Seminal MicroRNA in infertile men with Varicocele

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M.D. Degree in Andrology, Sexology & STIs

by

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Abstract

Objective: To assess seminal miRNA 122, 181a, 34c-5 in infertile men with varicocele.

Materials and Methods: This study included 79 men that were consecutively recruited from the Andrology Department, Kasr El-Aini Hospital, after institutional review board (IRB) approval and informed consent. They were divided into; fertile men without Vx (n=19), fertile men with Vx (n=14), oligoasthenotertatozoospermic (OAT) men without Vx (n= 23) and OAT men with Vx (n= 23). They were subjected to history taking, clinical examination and semen analysis. In their seminal plasma**,** *BAX* and BCl_2, malondialdehyde (MDA) and glutathione peroxidase (GPx) were estimated in addition to seminal plasma miRNA 122,181a, 34c-5 by quantitative real time –PCR.

Results: Seminal miRNA 122, 181a, 34c-5 demonstrated significant positive correlation with sperm count, sperm motility, sperm normal forms, seminal GPx, seminal BCl_2, and with each other and significant negative correlation with seminal *BAX*, MDA.Seminal miRNAs 122, 181a, 34c-5 demonstrated nonsignificant correlation with Vx grade.There was a significant increase in seminal Bax and significant decrease in seminal Bcl_2 in infertile men compared with fertile men being exaggerated in infertile men with Vx. There was a significant increase in seminal MDA and significant decrease in seminal GPx in infertile men compared with fertile men being exaggerated in infertile men with Vx. There was a significant decrease in mean levels of seminal miRNAs in infertile men compared with fertile men being the least in infertile men with Vx.

Conclusion: Seminal miRNA 122, 181a, 34c-5 have significant positive correlation with sperm count, sperm motility, sperm normal forms, seminal GPx, seminal BCl_2, each other, significant negative correlation with seminal *BAX*, MDA and nonsignificant correlation with Vx grade.

Keywords: Male infertility; varicocele; miRNA; OAT; *BAX*; BCl_2

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Last but certainly not least, I owe to the patients included in this study, may God alleviate their sufferings and may all our efforts be just for their own benefit.

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List of abbreviations

Ago	Argonaute
AKT1	v-akt murine thymoma viral oncogene homolog 1
AKT2	v-akt murine thymoma viral oncogene homolog 2
ART	Assisted reproductive techniques
ART	Assisted Reproductive Technology
BAX	BCl_2 associated X protein
BCL	B cell lymphoma 2
cDNA	Complementary deoxyribo nucleic acid
CDUS	Colour Doppler scrotal ultrasonography
Cyt C	Cytochrome C
DFI	DNA fragmentation index
DNA	Deoxyribo nucleic acid
ECL	Enhanced chemiluminescence
ERβ	Estrogen receptor β
FAM	Fluorescent dye
FoxO	Fork head box O
FSH	Follicular stimulating hormone
GnRH	Gonadotrophin releasing hormone
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Glutathione disulfide
HCG	Human chorionic gonadotropin
HRP	Horseradish peroxidase
HSPA1B	Heat shock 70kDa protein 1B
ICSI	Intracytoplasmic sperm injection
IRB	Institutional review board

