Assessment of expression profiles of three microRNAs and their clinical, laboratory, and histopathological correlations in non-obstructive azoospermia : A controlled study

Original Article

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ABSTRACT

Background: MicroRNAs (miRNAs) play essential roles in human spermatogenesis, but little is known about seminal plasma miRNAs in infertile men.

Aim: To assess miRNA-34b, miRNA-181a, and miRNA-429 expression profiles in seminal plasma of patients with nonobstructive azoospermia (NOA) and to correlate these expression profiles with their clinical, laboratory, and histopathological features.

Patients and Methods: This was a prospective case–control study which included two groups: 50 males with NOA and 50 healthy fertile males who attended the Andrology Outpatient Clinic, the Genetics Unit, and a private IVF Center. Semen analysis and assessment of the three miRNAs expression profiles in seminal plasma were done by real-time PCR for both groups. Hormonal profile assessment (follicle-stimulating hormone, luteinizing hormone, testosterone, and prolactin), testicular sperm extraction, and histopathology were done for the patients' group.

Results: In this study, there were statistically significant upregulations of both miRNA-429 and miRNA-181a in the patients' group compared with controls (P<0.001), which was not the case for miRNA34b (P=0.259). There were statistically significant relationships between the three miRNAs, testicular sperm extraction results, and histopathological patterns (P<0.05).There were significant positive correlations between miRNA34b, miRNA-429, and follicle-stimulating hormone(r=0.466, P=0.001; r=0.375, P=0.009, respectively) and significant negative correlations between miRNA34b, miRNA-181a, and Johnsen's score (r=-0.287, P=0.048; r=-0.351, P=0.015, respectively). There was statistically significant negative correlation between miRNA-429 and testosterone (r=-0.330, P=0.022).

Conclusion: The expression levels of miRNA 181aandmiRNA-429 only were upregulated in NOA patients compared with controls. Thus, they may represent useful noninvasive biomarkers for NOA.

Key Words: Male infertility, microRNA-181a, microRNA-429, microRNA34b, nonobstructive azoospermia, spermatogenesis.

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INTRODUCTION

Around half of couple's inability to conceive is contributed to male factor[1]. A significant proportion of these cases were diagnosed as azoospermic[2]. Current clinical diagnostic techniques for non-obstructive azoospermia (NOA) cases are inconclusive and frequently necessitate surgical intervention for a definitive diagnosis[3].

MicroRNAs (miRNAs) are small noncoding single-stranded RNA molecules that are physiologically produced to regulate or mostly downregulate genes[4,5]. MiRNA dysregulation plays an important role in the development of many diseases, and associations with some male reproductive disorders have been observed[6]. MiRNAs have been found in the human sperm and seminal plasma of men with NOA[7]. Changes in miRNA expression patterns have been associated with different testicular histopathologic patterns of azoospermia patients, implying that miRNAs could be used as specific biomarkers for male fertility[8].

We aimed to assess the level of a set of three miRNAs

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(miRNA-34b, miRNA-181a, and miRNA-429) in seminal plasma of patients with NOA and correlated their expressions profiles with the clinical data, hormonal profile, and histopathological patterns of patients to assess the potential role of each of these miRNAs as indicators of spermatogenic efficiency and their clinical relevance in infertile males with NOA as noninvasive markers.

PATIENTS AND METHODS

This case–control study was carried out in the Andrology Outpatient Clinic of Suez Canal University Hospital, the Genetics Unit in the Center of Excellence in Molecular and Cellular Medicine, Faculty of Medicine, Suez Canal University and private IVF center (Cleaopatra IVF center). After getting institutional board review clearance, the study was carried out in compliance with the Helsinki Declaration standards. All the participants signed a written informed consent form. With 50 people in each group, the study included two groups: patients and controls. The patients were chosen based on the following inclusion criteria: married males between the ages of 18 and 60 years and had NOA. The exclusion criteria included patients with leucocytospermia and hemospermia, hypergonadotrophic or hypogonadotrophic hypogonadism, history of cryptorchidism, varicocele, undescended testicles, history of testicular trauma, radiotherapy, chemotherapy, orchitis, testicular carcinoma, chronic urinary tract infection, occupational exposure to chemicals, insecticides, or excessive heat, presence of any chronic illness, long-term medication intake and surgical history of hernia, varicocelectomy, or orchidopexy. Fifty healthy fertile men with normal sperm parameters who had fathered at least one healthy child without assisted reproductive measures were selected as controls.

