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High Expressions of MicroRNA-143 in Patients With Methamphetamine Abuse Disorder: A Case-control Study

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ABSTRACT

Background: Chronic drug abuse changes microRNA (miRNA) expression in the brain, which may contribute to addictive behaviors. Many miRNAs play critical roles in developing drug addiction. Methamphetamine induces various alterations in different systems by affecting gene expression, but the effects of methamphetamine on miRNA profiles need to be elucidated. This study evaluated the expression of miRNA-183 and miRNA-143 in the blood of methamphetamine abusers and controls.

Materials and Methods: In this case-control study, the case group comprised 60 people with a methamphetamine addiction from Tabriz City, East Azerbaijan, Iran, and the control group comprised 60 healthy controls of comparable ages and ethnicities. Total RNA was extracted from peripheral blood samples, and then cDNA was synthesized. MicroRNA-183 and microRNA-143 expression levels were determined using real-time PCR.

Results: The results indicated that methamphetamine abusers had significantly higher blood levels of miRNA-143 than healthy controls ($P<0.05$); however, miRNA-183 expression was comparable between the two groups ($P>0.05$).

Conclusion: MicroRNA-143 may play a role in the pathology of methamphetamine abuse, so it may be used as an additional method to identify people with an addiction precisely.

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Introduction

In recent years, substance addiction has been recognized as a persistent and incapacitating brain disorder [1], which is regarded as one of the most financially burdensome illnesses in modern society. Methamphetamine, a psychostimulant, possesses properties that make it readily producible, broadly accessible, and highly susceptible to inducing addiction [2]. In addition, the production process is simple and can be done with inexpensive materials. In recent years, methamphetamine abuse has increased significantly, which needs a global response to combat its abuse [3]. According to the available data, approximately 34 million people consumed methamphetamine by the end of 2012 [4]. Consequently, developing effective diagnostic and treatment modalities has become a priority.

The analysis of blood samples is utilized to diagnose and monitor substance abuse in different industries. Generally, gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry techniques are used to detect and confirm the presence of abused drugs and their metabolites in the whole blood, serum, or plasma of drug users [5, 6]. However, executing these procedures requires a significant time commitment and specialized instruments. There is still a considerable need for objective and easily accessible blood biomarkers.

MicroRNAs (miRNAs) are a category of noncoding RNAs composed of 20 to 22 nucleotides. They bind to the 3'-untranslated regions (3'-UTR) of specific mRNA targets to regulate gene expression. MicroRNAs exert their regulatory function by inhibiting or halting the translation process of their target genes [7]. Due to their role as mediators in numerous neurobiological processes, including brain development, neuronal function, and synaptic plasticity, which has been linked to addiction, miRNAs have garnered considerable interest. According to recent studies investigating the correlation between miRNA dysfunction and addiction, miRNAs contribute to substance use disorders, such as alcohol, morphine, cocaine, and amphetamine addiction, as well as amphetamine-induced psychosis. However, the connection between miRNAs and methamphetamine use disorder has not yet been established [8-10].

MicroRNA-183 and microRNA-143 target the mediators of angiogenesis, inflammation, synaptic transmission, and neurological development [11, 12]. Previous studies have reported that the expression of both miRNA-183 and miRNA-143 are altered in patients with drug abuse disorder [13, 14]. However, detailed information about the expression pattern of miRNA-183 and miRNA-143 in patients with methamphetamine abuse is unavailable.

The investigation of miRNA-183 and miRNA-143 expression in Iran's methamphetamine-using population has not been exhaustively examined. This study examines the differential expression of miRNA-183 and miRNA-143 in the blood of methamphetamine-using Iranian-Azeri men. The research employed a case-control design.

Materials and Methods

Patients and sample collection

The case-control study was conducted on 120 male individuals from educational institutions in Tabriz, Iran, in 2018 and 2019. The study exclusively included male individuals within the age range of 20 to 40 years. The case group comprises a sample of 60 individuals who have recently been diagnosed with methamphetamine dependence and have not undergone any form of substance abuse treatment. The study excluded individuals with severe medical conditions, including chronic ailments, neurological diseases, significant psychological issues, or cardiovascular conditions. Individuals who engaged in substance abuse, excluding methamphetamine, were also deemed ineligible for participation. The control group comprised 60 individuals deemed physically fit and matched in gender and age. These individuals underwent a standardized physical examination and health assessment. The study sample consisted of individuals recruited from the East Azerbaijan Province in Iran. The participants were carefully matched based on age and ethnicity, ensuring they had no familial or genetic relationships. Questionnaires and interviews were utilized to collect data on the participants' clinical characteristics, lifestyle, and demographic information. The data collected encompassed various demographic variables such as age, gender, literacy levels, marital status, syphilis infection status, and history of substance use. All participants were presented with comprehensive details about the study and were requested to sign a consent form, adhering to the ethical guidelines outlined in the Helsinki Declaration.

