Scientific Journal for Damietta Faculty of Science **13**(1) 2023, 1-6
ISSN Print 2314-8594
ISSN Online 2314-8616

https://sjdfs.journals.ekb.eg/

Frequency of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Mutations in Azoospermic Egyptian Patients

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Received: 02 April 2023 /Accepted: 07 May 2023

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Abstract

Males with cystic fibrosis (CF) are infertile. Congenital bilateral absence of the vas deferens (CBAVD) is one of the causes. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations have also been found to be an additional cause of male infertility. Therefore, the aims of this study were to investigate the possible involvement of five common CFTR gene mutations (ΔF508, G551D, G542X, W1282X, and R117H) in azoospermia Egyptian infertile males. Materials and Methods: Blood samples were collected from 32 infertile males with Azoospermia In addition, 25 healthy and fertile individuals were included. Fresh semen samples were analyzed by using computer-assisted sperm analysis (CASA). The hormonal profile was investigated using Immulite 2000. Further, screening for CFTR gene mutations were detected by using the Amplification-Refractory Mutation System (ARMS)-PCR technique. Results: Heterozygous CFTR mutations were detected in 4 patients among the studied 32 (12.5%) azoospermic individuals. ΔF508 and R117H are the only 2 detected mutations that gave positive results. Their incidences were 9.4% and 3.1%, respectively. On the hormonal levels, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were increased with no simultaneous effects on the testosterone level; a finding which supports testicular insufficiency rather than the CFTR gene. Conclusion: Whether the latter negatively contributes to azoospermia or not still needs further investigations; on a large-scale male sample. At this time, the importance of CFTR gene mutations study in Egyptians will be more valuable.

Keywords: Cystic fibrosis, Azoospermia, CFTR mutations, Egyptian male infertility.

Introduction

Infertility is typically characterized by the inability to get pregnant after having frequent sex without contraception for a year **(Krausz *et al.*, 2000)**. Male factor infertility is mostly caused by azoospermia, asthenozoospermia, teratozoospermia, and oligozoospermia. Azoospermia refers to the absence of sperm in the ejaculate. Failure of spermatogenesis is typically the cause of the former **(Egozcu *et al.*, 2000; Jarow *et al.*, 1989)**. The CFTR gene is

located on chromosome 7 (7q31.2), and contains 27 exons, 26 introns, and 230 kb of the genomic DNA **(Tsui *et al.*, 2013)**. This protein (CFTR) acts as a channel that regulates the movement of salt and fluids into and out of the cells that generate mucus, perspiration, saliva, tears, digestive enzymes, and semen **(Farinha *et al.*, 2018)**. Patients with CFTR mutations have a restriction in the passage of salts into and out of cells, producing thick sticky mucus. This mucus builds up and leads to a variety of symptoms; including chronic lung infection and inflammation, pancreatic insufficiency, and infertility **(Quinton *et al.*, 2007)**.

The CFTR gene has more than 2,000 known mutations, which are responsible for the various clinical phenotypes of CF **(Elborn, 2016).** The CFTR gene has more than 2,000 known mutations, which are responsible for the various clinical phenotypes of CF **(Elborn, 2016)**. ΔF508 is the most common mutation for CF in the Caucasian population and occurs in the genomic DNA sequence that codes for the first nucleotide-binding domain, representing about 90 % of mutations observed in CF patients **(Riordan *et al.*, 2008).** But according to a present study, the most common mutations of the CFTR gene in all patients were followed by ΔF508 and R117H mutations.

ΔF508 mutation is present in exon 10 and occurs due to deletion of the phenylalanine at position 508, so occurs impairs CFTR protein folding **(Lukacs and Verkman, 2012)**. R117H mutation occurs result replacement of arginine by histidine at position 117 of the CFTR gene, in exon 4, which affects both the pore properties and the gating of the CFTR channel **(De Nooijer., *et al*., 2011; Sheppard, *et al*., 1993)**.

Data on azoospermic patients are insufficient in Egypt. Despite having a high rate of consanguineous marriages. The United Arab Emirates and Bahrain had CF frequencies of 1 in 15800 and 1 in 5800 live births, respectively. These numbers were frequently lower than those of the majority of European countries, where live birth rates ranged from 1 in 2000 to 1 in 4000, and the US (one: 3500 live births) **(WHO, 2004)**. Further, the relationship between CFTR gene mutations in cases of infertility other than CBAVD is unclear **(Asadi *et al.*, 2019)**.

The purpose of this study is to investigate the possible involvement of ΔF508, G551D, G542X, W1282X, and R117H as CFTR gene mutations markers.

