

Original Research: Expression Profile of CAPZA3 And TR-KIT Genes in Men with Globozoospermia & Asthenoteratospermia that Undergo ICSI Protocol

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ABSTRACT

Objective(s): Sperm-mediated oocyte activation depends upon suitable expression and assembly of sperm-borne oocyte-activating factors (SOAFs) during spermiogenesis. Several factors have been considered as candidates for oocyte activation in recent years. Globozoospermia is a severe sperm morphology disorder that is a rare type of teratozoospermia with an incidence of 0.1% among infertile individuals. testis-specific genes including CAPZA3 [capping protein (actin filament) muscle Z-line, alpha, which is considered as a nominee for sperm associated oocyte activating factors, an actin-capping protein, controlling actin polymerization during spermiogenesis. They contain a common bidirectional promoter. Another gene TR-KIT (a truncated form of the KIT receptor) which is a major sperm-associated oocyte-activating factor. The objective of this study was to investigate the expression profile of CAPZA3 and TR-KIT mRNA, in men with total globozoospermia, Asthenoteratospermia, and fertile individuals.

Materials and Methods: Semen samples were collected from three groups including 25 fertile men, 20 Asthenoteratospermia and 12 Globozoospermia that undergo intra-cytoplasmic sperm injection (ICSI), Expression of CAPZA3 and TR-KIT were assessed by Real time PCR.

Results: Individuals with Globozoospermia have presented significantly lower expression of CAPZA3 and TR-KIT mRNA when compared with fertile men. Asthenoteratospermia (male factor) showed significantly lower expression of CAPZA3 mRNA, whereas non-significantly of TR-KIT mRNA. Levels of CAPZA3 and TR-KIT mRNA in the spermatozoa of fertile men were significantly higher than the corresponding values of the globozoospermic and Asthenoteratospermia subjects.

Conclusion: Analysis mRNA of CAPZA3 gene maybe assist the researcher to identify individuals with a lack of ability to induce oocyte activation and make them a candidate for artificial oocyte activation and help researcher to identify genetic defects associated with failed fertilization. whereas, mRNA of TR-KIT gene appears inability to induce oocyte activation

Keywords- CAPZA3, mRNA, TR-KIT gene, fertile.

I. INTRODUCTION

The quantity of normal sperm cells is reduced in infertile men, which is one of the main problems in infertile(1). Among the factors included in male infertility, there is "globozoospermia" first described by Wolff, Schill, and Moritz (1976) (2). which is rare morphology disorder and the primarily, characterized by the presence of round-headed spermatozoa with cytoskeleton defects around the nucleus and no acrosome, despite other sperm parameters such as count, density, and motility being normal (3). The potential of genetic abnormalities being passed, on to future generations still exists. Genetic factors are thought to be responsible for roughly 35% cases of infertility in men.(1)

Nowadays, new strategies for treating spermatogenesis of infertility, such as assisted reproductive technologies (ART) including *Intracytoplasmic sperm injection* (ICSI), which is a procedure used to treat a myriad of male factor infertility cases.(4,5). Importantly, numbers of patients who repeatedly fail ICSI have also fail to stimulated egg activation (5).The first step of stimulation is usually provided by the sperm in most cases that have been studied, sperm triggers a remarkable chain of intracellular Ca²⁺ oscillations in oocytes which proliferate a series of biochemical events called 'Egg activation'.(6,7) Egg activation is a process that involves a multitude of cellular changes involve cortical and zona reactions to prevent polyspermy, resumption or completion of meiosis II, and metabolic activation of the egg that precedes the first embryonic cell division(8). The absence of sperm associated oocyte-activating factors (SAOAFs), in the posterior acrosomal region of the sperm head causes fertilization failure in roughly 1-3 % of instances (9). CAPZA3 is considered as a candidate for sperm associated oocyte activating factors and is located back-to-back with Phospholipase C ζ (PLCζ). CAPZA3 mRNA is transcribed during spermiogenesis in