RNA extraction, polyadenylation, and cDNA synthesis

After a 12-h overnight fast, 5 mL of peripheral blood was extracted from each participant in the case and con-

Table 1. The clinical features and demographic characteristics of the studied patients with methamphetamine abuse and healthy controls

Variables		Mean±SD/No. (%)		P
		Case (n=60)	Control (n=60)	
Age (y)		28.41±2.51	32.73±9.22	0.097
Body mass index (kg/m^2)		22.19±2.18	22.34±2.55	0.529
Marital status	Married	28(46.6)	42(70.0)	0.008
	Single	18(30.0)	12(20.2)	
	Divorced	14(23.3)	6(10.0)	
Educational degree	Under diploma and diploma	44(73.3)	36(60.0)	0.265
	Higher diploma	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	-	-
	Drug use (per day)	1.87±2.11	-	-
Drug manner	Injection	4(6.6)	-	-
	Oral inhalation	56(93.3)	-	-
Syphilis infection status	Positive	8(13.3)	0(0.0)	<0.001
	Negative	52(86.6)	60(100.0)	

Note: P<0.05 is statistically significant.

trol groups and deposited in vials containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). The RNA extraction reagent (GeneAll Biotechnology, Germany) was used to perform complete RNA extraction in accordance with the manufacturer's instructions. The quantity and purity of extracted RNA were determined using a NanoDrop device and 1% agarose gel electrophoresis. RNA samples were kept at -20°C until expression analysis. The poly-A polymerase enzyme was used to perform polyadenylation at 37°C for 30 minutes and 65°C for 20 minutes, following RNA extraction. With polyadenylated RNA and BON-RT adaptor primers, cDNA was then synthesized. For the synthesis of cDNA, the reaction mixtures were held at 4°C after being heated to 16°C for 30 min, 42°C for 30 minutes, and 85°C for 5 min.

Quantitative real-time RT-PCR

TaqMan probe-based RT-qPCR was used to validate the expression of miRNA-183 and miRNA-143 in triplicate blood samples from study participants. The reaction mixture contained 0.5 µL of each primer, 1.5 µL of cDNA, 4.5 µL of DEPC water, and 7.5 µL of PCR buffer for a total volume of 15 µL. One cycle (94°C for 1 min), 55 cycles (94°C for 10 s), annealing (30 s), and extension at 72°C for 20 s were performed in this order. Primers used were Has-miR-143-F-ACTGTTGAGATGAAGCACT and Has-miR-183-F-TGACTATGGCACTGGTAGA. The relative expression levels of miRNA-183 and miRNA-143 were normalized to U6 using the $2^{-\Delta Cq}$ method.

Statistical analysis

The acquired data were analyzed using GraphPad Prism software, version 6 and the SPSS software, version 21. The results are presented as the Mean±SE of the mean for miRNAs and the Mean±SD for all other variables. The expression levels of miRNA-183 and miRNA-143 in the case and control groups were normalized using U6 expression. The Pearson correlation analysis determined the miRNA-183 and miRNA-143 expression variation between the case and control groups. Using the independent sample t-test and the chi-square test, the difference between the case and control groups' demographic and