Materials and Methods

Subjects

After awarding written consent this study included 25 fertile control and 32 infertile males with Azoospermia. The specimens were classified according to the World Health Organization Laboratory Manual for examining and processing human semen, 5th Edition according to **WHO (٢٠١٠)**. They were selected from the andrology patient's clinic, at Mansoura main University Hospital, Egypt.

Inclusion criteria

Healthy controls had normal sperm count (not less than 15 million/ml) according to World Health Organization guidelines **(WHO, 2010)** requirements, normal morphology as well as normal sperm motility.

Azoospermic patients had no sperm in the seminal plasma. Also, there serum FSH and LH within the reported abnormal values WHO. Further, their partners were clinically, radiologically normal.

Exclusion criteria

Healthy controls with lower sperm count (15 million/ml, WHO). Azoospermic patients with normal FSH and LH.

Semen analyses

The diagnosis of primary infertility in all patients is based on clinical evaluation and semen analysis by using computer-assisted sperm analysis (CASA).

Hormonal analyses

Serum FSH, LH, and testosterone were performed by using the Immuno-chemiluminescent analyzer (siemens immulite 2000, Germany).

Extraction of DNA

A peripheral venous blood sample (3 – 5 mL) was collected from each patient into tubes containing Ethylenediaminetetraacetic acid (EDTA), For molecular testing. Genomic DNA was extracted from the leukocytes of each individual by using the spin-column procedure

(QIAamp Mini Kit; Qiagen, Hilden, Germany). The extracted DNA were stored in a freezer at -20°C.

CFTR gene mutations assay

The mutations analysis for CFTR gene were done by using Amplification-Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) after using the specific primers **(Table 2)** (Bilegio, Holland) to determine the genotype of common mutations in the CFTR gene (ΔF508, G551D, G542X, W1282X, and R117H) **(Table 1).** At the end of the last extension step, electrophoresis of the ARMS reaction products was performed using 1.5 % agarose gel containing ethidium bromide.

Statistical analyses

SPSS statistics software was used to conduct the statistical analysis. Every *p* value was calculated using two-sided comparisons. $p<0.05$ is considered as significant and $p<0.001$ highly significant

Table 1: PCR amplification conditions for CFTR gene mutations

Stage's name	Degree temperature (°C)	Duration	Number of cycles
1st denaturation	94	5 min	1
Denaturation	94	2 min	30
Annealing	60	2 min	30
Extension	72	2 min	30
Final extension	72	10 min	1

Table 2: Primers used for the detection of CFTR gene mutations.

Types of Mutations	Location	primers sequence used for single ARMS test (5'to 3')	size of base pairs
R117H	Exon 4	CACATATGGTATGACCCTCTATATAAACT	
		CCTATGCCTAGATAAATCGCGATAGAAC	237
		CCTATGCCTAGATAAATCGCGATAGAAT	237
W1282X	Exon 4	CCCATCACTTTTACCTTATAGGTGGGCCT	
		CCTGTGGTATCACTCCCAAGGCTTTCCAC	178
		CCTGTGGTATCACTCCAAAGGCTTTCCAT	178
ΔF508	Exon 10	GACTTCACTTCTAATGATGATTAGGGAG	
		GTATCTATATTCATCATAGGAAACACCAC	160
		GTATCTATATTCATCATAGGAAACACCAC	157
G542X	Exon 11	TAAAATTTCAGCAATGTTGTTTTTGAC	
		ACTCAGTGTGATTCCACCTTCTAC	256
		CACTCAGTGTGATTCCACCTTCTC	257
G551D	Exon 11	TAAAATTTCAGCAATGTTGTTTT	
		GCTAAAGAAATTCTTGCTCGTTGC	285
		AGCTAAAGAAATTCTTGCTCGTTG	286

Results

In the present study, we examined 32 infertile males and 25 fertile controls (sperm count >20 million/ml) for five different CFTR mutations. The Mean semen volume in Azoospermia patients was 2.2 ± 0.4 ml. This mean volume was highly significantly decreased when compared to the expected value of the normal individuals (3 ± 0.9 ml).

The hormones profile showed elevated FSH and LH levels in sera of patients with azoospermia compared with control. In spite normal testosterone mean level was found in sera of the 32 infertile males **(Table 3).**

Molecular analyses

Heterozygous CFTR mutations were observed in 4 of 32 (12.5%) infertile males and no mutations were observed in control. Only two mutations were observed in the patients' group. The most common mutation was ΔF508. It was observed in 3 cases (9.4%). Also, R117H was observed in only one case (3.1%) **(Table 4).**

Table 3: Semen analysis and hormones profile in infertile Egyptian males.

