characteristics have been noted between female and male OCD

patients.[56-58] Although there are not enough studies yet, it can be thought that miR-6740 may be related to the female, and further studies should focus on this issue.

There were some limitations in our study. First, the limited and relatively known miRNA expression levels were observed; as the knowledge of miRNAs increases, the relationship between more miRNAs and OCD could be revealed more clearly. Since comorbid conditions such as depression and anxiety disorders were not excluded, this may have been a confounding factor in miRNA levels. In the treatment of OCD, clomipramine is slightly superior to selective SSRIs in efficacy but worse in terms of tolerability. Therefore, SSRIs and clomipramine are accepted as first-choice drugs in OCD. In our study, we treated with various drugs considering the side-effect profiles of the drugs and the clinical characteristics of the patients. This may have caused a limitation by affecting the response to treatment. Last, a longitudinal study with a large sample size on this subject may be more enlightening to support our results.

In conclusion, our study revealed various miRNA expression levels are different in OCD compared to healthy controls. Besides, miR-106b-5p was found to be associated with treatment-resistance and miR-374b-3p with disease severity. It is increasingly essential to reveal epigenetic factors though miRNAs may be important in the development of OCD and understanding phenotypic differences. In further studies, miRNA studies may reveal data which may help us to determine the disease's course, predict treatment, and individualized response treatment methods.

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