Original Article

MicroRNA-127 and MicroRNA-132 Expression in Patients with Methamphetamine Abuse in East Azerbaijan, Iran: A Case-Control Study

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Abstract

Background: Addiction is a personal and social problem worldwide, and has physical and psychological effects on consumers' health. Recently, miRNAs have been described as noninvasive biomarkers. Currently, methamphetamine abuse (MA) is mainly diagnosed by chromatography. This study aimed to investigate the expression and diagnostic value of miR-127 and miR-132 in blood samples of patients with MA and non-user healthy controls.

Methods: A total of 60 patients with MA (case group) and 60 non-user healthy individuals (control group) were selected from Tabriz, East Azerbaijan, Iran. Peripheral blood was obtained and total RNA was extracted. Then, cDNA synthesis was performed and miR-127 and miR-132 expression was evaluated using real time polymerase chain reaction (PCR) method.

Findings: The results of this study demonstrated that miR-127 was significantly lower (0.042-fold change) in patients with MA than in the control group ($P<0.05$). However, miR-132 was significantly higher (7.1-fold change) in patients with MA than in the control group ($P<0.05$).

Conclusion: In general, expression of miR-127 and miR-132 may alter in patients with MA. Further studies are needed to identify underlying molecular mechanisms in patients with MA.

Keywords: Addiction, Methamphetamine, MicroRNA-127, MicroRNA-132, Biomarker

Introduction

Drug addiction is a chronic cerebral disorder and a major problem in the world.[1] Methamphetamine is a readily available and highly addictive psychostimulant. In recent years, methamphetamine abuse (MA) has become more popular worldwide.[2] Almost 1.8 million people in the United States had used methamphetamine at some point in their lives by the end of 2017.[3] Therefore, the introduction of biomarkers for diagnosis of MA has become an important issue.

Rapid, easy, and low-cost diagnosis of MA or other types of drug abuse using blood factors or molecular biomarkers is important in forensic laboratory. Currently, drug abuse diagnosis is made by liquid or gas chromatography of the whole blood serum.[4] However, these methods are time-consuming and require specific instruments. Thus, it is essential to identify and develop biomarkers for rapid and inexpensive diagnosis of addiction.

MicroRNAs are 20-22 nucleotide non-coding RNAs that regulate target genes expression.[5] In recent years,

miRNAs have attracted attention as regulators of neurobiological pathways associated with addiction. In this regard, previous studies indicated that numerous miRNAs are involved in amphetamine, cocaine, morphine, and alcohol abuse.[6,7]

In a study by Kenny, it was indicated that miRNAs, such as miR-127 and miR-132, modify motivational characteristics of substance abuse.[8] Moreover, Sánchez-Mora et al reported that miRNAs play an important role in drug intake through regulation of downstream signaling pathways that influence rewarding characteristics of addictive drugs.[9] However, the role of miRNAs in MA is still unclear. Several neurological mediators are introduced as targets of miR-127 and miR-132 that engage in synaptic transmission, angiogenesis, and inflammation.[10,11]

So far, no systematic study has been conducted in Iran to investigate miR-127 and miR-132 expression in MA. Therefore, this study aimed to investigate the differential expression of miR-127 and miR-132 in Iranian Azeri

patients with MA using a case-control study.

Methods
Participants and sampling
In the present case-control study, 120 men were selected from Alzahra hospital in Tabriz, Iran in 2018-2019. All studied participants aged 20-40 years. The case group included 60 patients newly diagnosed with MA who had not received any treatment for drug abuse. The patients with a chronic disease, cardiovascular disease (CVD), and major brain and psychiatric disorders were excluded from the study. Those abusing drugs and substances other than methamphetamine were also excluded. The control group consisted of 60 gender- and age-matched healthy participants who received routine physical examination and health check-up. All members of case and control groups were selected from East Azerbaijan in Iran and were homogeneous in terms of ethnicity and age. The demographic characteristics of patients with MA and those in the control group (age, gender, education level, marital status, syphilis infection status, and drug use history) were collected.

RNA extraction, polyadenylation, and cDNA synthesis
The peripheral blood was obtained from all participants after 12 hours of fasting and RNA was extracted using RNA extraction kit (GeneAll Biotechnology, South Korea) according to the manufacturer's instructions. The quantity and quality of RNA samples were assessed by NanoDrop (Thermo Scientific, USA) and electrophoresis on 1% agarose gel. The polyadenylation reaction was performed following RNA extraction by Poly (A) polymerase in 37°C (30 minutes), and then 65°C (20 minutes). Then, cDNA synthesis (Thermo Fisher, USA) was performed using polyadenylated RNA and BON-RT adaptor primers. Then, cDNA synthesis was repeated in 16°C (30 minutes), 42°C (30 minutes), and 85°C (5 minutes).