spermatid, which in clued acrosomal biogenesis, formation of sperm head morphology, capacitation and acrosome reaction. It is an actin-capping protein controlling actin polymerization (7). Therefore, genetic defects expected to affect the expression of CAPZA3 and localization and that influencing the activation potential of quality a semen fluid, like in globozoospermia. (10). the other candidate of sperm borne oocyte activation factors (SOAFs) is truncated *c-kit* gene product (*tr-KIT*) (11). Which is released into ooplasm upon incorporation of sperm with oocyte and due to intracellular calcium oscillation (12). Tr-KIT is present in the human sperm head at equatorial segment, midpiece and sub-acrosomal regions and its existents have been associated with sperm motility, acrosome reaction and oocyte activation in mammal (13). Considering the possibility roles of CAPZA3 and Tr-KIT during spermiogenesis and fertilization, we aimed to evaluate genes expression of both CAPZA3 and tr-KIT in seminal fluid samples of men with normal and abnormal parameters in cases of globozoospermia and Asthenospermia of infertile men that undergo ICSI protocol.

II. MATERIALS AND METHODS

Semen samples were obtained from 57 men which is classified into three groups; 25 fertile men, 20 Asthenoteratospermia and 12 Globozoospermia that undergo intra-cytoplasmic sperm injection (ICSI). Their ages ranged from (25-44) years.

Sample collection:

Freshly ejaculated seminal fluid samples, were obtained by masturbation after (3-5) days of sexual abstinence. Then samples were analyzed according to WHO criteria (WHO, 2010) for sperm concentration, progressive motility and normal morphology.

RNA extraction and cDNA synthesis:

Genomic RNA was isolation from collected semen (sperm) by using Trizol (BIOLINE). The integrity of extracted total RNA was evaluated by using Quantus Fluorometer in order to detect the concentration of RNA ratio (10-50) ng/ μ l. WizScript™ RT FDmix (Hexamer) Kit was used, for reversely transcribed of a total RNA to a complementary DNA (cDNA).

The procedure was executed in a reaction volume of 20 μ l according to the manufacturer instruction, first strand cDNA synthesis was carried out using template RNA (5 μ g) and RNase free water (up to 20 μ l) were added into each, the RT FDmix tubes. After transferred of tubes to a thermal cycler the following program, for cDNA synthesis was using [Primer annealing: 25°C, 10 min, cDNA 42°C, 30 min., Heat inactivation 85°C synthesis, 5 min., and Hold 4°C,

infinity]. Synthesized cDNA is immediately, used as template for PCR or store at -20°C.

Quantitative real-time PCR:

Quantitative real-time RT-PCR was performed by using a thermal cycler (Qiagen Rotor-Gene Q 5PLEX Technologies) with qPCR soft software in a 25ul reaction volume for expression levels of CAPZA3, Tr-KIT, and CAPDH genes as a target gene. Primers used for quantitative real time PCR are CAPZA3, [(F:5'CTGCTGCTTTTCATGGATAGAC3')(R:5'TACTCTTTCTTGTCCTTCTCTG3')], Tr-KIT[(F:CAGCCAGAAAATCCTCCTTACT3')(R:5'GCATCCACTTACAGGTAG3')], GAPDH[(F:GAAATCCCATCACCATCTTCCAGG),(R :GAGCCCCAGCCTTCTCCATG) (13 14 15).

qPCR Master mix (WizPure™ qPCR Master SYPR kit) were using. Master Mix, template DNA, and primers were thoroughly mixed before providing qPCR reactions. The reaction mix setup and amplification protocol with modification were applied as recommended by the manufacturer [(qPCR Master (SYBR) 12.5 μ l, 10 μ M Forward Primer1.0 μ l, 10 μ M Reverse Primer1.0 μ l, Template cDNA3 μ l)]. Thermal program of CAPZA3, Tr-KIT, and CAPDH genes expression was comprised of: 5 min at 95 °C followed by 40 repetitive cycles for 15 sec at 95 °C, 30 sec at 61 °C and 60 °C respectively for CAPZA3, Tr-KIT, and CAPDH and 20 sec at 72 °C. Melt curve was included of: 1min /95 °C- 30 sec /55 °C - 30sec/95 °C. The expression level of CAPZA3 and Tr-KIT mRNA was normalized to GAPDH expression level as a housekeeping gene. Calculation of relative expression was evaluated using, the $\Delta\Delta$ Ct method and final result was demonstrated as $2^{-\Delta\Delta Ct}$ (16, 17).