All the participants were subjected to history taking, general and genital examination. Semen analysis was carried out according to WHO 2010 guidelines [9]. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), and prolactin were measured in patients' serum from early morning samples by the ELISA method (Diagnostics Systems Laboratories, Webster, Texas, USA). All patients underwent testicular sperm extraction operation and histopathological examination as well as Johnson's score calculation for their testicular biopsies.

Assessment of miRNAs expression levels in seminal plasma of patients and controls: total RNA, including miRNAs, was isolated by centrifuging fresh semen samples for 10 min at 3500 rpm, followed by total RNA separation using 100 ml of the supernatant seminal plasma and an equal volume of Qiazol Reagent (provided with miRN easy extraction kit, Qiagen, Germany). The Qiagen miRN easy Mini kit (miRNeasy Mini kit, Cat No. 217004; Qiagen) was used to isolate total and small RNAs from the separated plasma, according to the manufacturer's procedure. A Nanodrop ND-2000 spectrophotometer was used to evaluate the concentration and purity of RNA samples (Thermo Scientific, USA). cDNA production consisted of the following steps: in a 20 ml reaction, 5 l of template RNA was added to 15 l of master mix and transcribed to cDNA using the miScript Reverse Transcription Kit (miScript II RT Kit, Cat no. 218161; Qiagen) according to the manufacturer's instructions. This system was used to perform selective conversion of mature miRNAs into cDNA in miScript HiSpec buffer, where miRNAs were polyadenylated by poly(A) polymerase and transcripted with reverse transcriptase with oligo-dT priming. The cDNA is then used to quantify mature miRNA expression using real-time PCR. The miScript Sybr Green PCR and miScript Primer Assays (miScript Sybr Green PCR kit, Cat No. 218073; Qiagen) were used to detect miRNA. For quantitative real-time PCR analysis of miRNA-34b, miRNA-181a, and miRNA-429, two and a half microliter reverse transcription products were used as templates in 20 ml reactions (miScript Primer Assays, Cat No. 218300; Qiagen). According to the manufacturer's instructions, with the following thermal cycling conditions: initial activation step of 95ºC for 15 min, followed by 40 cycles of 94ºC for 15 s (denaturation), 55ºC for 30 s (annealing), and 70ºC for 30 s (annealing) (extension). All reactions were performed on the StepOne TM Real-Time PCR Systems (Applied Biosystems, Foster City, California, USA) and the resulting cycle threshold (CT) values were compared with the RNU6B endogenous control (miScript Primer Assays, Cat No. 218300; Qiagen). MiRNA expression was reported as the CT value calculated by subtracting the CT values of RNU6B from the CT values of each of miRNA-34b, miRNA-181a, and miRNA-429 of patients and using the equation 2-ΔΔCT method to provide the fold change (FC) of each miRNA in each sample. FC represented the relative expression of miRNA in seminal plasma.

Statistical analysis

The sample size was calculated using the following formula[7,10]:

$$n=2\left[\left(Z_{\alpha/2}+Z_{\beta}\right)\times\sigma/\,\mu_1-\mu_2\right]^2$$

Data were collected and outcome measures coded, entered, and analyzed by using SPSS (Statistical Package for the Social Sciences), version 25 (IBM Corp., Armonk, New York, USA). The data was tested for normality using Kolmogorov–Smirnov test. According to the type of data and normality test result Chi-square Test ($\chi 2$) test, Student's t test, Mann–Whitney U test, McNemar test and Wilcoxon signed ranks test was used for comparison. Correlation testing between variables was done by Pearson's or Spearman's correlation according to normality distribution. Data were represented as number and percentage; the quantitative continuous group is represented by mean ±SD (for parametric data) median and interquartile range

(for nonparametric data). A *P* value of less than 0.05 was considered statistically significant for two-tailed tests.