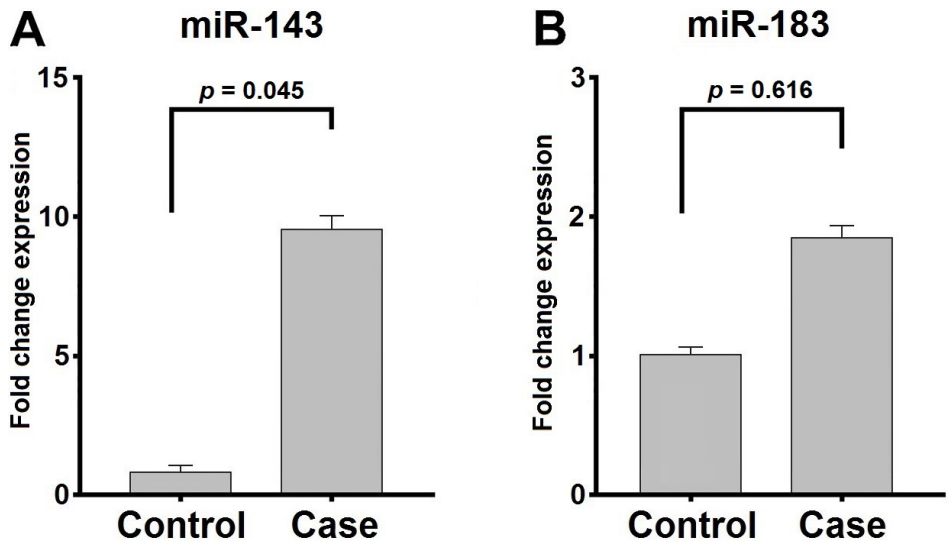

Figure 1. The relative expression levels of the miRNA-183 (P=0.045) and miRNA-143 (P=0.616) in the patients with methamphetamine abuse and healthy controls

clinical characteristics was analyzed. The statistical significance was set at P>0.05.

Results

Participants characteristics

Table 1 presents the demographic and clinical characteristics of the patients under study and the healthy control group. There were statistically significant differences between the patient group and the healthy control group regarding age, marital status, and the presence of syphilis (P<0.05). However, no statistically significant differences were found in body mass index (BMI) and educational attainment between the two groups (P>0.05).

Expression of miRNAs

Figure 1A compares the expression levels of miRNA-183 and miRNA-143 in individuals who use methamphetamine and healthy control. The expression level of miRNA-143 exhibited a statistically significant 9.3-fold increase in patients with methamphetamine misuse when compared to a control group of healthy control (P=0.045). Furthermore, despite lacking statistical significance (P=0.616), the methamphetamine abusers

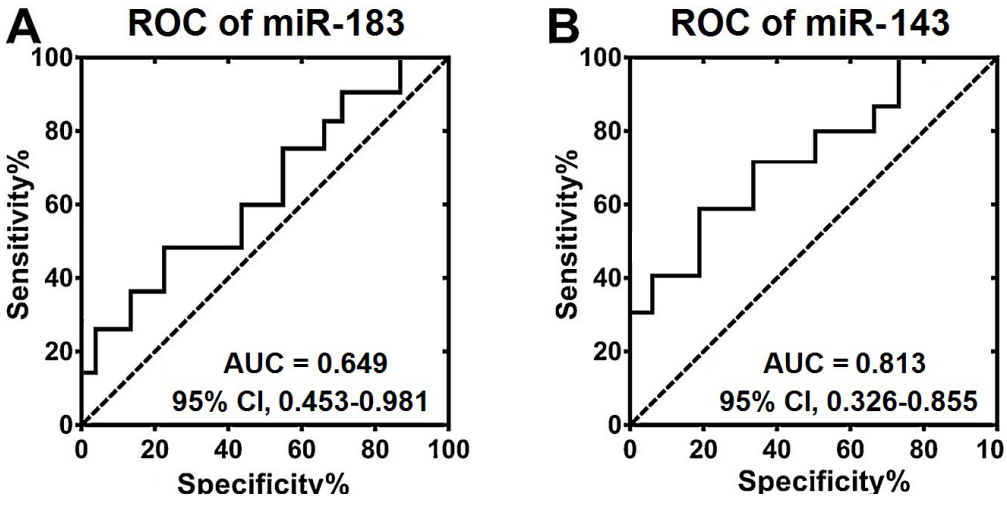

Figure 2. The discriminative ability of the altered expression in the patients with methamphetamine abuse and healthy controls by ROC analysis

A) miRNA-183, B) miRNA-143

exhibited a 1.8-fold increase in the expression level of miRNA-183 compared to the healthy control.

Diagnostic ability of miRNAs

Receiver operating characteristics (ROC) curve analysis was employed to assess the diagnostic efficacy of miRNA-183 and miRNA-143 in identifying methamphetamine addiction among patients. The investigation of the ROC curve for miRNA-143 in distinguishing methamphetamine-dependent participants from controls yielded an area under the curve (AUC) value of 0.712 (Figure 2A). Furthermore, a ROC curve analysis was conducted to assess the discriminatory ability of miRNA-183 in distinguishing individuals with methamphetamine dependence from control subjects. The AUC was calculated to be 0.75, as depicted in Figure 2B. The results of this study suggest that miRNA-143 has potential as a biomarker for the detection of methamphetamine users.