Statistical Analysis:

Data were statistically analyzed by utilizing SPSS for Windows, version 21 (SPSS Inc. Chicago, IL, United States). Data have appeared as mean \pm standard error of mean (SEM). Categorical variables were analyzed by Tukey's test and Post Hoc test for multiple comparisons were applied after ANOVA tests.

III. RESULTS

Semen parameters of the infertile patients under go ICSI protocol:

The mean age of men were (32.88 \pm 1.323), (34.12 \pm 1.45), and (37.15 \pm 1.16) years old in the fertile, globozoospermic and Asthenoteratospermia groups, respectively. Certain semen parameters appeared highly significant difference between the infertile and fertile control groups (P=0.001) as shown in Table (1).

Table 1: Comparison the certain Semen parameters between studied male infertility groups and the fertile control group.

Semen Parameters	Control Group N=25	Patients Groups		
		Globozoospermic N = 13	Asth. N = 20	P-Value
Sperm count (million/ejaculated)	69.04±5.62	49.16± 5.71	50.18± 3.77	0.07
Progressive motility (%)	45.5± 3.28 a	31.61± 2.81 B	15.5± 2.97 C	0.001**
Non progressive motility (%)	20.68±2.78 a	18.35± 1.36 A	28.02± 1.85 B	0.001**
Immotile (%)	38.24± 4.01 a	52.82± 3.75 b	57.82± 3.58 B	0.001**
Morphologically abnormal sperm(%)	60.89± 2.9 A	100±0.00 B	78.31± 2.83 c	0.001**

Data were expressed as mean ± SE; Statistical analyses were performed by ANOVA(2-tailed) and followed by Tukey's test for multiple comparisons. * P<0.05 = Significant values. ** P<0.01 = Significant values; a,b,c and d:Different letters mean there is a significant difference

Quantitative Expression of CAPZA3 and Tr-KIT mRNA in semen samples

Up to our knowledge, this is maybe the first study able to determine the expression of CAPZA3 and Tr-KIT genes in some Iraqi infertile males, which underwent ICSI protocol. Furthermore, to know of the effect of these genes expression on male-factors infertility. In current research, Total RNA was successfully extracted from all seminal samples. The concentration of total RNA (ng/ µl, shown as mean ±SE)

in fertile group was (33.19±3.25), and in patients groups was; globozoospermic (27.85±1.99), and Asthenoteratospermia (37.44±5.35).

The gene expression of CAPZA3 was normalized to the level of a housekeeping gene GAPDH and quantified by the ΔCt value and folding 2^{-ΔΔCt} method, as illustrated in Table (2). The (mean ± SE) Ct value, ΔCt (normalization Ct values) and the expression fold 2^{-ΔΔCt} of Tr-KIT mRNA in semen samples of the investigated groups were as shown in Table (3).

Table 2: Expression level of CAPZA3 in seminal fluid of the studied groups.

Parameters	Control Group, N= 25	Patients Groups		
		Globozoospermic N = 13	Asth. N = 20	P-Value
Ct CAPZA3 (mean± SE)	21.226±0.701	21.016±0.72	19.381±3.04	0.491 (NS)
Ct CAPDH (mean± SE)	21.68±0.691	21.09±0.901	20.76±4.37	0.922 (NS)
ΔCt CAPZA3 (mean± SE)	0.454±0.759	0.074±0.093	1.379±2.38	0.06 (NS)
Expression fold 2 ^{-ΔΔCt} (mean ± SE)	1	0.136±0.039	0.803±0.168	0.05 *

‡Univariate analysis of variances (ANOVA) significance test; † Post Hoc test (Tukey's test) for multiple comparison using. * P<0.05 = Significant values; NS, no significant difference.

Table 3: Expression level of *Tr-KIT* in seminal fluid of the studied groups.