RESULTS

The age of patients ranged from 24 to 55 years with a mean of 32.9±6.89, while the age in the control group ranged from 20 to 48 years with a mean of 30.72±7.32 with no statistically significant difference between the two groups (*P*=0.129). The majority in the patients' group and control group were non-smokers (34 and 31, respectively) with no statistically significant difference between the two groups (*P*=0.529). Semen volume in the patients' group ranged from 1.50 to 5 ml with a mean of 3.58±1.04, while in the control group it ranged from 1.5 to 7 ml with a mean of 3.61±1.36 with no statistically significant difference between the two groups (*P*=0.848).

MiRNA-34b FC median in the patients' group was 2.14 with no statistically significant difference between patients and controls (P=0.259). Meanwhile both miRNA181 FC and miRNA-429 FC were statistically significantly higher in patients than controls (*P*=0.003, *P*<0.001, respectively) (Table 1).

Semen analysis parameters in the control group were all in the normal range. Count ranged from 25 to 255 million/ml and its mean was 102.46±36.66. Progressive motility ranged from 35 to 55% and the mean was 44.66±5.39. Abnormal forms ranged from 40 to 85% and the mean was 65.50±9.44 and vitality ranged from 58 to 63% and the mean was 60.48±8 (Table 2).

Table 1: Characteristics of study groups

	Patients' group	Control group	*P* value
Age			
Mean ±SD (range)	32.9±6.89 (24.0–55.0)	30.72±7.32 (20.0–48.0)	0.129
Smoking [n (%)]			
Smokers	16 (32)	19 (38)	0.529
Nonsmokers	34 (68)	31 (62)	
Semen volume			
Mean±SD (range)	3.58±1.04 (1.5–5.00)	3.61±1.36 (1.50–7.0)	0.848
MicroRNA-34b FC			
Median (range)	2.14 (0.18–10.38)	1.0 (1.0–1.0)	0.259
MicroRNA181 FC			
Median (range)	2.01 (0.38–9.11)	1.0 (1.0–1.0)	0.003*
MicroRNA-429 FC			
Median (range)	5.98 (0.92–162.9)	1.0 (1.0–1.0)	<0.001*

FC, fold change.
*Statistically significant difference (Mann–Whitney test) *: at $P \leq 0.05$.

Table 2: Microscopic parameters in semen analysis in the control group

Semen analysis	
Count (millions/ml)	
Minimum–maximum	25–255
Mean ±SD	102.46±36.66
Progressive motility (%)	
Minimum–maximum	35–55
Mean ±SD	44.66±5.39
Abnormal forms (%)	
Minimum–maximum	40–85
Mean ±SD	65.50±9.44
Vitality (%)	
Minimum–maximum	58–63
Mean ±SD	60.48±8.00

Table 3: Hormonal profile of patients' group

Hormones	
LH	
Minimum–maximum	1.4–13
Mean ±SD	6.24±2.93
FSH	
Minimum–maximum	9.8–72.1
Mean ±SD	26.27±18.19
Testosterone	
Minimum–maximum	1.3–11.9
Mean ±SD	5.21±2.78
Prolactin	
Minimum–maximum	1.70–24.0
Mean ±SD	10.26±4.29

Values presented as mean±SD, FSH, follicle-stimulating hormone; LH, luteinizing hormone.

The hormonal profile in the control group was in normal range. In patients group LH mean was 6.24±2.93 and ranged from 1.4 to 13 (normal, 1.5–9.3 mIU/ml); FSH mean was high 26.27±18.19 and ranged from 9.8 to 72.1 (normal, 1.6–11 mIU/ml). TT mean was 5.21±2.78 and ranged from 1.3 to 11.9 (normal, 2.41–8.27 mIU/ml) and prolactin mean was 10.26±4.29 and ranged from 1.70 to 24.0 (normal, 2–18 mIU/ml) (Table 3).