Discussion

Evidence indicates that dysregulated miRNAs in neural and brain tissues affect various neurobiological processes, including neurogenesis and neuronal function, particularly synaptic plasticity, linked to substance addiction [15-17]. Several brain-enriched miRNAs, including miRNA-183 and miRNA-143, impact the development of dendritic spines, the remodeling of synapses, the pleasurable effects of drugs, the behavior of seeking drugs, and the rates of alcohol self-administration [18]. The relationship of miRNAs with addiction, which is a distinctive characteristic of the human species, holds substantial clinical implications. An expanding body of literature [19, 20] suggests that miRNAs present in both the brain and blood could potentially function as biomarkers for mental disorders and addiction-related conditions. Several studies [21, 22] have provided evidence indicating variations in miRNA levels in the bloodstream between individuals in good health and those diagnosed with different physical and mental conditions. The impact of methamphetamine use on miRNA expression has remained ambiguous. To gain a comprehensive understanding of the mechanisms underlying substance addiction and to devise effective therapeutic interventions for the substantial public health issue of methamphetamine use, it is imperative to ascertain the precise miRNAs that are intricately linked to this phenomenon.

In this study, we utilized quantitative reverse transcription polymerase chain reaction (qRT-PCR) to investigate the concentrations of miRNA-183 and miRNA-143 in the blood samples of Iranian Azeri individuals with methamphetamine dependence. Our study revealed a statistically significant association between methamphetamine use and elevated expression of the miRNA-143 gene in these addicts. Furthermore, the statistical analysis demonstrated that altered levels of miRNA-143 can be utilized as a serological index for the detection of methamphetamine consumption disease in forensic and clinical settings.

The impact of blood levels of miRNAs on methamphetamine and other substance abuse disorders has been noted to be significant [23-25]. In a recent study conducted by Gu et al., it was observed that individuals who use methamphetamine exhibited significantly elevated serum levels of miRNA-9-3p compared to a control group consisting of healthy controls [23]. According to Zhao et al., the plasma levels of miRNA-15b, miRNA-181a, miRNA-let-7d, and miRNA-let-7e were significantly reduced compared to those observed in individuals without health conditions [24]. Furthermore, Zhang et al. (2020) have provided evidence indicating that the long-term use of methamphetamine leads to a reduction in the expression of miRNA-181a [25]. The present investigation revealed that individuals who use methamphetamine exhibited elevated levels of miRNA-143, thereby implying a potential heightened involvement of miRNAs in the regulation of methamphetamine addiction. The specific pathogenic and physiological roles of miRNAs in individuals with methamphetamine dependence are not well understood. Further analysis of canonical pathways in this particular context has demonstrated that most miRNAs associated with methamphetamine addiction are linked to signaling pathways such as MAPK, CREB, G-protein coupled receptors, and GnRH [26, 27].

Due to the small sample size in this study, further investigation is necessary to ascertain the potential pathological or physiological functions of miRNA-143 in individuals who use methamphetamine. We also did not investigate the possible confounding factors such as diet, exercise, and other lifestyle factors that may affect miRNA expression. Moreover, we only examined the expression of two miRNAs, and there may be other miRNAs that are differentially expressed in methamphetamine abusers. In addition, we did not investigate potential mechanisms underlying the differential expression of miRNA-143 in methamphetamine abusers. It is imperative to undertake systematic longitudinal research to investigate potential biomarkers that can be utilized for the diagnosis and prognosis of diseases associated with methamphetamine addiction.

Conclusion

Our research results suggest that miRNA-143 could potentially contribute to the pathogenesis of methamphetamine addiction and serve as a viable peripheral blood biomarker for the detection of methamphetamine abuse. Further investigation is required to ascertain the genetic pathways or specific targets that are modulated by miRNA-143 in individuals who abuse methamphetamine. Exploring miRNA-143 as a potential target could aid in developing innovative therapeutic strategies.

Ethical Considerations

Compliance with ethical guidelines

All participants were informed about the study details and were requested to sign a consent form, following the ethical guidelines outlined in the Helsinki Declaration. The present study was approved by the Ethics Committee of Tabriz University of Medical Sciences (Code: IR.IAU.TABRIZ.REC.1398.082).

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Authors contribution's

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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