Parameters	Control	Azoospermia
Patients number	25	32
Mean age (years)	27.9 ± 3.8	29.7 ± 4.1
Semen analysis		
Volume (ml)	3 ± 1	2.2 ± 0.4**
count (million/ml)	51.3 ± 13.3	0
Total sperm motility (%)	76.8 ± 2.5	0
Abnormal sperm morphology (%)	16.2 ± 6	0
Hormonal assays		
FSH (mIU/ml)	6.4 ± 2.5	24.8 ± 9.2**
LH (mIU/ml)	6 ± 2.5	13.6 ± 5.4**
Testosterone (ng/dl)	5.1 ± 1.6	4.8 ± 1.3

*p<0.05 is considered as significant and **p<0.001 highly significant. FSH: follicle-stimulating hormone; LH: luteinizing hormone

Figure 1 Represented the Polymerase chain reaction (PCR) products for 4 cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations; namely, ΔF508, G551D, G542X and W1282X in one infertile male with Azoospermia using Amplification Refractory Mutation System (ARMS) PCR method. Lanes 1 optionally found the results with a heterozygous individual carrier for ΔF508 mutation. Lanes 2, 3, and 4 represent normal gene G551D and G542X, and W1282X, respectively.

Figure 2 Represented Polymerase chain reaction (PCR) products for the remaining cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations (R117H) for the four infertile males with Azoospermia. ARMS-PCR method were used. Lanes 3 showed a heterozygous individual carrier for R117H mutation. but Lanes 1, 2, and 4 represent normal R117H mutation.

Table 4: Frequency of CFTR mutations among patients with azoospermia

SN	Groups	CFTR mutations
1	Azoospermia	ΔF508 /-
2	Azoospermia	ΔF508 /-
3	Azoospermia	R117H /-
4	Azoospermia	ΔF508 /-

SN: Serial number

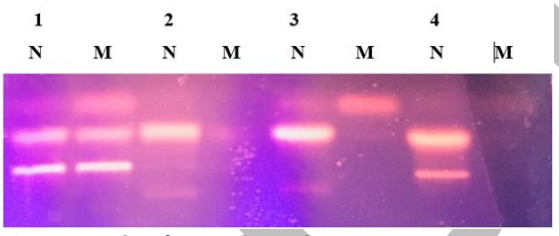

Figure 1: This figure showed CFTR gene mutations on 1.5% agarose gel electrophoresis (N: normal; M: mutant). lanes 1 represent ΔF508 Heterozygous mutations. Lanes 2, 3, and 4 represent normal gene for G551D, G542X, and W1282X, respectively.

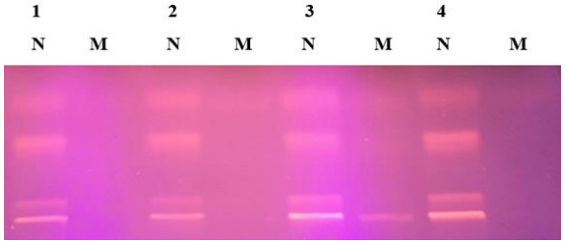

Figure 2: This figure showed CFTR gene mutations on 1.5% agarose gel electrophoresis (N: normal; M: mutant). Lanes 3 represent optionally found result with a heterozygous individual carrier for R117H mutation. Lanes 1, 2 and 4 represent of normal R117H.

Discussion

Several studies from many countries have demonstrated that CFTR gene mutations actually lead to the pathophysiology of male infertility, especially in patients with obstructive azoospermia, or testicular failure **(Chillón *et al.*, 1995; Costes *et al.*, 1995 and Dohle *et al.*, 2002)**. Unfortunately, it is unclear what types and how frequently CFTR gene mutations are found in the Egyptian population **(Fathy *et al.*, 2016)**. This study was conducted to investigate the frequency of CFTR gene mutations in infertile azoospermic Egyptian. In such patients' high incidences of CFTR mutations was observed. In this regard, **Van Der Ven *et al.* (1996)** found heterozygous CFTR mutations in 3 of 21 (14.3%) with azoospermia men and 14 of 80 (17.5%) in healthy men with infertility due to poor sperm quality. **Gallati *et al.* (2009)** Adde that CFTR mutations were found in 68% of men with CAVD, 31% of men with azoospermia, and 22% of men with oligospermia (Switzerland). In Germany, **Schulz *et al.* (2006)** conducted yet another large investigation; before pre-intracytoplasmic sperm injection (ICSI). Among their results, only nine CFTR mutations were examined in 597 infertile males. According to their results, CFTR mutations were observed in 7 of 67 (10.44%) patients with azoospermia. This result was slightly lower than the results of the current investigation (12.5%) in azoospermic Egyptian patients. According to **Sharma *et al.* (2014)**, the heterozygous CFTR mutations is about 11.6% in patients with non-CBAVD obstructive azoospermia. In patients with spermatogenesis defects, it was about 7%.