Results in Table 4 revealed that in the patients' group there was statistically significant relationship between miRNA34b and age (*P*=0.005) as in the age group above 30 years the median of miRNA34b was 6.7, which decreased to 0.34 in the age group below 30 years. Meanwhile, there were non-significant relationships between both miRNAs-181a and miRNAs-429 and age (*P*=0.197, 0.105, respectively) as well as there were statistically non-significant relationships between miRNA34b, miRNA181a, and smoking (*P*=0.512, 0.85, respectively). Meanwhile, miRNA-429 showed statistically significant relationship with smoking (*P*=0.042).

The testicular sperm extraction (TESE) results were positive in 33 patients and were negative in 15 patients and there were statistically significant relationships between the three studied miRNAs and TESE results (*P*=0.042, 0.03, 0.032, respectively) (Table 4).

Histopathological patterns of patients TESE were as follows: 14 cases showed germ cell aplasia (GA), 20 cases showed a mixed pattern, 13 cases showed hypospermatogenesis with only one case with tubular halynization and there were statistically significant relationships between the three studied miRNAs and histopathological pattern (*P*=0.037, 0.034, 0.049) (Table 4).

Table 4: Relation between microRNA-429 fold change and characteristics in patients' group

		Fold change					
		MicroRNA-34b		MicroRNA181		MicroRNA-429	
	n (%)	FC	P	FC	P	FC	P
Age			0.005*	w	0.197		0.105
>30	24 (100)	0.34 (5.92–62.4)		3.61 (0.36–13.8)		12.67 (1.16–658.77)	
≤30	24 (100)	6.7 (0.299–2.8)		1.35 (0.42–4.34)		4.05 (0.49–18.67)	
Smoking							
Smokers	15 (31)	2.14 (0.11–6.17)	0.512	2.56 (0.097–4.83)	0.850	1.7 (0.14–124.45)	0.042*
Nonsmokers	33 (69)	2.29 (0.25–24.51)		1.46 (5.23–11.16)		10.03 (1.88–230.28)	
TESE results							
Positive	33 (69.6)	0. 502 (0.016–8.198)	0.042*	1.19 (0.29–5.46)	0.030*	3.5 (0.66–30.8)	0.032*
Negative	15 (30.4)	4.07(1.99–95.7)		2.77 (1.36–28.8)		12.67 (6.25–5231.2)	
Histopathological patterns of TESE cases							
Tubular hyalinization	1 (2.1)	–		–			
Germinal cell aplasia	14 (29.2)	3.32 (1.69–33.4)	0.037*	3.21 (1.36–34.13)	0.034*	12.35 (5.09– 5607.7)	0.049*
Mixed pattern	20 (41.6)	2.05 0.25–23.97		3.27 (0.11–6.45)		5.085214 (0.923–137.53)	
Hypospermatogenesis	13 (27.1)	0.163 (0.003–2.53)		0.823 (0.008–2.68)		2.93 (0.33–11.8)	

FC, fold change, fold change data are presented either in median (range) or mean +SD (range), TESE, testicular sperm extraction, Two samples in each group yielded no extract, so fold change and relations were calculated for 48 samples, *Statistically significant difference (Mann–Whitney test) *: at *P*≤ 0.05.

MiRNA-34b showed statistically significant negative correlations with age and Johnsen's score (r=–0.418, *P*=0.003; r=–0.287, *P*=0.048, respectively) and significant positive correlation with FSH (r=0.466, *P*=0.001); meanwhile, the correlations with LH, TT, and prolactin were not statistically significant. MiRNA-181a showed statistically significant negative correlation with Johnson's score (r=–0.351, *P*=0.015). Meanwhile the correlations with age, smoking, and hormones were statistically nonsignificant (*P*>0.05). MiRNA-429 showed a statistically significant positive correlation with FSH (r=0.375, *P*=0.009) and significant negative correlation with testosterone (r=–0.330, *P*=0.022), while there were statistically nonsignificant correlations with the other variables (*P*>0.05) (Table 5).

Receiver-operating curve (ROC) was used to determine the ability of miRNA- 34b, miRNA-181a, and miRNA-429 to differentiate NOA cases from controls. Our ROC results revealed that the area under the curve (AUC) for miRNA-34b was 0.566 indicating that it is not a good predictor for azoospermia. Meanwhile the AUC for miRNA-18a1 and miRNA-429 were 0.838, 0.867, respectively indicating that they are good predictors for azoospermia (Table 6) (Fig. 1a–c).