Parameters	Control Group N= 25	Patients Groups		
		Globozoospermic N = 13	Asth. N = 20	P-Value
Ct <i>Tr-KIT</i> (mean± SE)	21.6426±0.95	20.766±1.741	21.19±0.818	0.491 (NS)
Ct <i>CAPDH</i> (mean± SE)	21.878±0.49	21.04±1.401	21.896±1.09	0.922 (NS)
ΔCt <i>Tr-KIT</i> (mean± SE)	0.454±0.759	0.605±0.415	0.706±2.72	0.06 (NS)
Expression fold 2 ^{-ΔΔCt} (mean ± SE)	1	0.793±0.12	2.041±1.13	0.05*

‡Univariate analysis of variances (ANOVA) significance test; † Post Hoc test (Tukey's test) for multiple comparison using. * P<0.05 = Significant values; NS, no significant difference.

IV. DISCUSSION

For proper and complete spermatogenesis, a high number of coding and non-coding genes must be expressed, at the same time. As a result, stopping or altering each one's expression can cause the spermatogenesis process to be disrupted. Therefore, this side needs to be considered for the fulfillment of infertile men. The major emphasis for molecular valuation and use of RNA sperm is the wide range contribution of male factor in infertility, which is a trouble in the study of infertility men (18). The amount of sperm RNA is tiny, but it is essential for the diagnosis and investigation of male infertility (19).

The mean of age men in this research was the close on average; therefore, no significant difference between the studied groups showed. This means that is no age effect on male infertility. It was agreed with reported by (Dhuha *et al.*, 2021), who found that this age had no effect on male fertility status (15). Bioinformatics studies display that CAPZA3 is located back-to-back with PLCζ, another testis-specific gene. These two genes show to have a common bidirectional promoter (13,20). Because of the presence of several CAPZA3 were found on humans of multiple studies, in the acrosomal, equatorial, and post-acrosomal regions of the sperm head, with a potential tail localization, as well as, Tr-KIT is present in the human sperm head at equatorial segment, midpiece and sub-acrosomal regions(13). This notice is coordinate with role of the CAPZA3 in shaping the sperm head, and especially in formation of acrosome (21). The globozoospermia has absent of acrosome region, which was leading to reduce or absent of expression level (22). Therefore, Quantitative analysis of CAPZA3 and Tr-KIT mRNA revealed that the

expressions of these transcripts were significantly lower in individuals with globozoospermic compared to fertile individuals. Other reason, RNA depleted during spermiogenesis, the rRNA cleavage is responsible for preventing spurious translation following spermiogenesis and that primary means of preventing translation of mRNAs stored in mature spermatozoa and delivered at fertilization. (23,24).

CAPZA3 an actin-capping protein controlling actin polymerization during spermiogenesis. Actin may play a continuing role in both post testicular sperm maturation and acrosomal exocytosis. One of the actin protein studies by Howes and colleagues .in addition, these actins play a role both in the placement of cell shape during spermiogenesis and mis-shapen spermatozoa, defeat to fertilize because of impaired motility (13,25).

Our results showed that expression of tr-KIT mRNA in spermatozoa was significantly lower in sperm of men with globozoospermia, whereas, no significantly with Asthenoteratospermia. In this regard Muciaccia *et al.* (14,26) also observed significant correlations between expressions of sperm tr-KIT with sperm motility and morphology, but not sperm concentration and that difference between current study. Evaluation of sperm tr-KIT could be considered as an additional parameter along with classic seminal fluid analysis for assessment of sperm quality (27). Defects in spermatogenesis may be due to misregulation of testis-specific genes that could affect spermatogenesis and then expression of these genes, which lead to poor sperm quality. Evaluation of sperm TR-KIT could be considered as an additional parameter along with classic seminal fluid analysis for assessment of sperm quality (27, 28).

V. CONCLUSION

The result of this study show demonstrated that analysis mRNA of CAPZA3 gene maybe assist the researcher to identify individuals, with a lack of ability to induce oocyte activation and make them a candidate for artificial oocyte activation and help researcher to identify genetic defects associated with failed fertilization .as well as, the sperm TR-KIT mRNA has an important association with fertilization in humans, and its expression is decreased in individuals with globozoospermia and inability to induce oocyte activation.

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