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Evaluation of several micro RNA (miRNA) levels in children and adolescents with attention deficit hyperactivity disorder

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HIGHLIGHTS

- miRNA 18a-5p, 22-3p, 24-3p, 106b-5p and 107 levels were decreased in ADHD.
- miRNA 155a-5p levels were increased in ADHD.
- miR-107 may be a candidate biomarker for ADHD.
- Dysregulation of circulating miRNAs may affect ADHD etiology and treatment.

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ABSTRACT

Attention-deficit/hyperactivity disorder (ADHD) is one of the most prevalent childhood disorders, although disorders etiology and pathogenesis remains unknown, several theories about ADHD development have been proposed and many researchers believe that it is caused by both genetic and environmental factors. In this study we evaluated miR18a-5p, miR22-3p, miR24-3p, miR106b-5p, miR107, miR125b-5p and miR155a-5p levels in child and adolescent ADHD patients. The research sample consisted a group of 52 ADHD patients, and 52 healthy volunteer controls. There was no significant difference in age and sex between the two groups ($p > 0.05$). miRNA 18a-5p, 22-3p, 24-3p, 106b-5p and 107 levels were statistically significantly decreased in ADHD patients($p < 0.05$). miRNA 155a-5p levels were increased in patients group ($p < 0.05$). The positive predictive value (PPV) and negative predictive value of miR107 was estimated for the cutoff point of 0.4480. PPV was 70% and NPV was 86.5% for the taken cut off point. There could be a close relationship between levels of circulating miRNAs and ADHD. If we could understand how the signaling pathways arranged by miRNAs, impact on CNS development, function and pathology this can improve our knowledge about ADHD etiology and treatment.

1. Objective

Attention-deficit/hyperactivity disorder (ADHD) is characterized by developmentally inappropriate levels of inattention, hyperactivity, and impulsivity [1]. It is one of the most prevalent childhood disorders, occurring in 3–7% of school-aged children and representing one third to one half of referrals to child mental health services [2]. ADHD is more common in boys than girls, with ratios ranging from 3:1 to 10:1 [3]. A large proportion of children with ADHD are diagnosed with another psychiatric disorder [4]. Overwhelming evidence suggests that ADHD is not a childhood condition but a lifelong disorder [5].

Although the etiology and pathogenesis of ADHD remain unknown, several theories have been proposed, and many

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Table 1
Some of the features of the studied miRNAS.

miRNA	Rationale for study	Previous studies	Reference
miR18a-5p	Believed to be involved in DNA damage in ADHD	Altered in DNA damage response	[41]
miR22-3p	Believed to regulate four candidate genes: BDNF, HTR2C, MAOA, and RGS2	Found to be altered in Panic Disorder	[22]
miR24-3p	Believed to be related with oxidative stress which is a potential neurobiological mechanism in ADHD	Found to be altered in oxidative DNA damage and lipid peroxidation	[42]
miR106b-5p	Believed to be related with oxidative stress which is a potential neurobiological mechanism in ADHD	Down regulated in resveratrol treatment that has antioxidant properties	[43]
miR107	Believed to be related with minimal brain change in ADHD	Altered in traumatic brain injury and neurodegenerative diseases	[30]
miR125b-5p	Believed to be related with hypoxia in ADHD	Altered in high altitude sickness	[44]
miR155a-5p	Believed to be related with prefrontal cortex pathophysiology ADHD	Altered in depression	[32]

researchers believe it is caused by both genetic and environmental factors [6]. Segregation analysis of data from neurochemical studies in families comprising genetic, twin, and adoption relationships strongly suggest a genetic etiology [7]. Substantial genetic influence on the disorder has been identified, with heritability estimates ranging from 60 to 90% [8]. Studies have identified abnormal regulation of neurotransmitter systems, particularly dopamine [9]. Preliminary molecular genetic studies have implicated several candidate genes, including the dopamine D_2 and D_4 (DRD4-7) receptors as well as the dopamine transporter (DAT-1) [10]. The presence of the L allele of the serotonin transporter gene 5-HTTLPR is associated with decreased serotonin levels and increased risk of ADHD [11].