Table 5: Correlation between fold change of microRNA-34b, microRNA181 and microRNA-429 and different parameters in patients' group

	Fold change					
	MicroRNA-34b		MicroRNA181		MicroRNA-429	
	r	*P*	*r*	*P*	*r*	*P*
Age	-0.418*	0.003*	-0.243	0.096	0.081	0.585
Smoking	-0.096	0.518	-0.096	0.518	-0.297	0.41
FSH	0.466	0.001*	0.212	0.147	0.375	0.009*
LH	0.057	0.701	-0.085	0.565	-0.032	0.829
Testosterone	0.065	0.663	-0.159	0.282	-0.330	0.022*
Prolactin	0.168	0.254	-0.222	0.129	0.116	0.434
Johnsen's score	- 0.287	0.048*	-0.351	0.015*	-0.226	0.122

FC, fold change, fold change data are presented either in median (range) or mean ±SD (range).
TESE, testicular sperm extraction.
Two samples in each group yielded no extract, so fold change and relations were calculated for 48 samples.
*Statistically significant difference (Mann–Whitney test) *: at $^{P} \leq 0.05$.

Table 6: Area under the curve values and agreement (sensitivity, specificity) for the studied microRNAs

	AUC	P value	95% CI	Sensitivity	Specificity
MicroRNA34b	0.566	0.269	0.450–0.681	89.58	27.08
MicroRNA181a	0.838	<0.001*	0.760–0.916	76.25	68.75
MicroRNA-429	0.867	<0.001*	0.790–0.943	79.83	64.58

AUC, area under the curve; CI, confidence intervals.
*Statistically significant at *P* value less than or equal to 0.05.

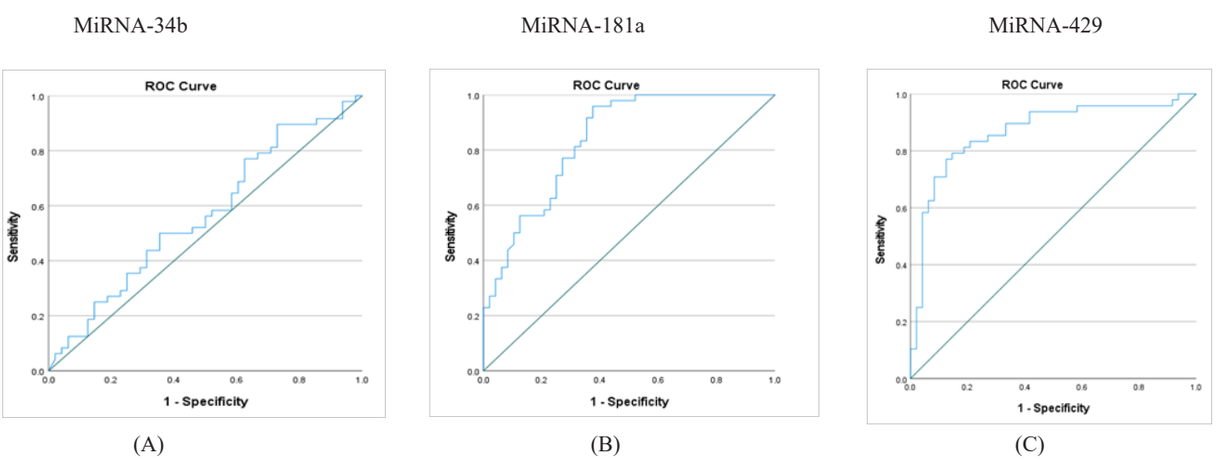

Fig. 1: ROC curve for the ability ofmiRNA-34b, miRNA-181amiRNA-429 expression in the seminal plasma to differentiate NOA cases from controls (A,B,C)

DISCUSSION

This study included two groups: NOA patients' group and control group. Expression profiles of miRNA-34b, miRNA-181a, and miRNA-429 in seminal plasma were assessed and correlated with clinical, hormonal, and histopathological features of patients.