IRF1 Interferon regulatory factor-1

IVF In-vitro fertilization

JC-1 Fluorescent carbocyanine dye

MDA Malondialdehyde

MEST Mesoderm-specific transcript homolog protein

MGB A minor groove binder

MHZ Mega Hertz

miRNA Micro ribonucleic acid

MMP Mitochondrial membrane potential

mRNA Messenger ribonucleic acid

NADPH Nicotinamide adenine dinucleotide phosphate

NF-kB Nuclear factor kappa-light-chain enhancer of B cells

NFQ A non-fluorescent quencher

NO Nitric oxide

NOA Nonobstructive azoospermia

NOTCH1 Notch gene homologue 1

OA Oligoasthenozoospermia

OAT Oligoasthenotertatozoospermia

PLAG1 Pleomorphic adenoma gene 1

PTEN Phosphatase and tensin homolog

RISC RNA-induced silencing complex

RNA Ribonucleic acid

ROS Reactive oxygen species

RT-PCR Reverse transcriptase – polymerase chain reaction

SDS-PAGE Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Sirt 1 Sirtuin 1

SOD Superoxide dismutase

SREBP Sterol regulatory element binding protein

TAC	Total antioxidant capacity
TaqMan	Taq polymerase plus pacMan
TBHP	Tertiary-butyl hydroperoxide
TBST	Tris-buffered saline/Triton X-100
TC^{99}	Technetium99
TGCTs	Tesicular germ cell tumors
TGF- Beta 1	Transforming growth factor B
TNF-α	Tumor necrosis factor-α
Tnp2	Transition protein 2
Vx	Varicocele
WHO	World health organization

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Introduction

Varicocele (Vx) is defined as a vascular abnormality in the veins within the pampiniform plexus **(Cil et al., 2015)**. Vx and its impact on male infertility is still a subject of debate. Approximately 15% of adult men are believed to have clinical or subclinical Vx, although the prevalence in infertile men is as high as 40% **(Shafi et al., 2014)**.

Vx can be categorized as; grade I, enlargement of the venous plexus of spermatic cord evident only by Valsalva maneuver; grade II, enlargement of the venous plexus of spermatic cord by palpation at upright position; and grade III, visual enlargement of the venous plexus of spermatic cord. Non-palpable enlargement of the venous plexus of the spermatic cord diagnosed by ultrasound is defined as subclinical Vx **(Mostafa et al., 2012; Mostafa et al., 2015)**.

Several theories explained the mechanisms by which Vx impairs male fertility including; scrotal hyperthermia, retrograde flow of metabolites, Leydig cell dysfunction, hypoxia due to venous stasis or impaired testicular artery perfusion and disrupted blood-testis barrier **(Mostafa et al., 2009)**. **Mostafa et al. (2001; 2009)** added that Vx has an oxidative stress effect on semen even in fertile normozoospermic men.

MicroRNAs (miRNA) are a family of small non-coding RNAs of about 22 nucleotides that play important roles in regulating post-transcriptional gene silencing via base pair binding to the untranslated region of their target mRNAs **(Stark et al., 2008)**. Several miRNAs have been implicated in the regulation of B-cell differentiation and T-cell receptor signaling **(Chen et al., 2004)**. Others are associated with inflammation and innate immune responses, in which it regulates the response to many microbial components and pro-inflammatory cytokines. In addition, modulation of miRNAs is related to apoptosis processes **(Taganov et al., 2006)**.

MiRNAs were first detected in human spermatozoa by **Ostermeier et al. (2002).** MiRNAs may also play important roles in mammalian spermatogenesis where a number of miRNAs are produced abundantly in male germ cells throughout spermatogenesis **(He et al., 2009)**. However, the molecular features of miRNA in spermatogenesis and male fertility are not well defined **(Abhari et al., 2014)**. Lately, miRNAs have great potential for forensic body fluid identification because they are expressed in a tissue specific manner and are less prone to degradation **(Park et al., 2014)**.

Aim of the Work

To assess seminal plasma microRNA 122, microRNA 181a and microRNA 34c-5P in infertile men with varicocele (Vx).

Varicocele and Male Infertility

Varicocele (Vx) is a major cause of male infertility, as it may impair spermatogenesis through several distinct physiopathological mechanisms. With the late advances in biomolecular techniques and the development of novel sperm functional tests, it has been possible to better understand the mechanisms involved in testicular damage provoked by Vx and, therefore, propose optimized ways to prevent and/or reverse them.

Approximately 8% of men in reproductive age seek medical assistance for fertility-related problems. Among them, 1%–10% carry a condition that compromise their fertility potential whereas Vx alone accounts for 35% of these cases. While Vx has an incidence of 4.4%–22.6% in the general population, 21%–41% of men with primary infertility and 75%–81% of men with secondary infertility have this condition **(Sadek et al., 2011; Mostafa et al., 2012)**.

Epidemiology

Vxs is identified in 7% and 10%–25% of pre-pubertal and post-pubertal men, respectively **(Akaby et al., 2000)**. The higher frequency in elderly males and in men with secondary infertility suggested that it is a progressive disorder **(Canals et al., 2005)**. Anecdotal experience