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Original Article

miR-508-5p and mir-510-5p expressions and their relationships with spermatozoa motility and morphology

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Abstract

Background and aims: miRNAs have regulatory functions in developmental processes. The stages of spermatogenesis can also be affected by miRNAs. We tried to detect a relationship between the expression of miR-508-5p and miR-510-5p and male infertility. The purpose of this study was to investigate the association between expression of miR-508-5p and mir-510-5p in ejaculated sperm from patients with idiopathic asthenozoospermia, teratozoospermia, and teratoasthenozoospermia.

Materials and Methods: We enrolled 18 men with asthenozoospermia, 17 men with teratozoospermia, 18 men with teratoasthenozoospermia, and 18 individuals with normozoospermia based on the clinical criteria. Then, the expressions of the mentioned miRs in the spermatozoa were evaluated by quantitative real-time PCR (qRT-PCR). Kruskal-Wallis was used to compare their expressions in the studied groups.

Results: The expression of mir-508-5p did not show any statistical significance in all groups. On the other hand, the expression of miR-510-5p in teratozoospermia groups ($P<0.05$) and the asthenozoospermia group ($P<0.05$) demonstrated a significant downregulation compared with the control and teratoasthenozoospermia groups.

Conclusion: By analyzing the expression profile of miRNAs, we concluded that the expression level of miR-510-5p changed in patients with abnormal motility and morphology of spermatozoa; therefore, it may affect infertility by down-regulating the expression of mir-510-5p which shows the role of it in abnormal morphology and motility defects in infertility cases.

Keywords: Asthenozoospermia, MicroRNAs, Teratoasthenozoospermia, Teratozoospermia

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Introduction

Studies show that 15% of couples are affected by infertility and 50% of the infertility cases are attributed to men (1). It is a complex disorder with multiple genetic/epigenetic and environmental causes (2).

A wide range of infertility genes have been identified in several studies (3,4). Generally, gene expression is affected by both genetic and epigenetic mechanisms (5). MicroRNAs, short non-coding RNAs, have the potential to regulate the level of protein expression through epigenetic mechanisms (6). It has been reported that microRNAs are involved in the process of spermatogonia differentiation (7-9). Ejaculated spermatozoa consist of a different pattern of RNAs that help to clarify the state of fertilization. The miRNAs can act as a molecular instrument in spermatogenesis, consequently leading to identifying valuable information of gene expression during the spermatogenesis process (10). MiR-493-5p is highly expressed in non-small cell lung cancer (NSCLC), and it has a positive association with clinical prognosis. The up-regulation of miR-493-5p in NSCLC may be used as tumor biomarkers for the diagnosis of patients (11). It has been proven that miR-493-5p triggers AKT/GSK-3β/Snail signaling in prostate cancer to inhibit the expression of downstream proliferative genes, particularly CREB1 and EGFR. Concerning the high rate of osteogenesis in human dental pulp cells (hDPCs), miR-508-5p can inhibit expression of GPNMB that has a pivotal role in hDPC differentiation (12). The expression of miR-508-5p in patients with congestive heart failure (CHF) correlates with the clinical features of ICM and NICM, resulting in the provision of novel targets for treatment (13). In thyroid cancer, miR-510-5p can directly bind to SNHG15 and negatively regulate the expression of this gene (14). NSCLC cell proliferation and invasion can be inhibited by downregulation of miR-510. SRCIN1 is recognized as the target gene of mir-510-5p (15). According to bioinformatics studies, miR-508-5p and mir-510-5p target CRISP3 which affects male infertility. Accordingly, there is a possible association between abnormal expression of Crisp3 and the role of miR-508-5p in spermatogenesis (16-18). In the present study, we intend to examine

the association between the expression of miR-508-5p and mir-510-5p in ejaculated sperm from patients with idiopathic asthenozoospermia, teratozoospermia, and teratoasthenozoospermia.

Materials and Methods

Study population and groups

The study group consisted of 71 infertile men referred to the Infertility Clinic of Hazrat Zahra (Shahrekord, Iran) from September 2018 to January 2019. A consent form was obtained from all participants. Then, semen analysis was done as a part of the process of infertility investigations. The study group was divided into 4 groups according to their semen analysis (19) as follows:

- Normozoospermia (control; perm motility >32%, sperm morphology ≥4%, n=18),
- Asthenozoospermia (sperm motility <32%, n=18),
- Teratozoospermia (sperm normal morphology <4%, n=17),
- Teratoasthenozoospermia (sperm morphology <4%, sperm motility <32%, n=18).

Participants with genetic abnormalities, reproductive system infection, varicocele, anti-sperm antibodies, and the history of cryptorchidism and mumps in childhood were excluded from the study. Abnormal pH, fructose level, and presence of white blood cells in the semen which were also considered as abnormal parameters.

Collection and preparation of semen samples

Demographic data and medical history of patients were recorded from their medical records. After 2-7 days of sexual abstinence, semen samples were obtained according to the standard semen collection technique. The World Health Organization (WHO) guidelines (2010, 5th edition) was the main pattern for analyzing the samples (19). Video test Image Analysis-Sperm 2.1 (Video test, Moscow, Russia) was used to evaluate the concentration and motility of sperm by CASA (computer-aided sperm analysis) method. After Papanicolaou staining, sperm morphology was assessed by standard manual microscopic method.