In our study, the means of age, smoking, and semen volume in both groups were comparable. We found a statistically significant upregulation in miRNA-181a and miRNA-429 FC in the seminal plasma of NOA patients' compared with controls (P=0.003, $P \leq 0.001$, respectively). In accordance with our results Salas-Huetos *et al.*[11] compared the expression levels of many miRNAs in spermatozoa from 8 infertile males to data from 10 control normozoospermic fertile males. They discovered a 3.27-fold increase in miRNA-181a expression in infertile males compared with controls (P=0.0061). In a prospective case–control study of 100 NOA patients and 100 fertile controls, miRNA-429 was significantly upregulated in seminal plasma of NOA patients compared with fertile controls (P=0.014), whereas miRNA-34b was not[12].

Meanwhile, Abu-Halima *et al.*[13] discovered that miRNA-429 expression was not significantly altered in NOA patients compared with controls, but this was for testicular tissue rather than seminal plasma. In another study for seminal miRNA expression in azoospermic patients, there was no statistically significant difference in miRNA-181a expression between patients and controls (P=0.459) [14]. This is probably due to the relatively small number of participants in that study. Meanwhile Wang *et al.*[15] found that miRN-181a levels were significantly lower in azoospermic patients compared with controls. This could be explained by ethnic variation, which could result in differential miRNA expression in opposite directions[16] and, to the best of our knowledge, this is the first study to explore seminal miRNA in Africans.

In a study for 272 miRNA in NOA seminal plasma, Wu and his colleagues found a nonsignificant difference in miRNA-34b expression from controls. This is consistent with our findings, which showed that miRNA-34b was not significantly deregulated in the patient group compared with controls[12].

In the studies of Abu-Halima *et al.*[7], the findings of significantly decreased miRNA-34b were either in testicular tissues or in sperm containing samples and the same were for Zhang *et al.*[18] and Salas-Huetos *et al.*[11]; meanwhile, we assessed the expression profile in NOA semen samples[8,17].

Also Barceló *et al.*[14] found a significant decrease in miRNA-34b but their assessment was for only exosomal miRNA in azospermic patients and not for the whole seminal miRNA.

ROC analysis and AUC values of miRNA18a1 and miRNA-429 revealed that they are good predictors for azoospermia (AUC=0.838 and 0.867, respectively) which was not the case for miRNA-34b. Other studies have shown similar results for miRNA-181a and miRNA-429[12,15].

In disagreement with our findings, the study of Abu-Halima *et al.*[7] showed a very weak AUC value for miRNA-429 and good AUC value for miRNA-34b. This contrast may be due to the assessment of mi-RNA in testicular tissues and not in seminal plasma of NOA patients.

The current study revealed that there were neither relationship (P=0.197, 0.105, respectively) nor correlation (r=–0.243, P=0.96; r=0.081, P=0.585) between age and both miRNA-181a and miRNA-429 expression levels. Meanwhile, we found a significant relationship (P=0.005) and significant negative correlation between age and miRNA-34b (r=–418, P=0.003) in the patients' group. According to our knowledge, there are no studies concerning the relationship between age and miRNA-429 in NOA patients. In correspondence Wang *et al.*[15] assessed miRNAs expression in infertile patients and reported that there was no statistically significant relationship between miRNA-181a and age (P=0.5704). Salas-Huetos *et al.*[11], in their comparative study on 30 infertile individuals, demonstrated that miR-34b seemed to be negatively correlated with age, which agreed with our results. Multiple genes that are targets of miRNA-34b play a role in aging-related processes by directing cell cycle progression and apoptosis[11]. These results, on the other side, reflect the light on the age-related effects on spermatogenesis on a molecular basis.

Smoking causes epigenetic alternations in male reproductive function[19]. We found statistically nonsignificant relationships between our studied miRNAs and smoking (P>0.05) except for miRNA-429 (P=0.042) and no significant correlations were found at all. To date, there is paucity in studies concerning the relationship between smoking and these three miRNAs in semen. In a community-based prospective study, the results revealed that blood-derived miRNA-181a expression level was downregulated in smokers versus nonsmokers by 0.83-fold (P=0.009)[20].