In Iran **Asadi *et al.* (2019)** examined 50 infertile males with non-obstructive azoospermia and 50 CBAVD for the presence of five CFTR mutations (ΔF508, G551D, G542X, N1303K, and R117H). In their non-obstructive azoospermia patients (NOA) no mutations were observed. Thus, a higher prevalence of CFTR mutations in Egyptian infertile patients was observed compared with the Iranian individuals with the same Pathology.

The only two research groups conducting Egyptian infertile men but with CBAVD include **Hussein *et al.* (2011)** who examined 30 infertile males having CBAVD. In their results ΔF508 mutation was found in 12 patients (40%)

20% of the alleles. Also, 5T variant was found in 46.6% (27% of alleles). The second research group **[Fathy *et al.* (2016)** investigated the occurrence of the 5T variant, p. Ser1251Asn mutations, and ΔF508 in 14 Egyptians with CBAVD. They found that, ΔF508 and p. Ser1251Asn, heterozygous CFTR mutations in 3/14 (21.4%) and 1/14 (7.1%) of the patients, respectively.

In a saudian study by **AlMaghamsi *et al*, (2020)** 7 of 50 (14%) infertile individuals with OAT or Azoospermia, were reported such patients were with CFTR mutations. Unfortunately, the studied mutations which are not included in the present study. Their results were slightly higher than those reported in our Egyptian infertile patients with azoospermia, having CFTR mutations.

Conclusion

This study identified the frequency of CFTR gene variations in Egyptians azoospermic males. CFTR mutations were presented in 12.5% of the research participants. Therefore, we can stop the transmission of this mutation to the following generation if more research reveals a connection between the CFTR gene mutation and extremely azoospermia.

Acknowledgment

We are extremely grateful to participating patients.

Ethics committee approval.

The research has complied with all relevant national regulations and institutional policies and has been approved by the author's institutional review board or equivalent committee.

Copies of the guidelines and policy statements are available for review by the Managing Editor if necessary.

The editors have the right to seek additional information or guidance from reviewers on any cases in which concerns arise.

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الملخص العربي

عنوان البحث: معدل تكرار الطفرات الجينية للتحور الجيني المنظم للتوصيل عبر الغشاء في التليف الكيسي في المرضى المصريين الذين يعانون من عدم وجود حيوانات منوية

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نبذة تاريخية: وجد أن الرجال الذين يعانون من تليف كيسي يعانون أيضا من العقم. ويعتبر الأشخاص الذين يعانون من تشوه ثنائي في الوعائين الناقلين للحيوانات المنوية سبب من هذه الأسباب. ووجد أيضا أن الطفرات الجينية للتحور الجيني المنظم للتوصيل عبر الغشاء في التليف الكيسي تعتبر سببا أضافيا للعقم عند الرجال. ولذلك فان الهدف من هذه الدراسة هو البحث عن الطفرات الخمسة الشائعة لجين التليف الكيسي وهي (ΔF508, G551D, G542X, W1282X, and R117H) ومدى تكرارها في الرجال المصريين الذين يعانون من عدم وجود حيوانات منوية في السائل المنوي.

الطرق والمواد المستخدمة: تم تجميع عينات دم من ٣٢ شخص ممن يعانون من عدم وجود حيوانات منوية في سوائلهم المنوية . أضف الى ذلك فقد ت حب عينات دم من ٢٥ شخص من الأصحاء .وذلك بعد أخذ موافقة كتابية من الجميع . وفي ذات الوقت تم أخذ لعينات السائل المنوي لكلا بعد أخذ الشروط المنشودة في هذا الصدد وتوصيفها باستخدام الكمبيوتر. تم عمل الهرمونات الأتية . الهرمون المنشط لأنتاج الحيوانات المنويه (FSH) والهرمون المنشط لإنتاج التيستوستيرون (LH) وكذلك هرمون التيستوستيرون نفسه. . (Testosterone) تلي ذلك فحص طفرات جين التليف الكيسي في مستخلص الحامض النووي الديكوسي ريبوزي لكريات الدم البيضاء لمجموعتي المرضى والأصحاء باستخدام تقنية(ARMS-PCR method)

النتائج : تم الحصول على الطفرتين الجينيتين واللتان سميتا ΔF508 , R117H وذلك بطريقة غير متماثلة (Heterozygous) وبنسب ٤.٩ % و ١. ٣ % على الترتيب. وعلى مستوى الهرمونات وجد أن هناك زيادة ف مستوى الهرمون المنشط لأنتاج الحيوانات المنوية (FSH) وكذلك في مستوى الهرمون المنشط لانتاج التيستوستيرون (Testosterone) ورغم زيادة الأخير فانه لم يصاحب هذه الزيادة اي زيادة في مستوى الهرمون الذكري مما يؤكد أن سبب العقم ربما يرجع لأسباب تتعلق بالخصية ذاتها.