MicroRNAs (miRNAs) are evolutionally conserved small non-coding RNAs that regulate approximately 30% of human protein coding gene expression at the post-transcriptional level, and they play important roles in a wide variety of biological functions [12]. miRNAs control gene expression by inhibiting translation or facilitating degradation of their target mRNAs. Computational predictions indicate that thousands of genes could be targeted by miRNAs in mammals [13]. miRNAs are important for maintaining homeostasis at the neuromuscular junction. They also play an important role in synaptic plasticity in the central nervous system, and are involved in memory and mental retardation [14].

The ability of miRNAs to affect the activity of all biological pathways may underlie some of the difficulties associated with linking psychiatric disorders to specific causative genes [15]. The involvement of multiple signaling pathways in psychiatric disease complicates both the investigation of the underlying biological causes and efforts to identify effective therapies. Focusing on the roles of miRNAs in psychiatric diseases may lead not only to an explanation of the dysregulation of multiple pathways but also to novel therapies that can target entire gene networks. Perkins et al. examined the expression of 16 miRNAs in the prefrontal cortex in subjects with schizophrenia or schizoaffective disorder and found decreased expression of 15 miRNAs in patients with schizophrenia [16]. However, although miRNAs have been shown to be particularly abundant in the brain, their role in the development and activity of the nervous system remains largely unknown. In this study, we evaluated miR18a-5p, miR22-3p, miR24-3p, miR106b-5p, miR107, miR125b-5p, and miR155a-5p levels in children and adolescents with ADHD. In selection of the miRNAs previous literature pointing out the potential underlying neurobiology (enzyme, carrier molecule, receptor etc.) of the disease were reviewed and potential miRNAs from the miRNA database (mirbase.org) that had both higher target scores and were available in our lab were chosen. Table 1 shows the features of the studied miRNAs.

2. Method

The research sample consisted a group of 52 patients from Harran University Faculty of Medicine Research Hospital, Child and Adolescent Psychiatry Clinic who were referred. The clinic for the first time and diagnosed with ADHD, and 52 healthy volunteer controls. All patients were diagnosed as ADHD by a child psychiatrist according to DSM-IV-TR diagnostic criteria and they were treatment naive. In this study, the ADHD module of K-SADS-PL was used to make the diagnosis of ADHD [17]. Patients with a history of cardiovascular disorders, epilepsy, diabetes mellitus, psychotic disorders, pervasive developmental disorders or severe head injury were excluded. The healthy controls were recruited from healthy child outpatient unit. After complete description of the study to the subjects, a written informed consent was obtained from the parents as well as the assent of children and adolescents. All of the study procedures were in accordance with the Declaration of Helsinki. Ethics committee of the Harran University Medical School approved the trial. Also a semi-structured form was used to detect several socio demographic and clinical variables of the patients. The final patient and control groups displayed similar distribution in age and gender. Study was funded by Harran University Board of Scientific Research Projects (Funding Number: 12010). This study was a cross-sectional study and blood sampling was made once from both controls and patients.

Total RNA was extracted from Peripheral Whole Blood using Tri-Reagent (Sigma). Reverse transcriptase reactions contained 5 μl of extracted total RNA, 50 nM stem–loop RT primer, 1× RT buffer, 0.25 mM each of dNTPs, 50 unit of modified M-MuLV Reverse Transcriptase (Thermo Scientific, Vilnius, Lithuania), 25 unit of RiboLock RNase inhibitor (Thermo Scientific, Vilnius, Lithuania) and nuclease-free water to a total reaction volume of 15 μl. The reaction was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). RT-PCR conditions for 30 min at 16 °C, 30 min at 42 °C, 5 min at 85 °C and then held at 4°. Quantitative-Comparative C_T ($\Delta\Delta C_T$) Real-time PCR was performed in an ABI Prism 7500 Real-Time PCR System (Applied Biosystems) using the SDS 2.0.6 software. The specific primers and fluorogenic ZNA™ probes for the microRNAs were designed using Primer Express 3.0 software (Applied Biosystems) and are listed in Table 2. The hsa-miR-26b-5p is used as control according to the Applied Biosystems application note cms_044972 (Applied Biosystems: http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_044972.pdf). The mixed RNAs generated from the control group was used as a Reference RNA sample. Primers and probes were purchased from Metabion International AG, D-82152 Martinsried/Deutschland.