After the samples were prepared for molecular tests, spermatozoa had to be isolated from the seminal plasma. The samples were centrifuged at 4°C for 10 minutes at 2000 g. Then, the sperm sediments were washed twice with phosphate-buffered saline (PBS) and were kept at -80°C.

Extraction of RNA and q-PCR

The total RNA was extracted from spermatozoa using the RNX Plus™ kit (Cat. no: RN7713c/EX6101; Sinaclon, Iran). The RNA concentration of each sample was evaluated at 260/280 nm absorbance ratio, using Thermo Scientific™ NanoDrop 2000, and the RNAs were stored at –70°C until performing qRT-PCR. Specific microRNA primers and cDNA Synthesis Kit (Parsgenom Co., Cat.no: PG4487-01 and PG4487-03, Iran) were applied to prepare cDNA from 2 µg of total RNA by adding poly-A tail. Real-time PCR was performed to show the quantification of mRNA using the SYBR Green kit (Takara Co., SYBR Premix Ex Taq II, Cat.no: RR820L) according to the protocols of the manufacturers.

Quantitative real-time PCR (qRT-PCR)

The qRT-PCR was performed using 1 µL of 1:10 diluted cDNA in 10 µL reaction volume. The qPCR reaction mixture contained 5 uL of SYBR Green Real-time PCR Master Mix Kit (TAKARA, Japan, catalog number. RR820Q) and 0.3 µL of 100 nM of each forward and reverse primer for miR-508-5p that was designed by Parsgenom Co. Quantitative RT-PCR was performed using Rotor-Gene RG-300 (Corbett Research, Sydney, AU).

Reaction steps were as follows: denaturation at 95°C for 5 minutes, followed by 40 cycles of replication (denaturation at 95°C for 5 seconds, primer annealing at 63°C for 20 seconds, and primer extension at 72°C for 30 seconds) and terminated by melting curve analysis (65 to 95°C; with 0.5°C increments) to confirm the specific amplification. The amplification of miR-493-5p in all samples was analyzed versus U6 snRNA as the internal control for data normalization. The Pfaffl method was used to calculate the fold change.

Data analysis

Data were expressed as mean ± SD. The data were analyzed using the Kruskal-Wallis test after assessing the normality or non-normality of the data by the Kolmogorov-Smirnov test. The statistical analysis was performed using REST 2009 software (for qPCR data analysis) and GraphPad Prism 5 software. $P<0.05$ was considered statistically significant.

Results

The features of semen in all groups were characterized to differentiate each group in terms of age, pH, morphology, motility, and concentration (Table 1). All the studied groups were normal with respect to age, pH, and sperm count. The results indicated that sperm morphology in teratoasthenozoospermia and teratozoospermia groups was abnormal in terms of maturity and formation. In these groups, the sperm morphology was different from that in asthenozoospermia and normozoospermia groups, which was normal. Furthermore, the sperm progressive motility in the teratoasthenozoospermia and asthenozoospermia groups were not normal compared to the teratozoospermia and normozoospermia groups (Table 1).

The expression of miR-510-5p in asthenozoospermia and teratozoospermia groups was 3.18 and 20.8 times lower than in the control group (P=0.0004 and

Table 1. Semen Analysis Results of the Studied Patients

Semen parameters	Study population				*P* value
	NZ (n=18)	TZ (n=17)	AZ (n=18)	TAZ (n=18)	
Age (y)	27.54±0.28[a]	28.17±0.77[a]	28.12±0.74[a]	28.18±0.93[a]	0.45
Semen pH	7.52±0.41[a]	7.49±0.31[a]	7.61±0.82[a]	7.43±0.45[a]	0.654
Sperm count (million/mL)	61±2. 7[a]	57.33±3.26[a]	58.19±3.53[a]	54.42±1.02[a]	0.245
Sperm motility (%)	64.56±1.84[a]	62.79±2.09[a]	21.07±1.94[b]	18.9±1.46[b]	0.0001*
Normal sperm morphology (%)	6.67±0.56[a]	1. 6±0.35[b]	6.1±0.49[a]	1.82±0.92[b]	0.0001*
Volume (mL)	3.8 ±0.42[a]	3.61±0.18[a]	3.52±0.54[a]	3.4±0.63[a]	0.947

NZ: Normozoospermia, TAZ: Teratoasthenozoospermia, AZ: Asthenozoospermia, TZ: Teratozoospermia.
Results are expressed as Mean ± SD. Values with different letters within the same rows are significantly different.
* significant value.

P=0.0001), respectively. The expression of miR-508-5p in teratoasthenozoospermia and teratozoospermia decreased slightly compared to the normal group; however, this change did not show any statistical significance. The expression of miR-510-5p shows down-regulation in asthenozoospermia, and teratozoospermia groups (Figure 1).

The next analysis illustrated that there was no significant relationship between miR-508-5p and miR-510-5p expressions and the sperm parameters including normal sperm morphology, motility, and sperm concentration in all groups.