Another study showed a negative correlation between miRNA-34b expression and its promoter methylation which was associated with smoking[21].

Measurements of FSH, LH, and prolactin are useful tests in the management of male infertility[22].

In the current study, we found nonsignificant correlations between LH, prolactin, and the studied miRNAs in the patients' group (P>0.05). Meanwhile, there were significant positive correlations between FSH, miRNA-34b, and miRNA-429 (r=0.466, P=0.001; r=0.375, P=0.009, respectively); and there was a significant negative correlation between TT and miRNA-429 (r=–0.330, P=0.022). On the other hand, Zhang and colleagues found that miRNAs-34b expression in testicular tissues of NOA

patients negatively correlated with the FSH, LH level, and was positive for testosterone. This could be due to ethnic differences in miRNA expression, which could lead to differential expression in opposite directions[16]. There is a lack of studies concerning the relationship between hormonal profile, miRNA-181 a, and miRNA-429. These results reflect the relation between negatively affected spermatogenesis reflected through elevated FSH and miRNAs dysregulation.

The testicular expression of miRNAs varies according to the stage of spermatogenesis[8]. In a controlled study that investigated the expression profile of miRNAs in 24 testicular biopsies from NOA patients with hypospermatogenesis, Sertoli cell-only syndrome and maturation arrest it has been reported that miRNA-181a was upregulated in patients with Sertoli cell-only syndrome and also in maturation arrest patients compared with controls[23].

The current study revealed similar findings as we found that miRNA-34b, miRNA-181a, and miRNA-429 showed significant relationships with both TESE results (P=0.042, 0.03, 0.032, respectively) and histopathological patterns in the patients' group (P=0.037, 0.034, 0.049, respectively).

Harmoniously, the results of our study showed that there were statistically significant correlations between Johnson's score, miRNA 34-b, and miRNA-181 expression levels (r=–0.287, P=0.048; r=0.351, P=0.015). Meanwhile there was a statistically nonsignificant correlation between miRNA-429 and Johnson's score in NOA patients.

To our knowledge, there were lack of studies concerning relation between miRNA-181a, miRNA-429 FC, and Johnson's score but in another study Zhang *et al.*[18] found significant relationship between miR-34b expression levels and Johnson's score ($P \leq 0.001$.). In the current study though miRNA-34b showed a statistically nonsignificant difference between the patients and controls, but it showed a statistically significant negative correlation with Johnsen's score. This demonstrates that the level of expression of this miRNA was related to the progression of spermatogenic defects in NOA patients. This supports the previous reports about the downregulation of miR-34b in Sertoli cell-only, GA, and maturation arrest[8], giving a cue on the role of this miRNA in NOA patients.

The limitations of our study were first, the expression levels of these miRNAs were assessed only in the seminal plasma and not in the testicular tissue. Second, we conducted our study on a limited number of cases; a larger sample size in future studies will serve a useful purpose for confirming our results.

CONCLUSION

In conclusion, our study revealed upregulation in miRNA-181a and miRNA-429 expression levels in seminal plasma of NOA patients compared with fertile males, which may add further insights into the role of their expression patterns in the development and/or regulation of spermatogenesis, and alterations of their expression could directly or indirectly contribute to reproduction abnormalities and thus why they may be used in the future as noninvasive markers for NOA.

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***Criteria for inclusion in the authors'/contributors' list*:**

Rashad M. Mostafa: idea of the research, supervision on the practical part and revision of paper writing. Hoda Y. Abdallah: participation in the idea of the research, participation in the practical part and collection of the scientific material. Heba M.Eissa: participation in writing of the scientific background, performing the practical part, collection of references, analysis of results. Donia R. Ibrahim: participation in writing of the scientific background, performing the practical part, collection of references, analysis of results. Sherif M. Ibrahim: participation in writing of the scientific background, performing the practical part, collection of references, analysis of results. Hany M. Saad: participating in the idea of the research, the practical work, collecting, drafting scientific material, collecting references, and analyzing results. Writing, reviewing, and submission of the paper.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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