MICRORNA 210 AND 34C-5P IN SEMINAL PLASMA IN PATIENTS WITH NON OBSTRUCTIVE AZOOSPERMIA

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Introduction

Infertility is failure of a couple to conceive after 12 months of regular unprotected sexual intercourse. Infertility could be either primary or secondary.(1) The male factor is responsible for 20-40% of cases. Causes of male infertility are divided into pre-testicular causes (hypothalamic-pituitary abnormality), testicular causes and post testicular causes. The testicular causes are classified into hypospermatogenesis, maturation arrest (MA), Sertoli cell only syndrome (SCO) and tubular hyalinization.(2)

Azoospermia is defined as complete absence of sperm in ejaculate. It is classified into obstructive azoospermia (OA), which is complete absence of spermatozoa in the ejaculate despite of normal spermatogenesis and non-obstructive azoospermia (NOA), which is absence of sperm due to testicular failure or hormonal disturbance.(3) Testicular biopsy is one of the main tools used for diagnosis of testicular causes.(2)

Aim of the work

The aim of the present work was to study the expression levels of microRNAs 210 and 34c5-p in seminal plasma of patients with idiopathic non obstructive azoospermia compared to their fertile counterparts and their correlation with testicular biopsy results.

Subjects and Methods

Subjects:

This study was conducted on 80 male individuals, categorized into 2 groups:

Group A: includes 40 infertile adult male patients with NOA.

Group B**:** The control group includes 40 fertile adult males who fathered children in the last 2 years and have normal semen parameters by computer assisted semen analysis (CASA).

Methods:

-Expression levels of miRNA 210 and 34C-5P was carried out using real time quantitative polymerase chain reaction (RQ-PCR). Semen samples were centrifuged and the seminal plasma was separated and stored at -80C° till the examination.

The molecular study included:

I. RNA extraction.

II. Reverse transcription to obtain complementary DNA (cDNA).

III. Relative gene expression analysis of miRNA 210 and miRNA34c-5p by (RQ-PCR).

Results

Table 1: Comparison between the two studied groups according to miRNA 210 expression and miRNA34c-5p expression.

	NOA (n = 40)	Controls (n = 40)	U	P
miRNA 210 expression				
Min. – Max.	0.01 – 9.52	0.29 – 4.14	360*	<0.001*
Median (IQR)	0.31 (0.19 – 0.69)	4.(0.42 – 1.96)		
miRNA 34c-5p expression				
Min. – Max.	0.002 – 0.92	0.09 – 5.4	74*	<0.001*
Median (IQR)	0.02 (0.01 – 0.05)	1.31 (0.32 – 3.14)		

IQR: Inter quartile range
U: Mann Whitney test
p: p value for comparing between the studied groups
*: Statistically significant at p ≤ 0.05 NOA: Non obstructive azoospermia.

Table 2: Relation between testicular biopsy and miRNA 210 and miRNA 34c-5p expression levels in NOA (n = 40)

	Testicular biopsy					H	p
	MA (n = 7)	SCO (n = 6)	Tubular Hyalinization (n = 4)	Hypo spermatogenesis (n = 21)	MA/Tubular hyalinization (n = 2)		
miRNA 210 expression							
Median (Min. – Max.)	0.60 (0.14 – 1.67)	0.13 (0.01 – 9.52)	0.25 (0.05 – 8.01)	0.36 (0.08 – 1.91)	0.230 (0.227– 0.233)	4.143	0.387
miRNA 34c-5p expression							
Median (Min. – Max.)	0.03 (0.01 – 0.04)	0.01 (0.002– 0.52)	0.02 (0.01 – 0.92)	0.02 (0.003 – 0.46)	0.012 (0.011 –0.012)	3.308	0.508

H: H for Kruskal Wallis test
p: p value for comparing between different categories
MA: Maturation arrest
SCO: Sertoli cell only

- There was a statistical significant difference between miRNA 210 and miRNA 34c-5p expression levels between the two groups.
- The median of miRNA 210 was highest in MA group, while the least in SCO. The median of miRNA 34c5-p was highest in MA group, while it was the least in SCO group.
- There was no statistical significant difference between miRNA 210 and miRNA 34c-5p expression levels in different types of testicular biopsy.

Conclusion

- miRNA 34c-5p and miRNA 210 expression levels are decreased in NOA than controls.
- miRNA 34c-5p expression is lower than miRNA 210 expression in seminal plasma of NOA patients.
- There is no significant association between seminal miRNA 34c-5p and miRNA 210 and different types of testicular biopsies in NOA patients.

References

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