Discussion

In the present study, we assessed the expression of miR-508-5p and mir-510-5p in spermatozoa of men with idiopathic infertility. Our results showed that the expression of mir-508-5p did not have a remarkable difference in all groups. Our analysis displayed a positive correlation between miR-508-5p and progressive motility in asthenozoospermia and teratozoospermia but it was not statistically significant. On the other hand, the expression of the miR-510-5p showed downregulation in Asthenozoospermi and Teratozoospermia groups and a positive correlation between miR-510-5p and progressive motility was observed in asthenozoospermia and teratoasthenozoospermia, but it was not statistically significant.

The regulatory function of miRNAs in post-transcriptional levels is involved in multiple developmental processes in many organisms. The ability of miRNAs to regulate mammalian spermatogenesis has been proved in several studies (20,21). Transcription is exclusively the major activity in meiotic and haploid phases of spermatogenesis rather than translational activity. In these phases, miRNAs target 5' and 3'-untranslated regions of mRNAs to control gene expression. This performance indicates the important roles of miRNAs in spermatogenesis (22).

The molecular basis of the human reproductive system dysfunction has remained obscure; however, the relationship of miRNAs with the male reproductive system has been shown in several studies. The high expression of miRNAs has the potential to retard spermatogenesis at an early stage of proliferation and/or early differentiation in Dicer-deleted testis (23,24). Furthermore, miRNA profiles are altered in the testicular tissues or reproductive cells of infertile patients (25,26). For instance, miR-20 and miR-106a were recognized as the dominant miRNAs in mouse SSCs, compared to differentiated spermatogonia or adult male germ cells (27). In adult pachytene spermatocytes and round spermatids, a high level of miR-34c was also reported (28). The meiotic process is dependent on miR-10a through targeting Rad51 which leads to the male germ cell development and spermatogenesis in both mouse and human (29). Therefore, miRNAs may be related to human infertility based on their functions in spermatogenesis and a different pattern in developmental stages.

These miRNAs can be exploited as the potential biomarkers in male infertility. The alterations of miRNA expression in patients with different spermatogenic and histopathologic impairments have the potential to be used as new biomarkers for diagnostic purposes. The expression profile of miRNA will not only increase the biomarker options for molecular diagnostics in infertility but also provide a better insight into the spermatogenetic processes, and even infertility treatment (30-32). A better knowledge of miRNAs requires more infertility cases and

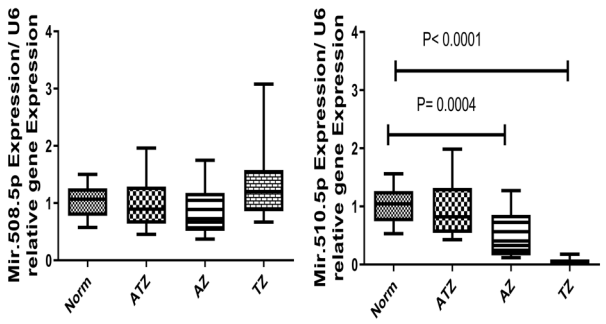

Figure 1. Comparison of miR-508-5p (A) and miR-510-5p (B) expression in different groups.
TAZ: Teratoasthenozoospermia; AZ: Asthenozoospermia; TZ: Teratozoospermia

reliable statistical analyses such as the ROC curve.

Regarding the regulatory functions of miRNAs on several genes, the heterogeneity or alteration in the pattern of miRNAs can cause specific features and apparent phenotypes of diseases. Besides, further studies with larger sample sizes are needed to confirm our results. It should be considered that a limited number of samples were utilized from men of couples with an inability to interpret our data. In our cases, the laboratory test displayed abnormal results for male and female partners who had a normal state. However, the infertility of these couples is most likely attributed to the male partner. To implement the clinical procedures, identifying the infertile men was a crucial and problematic duty. The evaluation of data was so complicated that increased or reduced expression of each of the miRNAs should be carefully correlated or defined. In addition, the candidate genes are usually regulated by several factors, and it is better to assess the role of miRNAs exactly.

Conclusion

By analyzing the expression profile of miRNAs, we concluded that downregulation in the expression of miR-510-5p in Asthenozoospermia and Teratozoospermia groups can represent an effective role of miR-510-5p in the regulation of motility and morphology of spermatozoa and its effect on male infertility.

Therefore, further studies on miR-508-5p and miR-510-5p are required to understand their functions in sperm development.

Conflict of interests

The authors declare no conflict of interest.

Ethical Approval

This study was carried out with the cooperation of the Infertility Clinic of Hazrat Zahra affiliated to Shahrekord University of Medical Sciences from September 2018 to April 2019. This study was approved by the Ethics Committee of Shahrekord University of Medical Sciences (IR.SKUMS.REC.1397.172). All patients were informed about the ethical considerations before entering the study. Then, they gave informed consent to participate in the study.

Authors Contribution

HT designed the study, TH and MS prepared and analyzed the data, TH and MS wrote the manuscript, and all authors read and approved the final manuscript.

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