Quantitative real-time polymerase chain reaction (PCR)
TaqMan probe-based RT-qPCR was applied (in triplicate) to verify miR-127 and miR-132 expression in blood samples of the participants. The reaction was carried out in 15 μL total final volume: 1.5 μL of cDNA, 7.5 μL of master mix buffer (containing DNA polymerase, dNTPs, SYBR Green, and $MgCl_2$), 0.5 μL of each primer, and 4.5 μL of DEPC water. The thermal cycles are as follows: 1 cycle for initial denaturation (94°C for 1 minute), 45 cycles for denaturation (94°C for 10 seconds), annealing (for 30 seconds), and extension (72°C for 20 seconds). The used primer sequences are Has-miR-127-F-3'-GAACTGAATCTCAGAGGG-5' and Has-miR-132-F-3'-GCGTAACAGTCTACAGCC-5'. Threshold cycle (CT) was determined for each sample. Expression levels of both miR-127 and miR-132 were normalized to U6.

Statistical analysis
The data were analyzed using SPSS software (version 21.0) and GraphPad Prism software and the results were presented as mean±SEM or mean±SD. The expression levels of miR-127 and miR-132 in patients with MA and those in the control group were normalized to U6. The difference between miR-127 and miR-132 expression levels in both groups were analyzed by Pearson correlation coefficient. Moreover, the difference between demographic and clinical characteristics in both groups were analyzed using t test and chi-square test. The level of significance was set at $P < 0.05$.

Results
Participant characteristics
The demographic and clinical characteristics of the studied patients with MA and non-user controls are demonstrated in Table 1. The results showed there was a significant difference between the two groups in marital status as well as syphilis infection status ($P < 0.05$) while no significant difference was observed between patients with MA and those in the control group in body mass index (BMI) and education level ($P > 0.05$).

Expression of miRNAs
The results of comparing miR-127 and miR-132

Table 1. Demographic characteristics of patients with MA and controls

Variable	Case group (n = 60)	Control group (n = 60)	*P* value
Age, years	28.41±2.51	32.73±3.22	<0.001
BMI, kg/m²	22.19±2.18	22.34±2.55	0.529
Marital status			
Married, No. (%)	28 (46.6%)	42 (70.0%)	
Single, No. (%)	18 (30.0%)	12 (20.2%)	0.008
Divorced, No. (%)	14 (23.3%)	6 (10.0%)	
Educational degree			
High school diploma or lower, No. (%)	44 (73.3%)	36 (60.0%)	0.265
Higher education, No. (%)	16 (26.6%)	24 (40.0%)	
Drug use history			
Onset age of drug use (y)	24.78±2.28	-	-
Drug use duration (y)	4.56±3.24	-	-
Frequency of drug use (per day)	1.87±2.11	-	-
Drug manner			
Injection, No. (%)	4 (6.6%)	-	-
Oral inhalation, No. (%)	56 (93.3%)	-	-
Syphilis infection status			
Positive, No. (%)	8 (13.3%)	0 (0.0%)	<0.001
Negative, No. (%)	52 (86.6%)	60 (100.0%)	

BMI, body mass index.
$P < 0.05$ is statistically significant.

expression in patients with MA and non-user controls are presented in Figure 1. The results showed there was a significant difference between patients with MA and controls in miR-127 and miR-132 expression ($P < 0.05$). In case group, miR-127 expression significantly decreased as compared to control group ($P = 0.026$) and miR-132 expression significantly increased ($P = 0.048$).

Diagnostic potential of miRNAs

The potential diagnostic values of miR-127 and miR-132 were evaluated by receiver operating characteristic (ROC) curves. In ROC curve analysis of miR-127, the score of the area under the curve (AUC) was 0.712 to discriminate patients addicted to MA from controls (Figure 2A). In ROC curve analysis of miR-132, AUC score was 0.753 (Figure 2B). The results suggested that miR-127 and miR-132 could not be used to definitively diagnose MA but could be used as profiles in treatment and recovery of these patients.

Discussion

Evidence has demonstrated that several miRNAs influence the neurobiological function (synaptic plasticity and neurogenesis) which is important for potential diagnosis and treatment of drug abuse.[10-12] In addition, numerous brain-enriched miRNAs play a critical role in drug abuse through several pathways such as synaptic remodeling and dendritic spine morphogenesis.[9,13] Therefore, the development of miRNAs that are associated with drug abuse can be important for management of addiction. Evidence has also suggested that brain-related miRNAs can be used as diagnostic biomarkers for drug abuse and various mental disorders.[14,15] Particularly, numerous studies have reported significant differences in miRNA between healthy individuals and patients with physical or psychiatric disorders.[16,17] However, alterations of miRNAs expression caused by MA are still unclear. Therefore, identifying miRNAs that are associated with MA can be helpful for development of various therapeutic approaches for this public problem.

This study analyzed the levels of miR-127 and miR-132 in Iranian Azeri patients with MA using Quantitative real-time PCR. It was shown that miR-127 expression significantly decreased and miR-132 expression significantly increased in Iranian Azeri patients with MA. Moreover, the results indicated that altered miR-127 and miR-132 expression can be a potential diagnostic biomarker for MA disorder.

Previous studies showed that expression of miRNAs altered significantly in patients with MA or other types of drug abuse.[18-20] Gu et al reported that miRNA-9-3p significantly increased in patients with MA compared with non-user healthy controls.[18] Zhao et al indicated that expression of plasma levels of miRNA15b, miRNA181a, miRNA-let-7d, and miRNA-let-7e in patients with MA
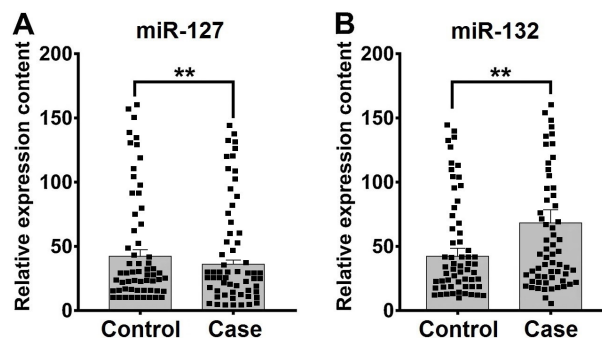

Figure 1. Relative expression levels of miR-127 (A) and miR-132 (B) in patients with MA and non-user healthy controls (**$P < 0.01$)
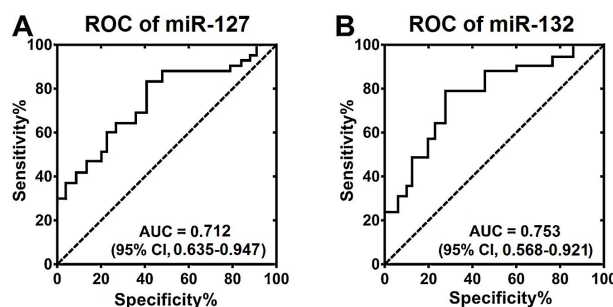

Figure 2. Potential diagnostic values of miR-127 (A) and miR-132 (B) for MA based on ROC analysis

significantly decreased compared to non-user healthy controls.[19] Furthermore, Zhang et al demonstrated that chronic methamphetamine use downregulates miRNA-181a expression.[20] Similar alterations in miR-127 and miR-132 expression in patients with MA in the present study and other studies reflect the potential role of these miRNAs in regulation of MA. However, the exact role of miRNAs is still unknown in patients with MA. Accordingly, some studies showed several miRNAs are associated with MA through numerous signaling pathways such as CREB, GnRH, and MAPK.[21] However, further investigations are required to determine the role of miR-127 and miR-132 in patients with MA. Moreover, future longitudinal studies are needed to explore the potential diagnostic value of miRNAs for MA disorders.

Conclusion

The results of the present study showed that expression of miR-127 and miR-132 may alter in patients with MA and serve as a diagnostic biomarker. However, further studies are needed to identify the genetic pathways or specific targets of miR-127 and miR-132 in patients with MA. Targeting miR-127 and miR-132 may be a useful approach for developing novel therapeutic methods.

Author Contributions
HS conceived the study; HS and HM designed the study; SRM

and ZA wrote the manuscript; HS and SF edited and revised the manuscript; all authors approved the final version of the manuscript.

Conflict of Interests
The authors declare no conflict of interest.

Ethical Approval
This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.IAU.TABRIZ.REC.1398.082).

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