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Research Article

Detection of Y chromosome microdeletion in AZFb and AZFc loci using sequence tagged sites (STSs)

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Abstract: This study is intended to find out the frequency of Y chromosome AZF (Azoospermia Factor) subregions microdeletions in Sudanese men complain of not been fathers after one year of unprotected sexual intercourses. Study participants were thirty two males who referred to fertility clinics for assisted reproduction techniques (ART). Using semen analysis, eighteen were found azoospermic and fourteen were found oligospermic. Four couples; males and females who achieved pregnancy during the first year of marriage were used as positive and negative control correspondingly. Testicular sperm extraction was done for ten azoospermic participants, 40% failed to retrieve sperms from both testes. DNA was isolated from blood samples. Polymerase chain reaction (PCR) was performed on six loci spanning the AZFb and AZFc subregions of the Y chromosome. Microdeletions of the Y chromosome were found in 12 out of 32 participants 37.5%. Microdeletion at AZFc subregion was found to be most frequent (50%). The microdeletion at AZFb loci was found to be 33.3%, and deletion at both loci AZFb+c were 16.7%. In conclusion, couples who attend fertility clinics and plan to use assisted reproductive techniques (ART) it is preferable to assess Y*q*11 microdeletions previous to deciding for ART because these microdeletion will transferred to the produced progeny.
Keywords: male infertility, AZF microdeletion, AZFb, AZFc, Y chromosome microdeletion, Azoospermia, Oligozoospermia

INTRODUCTION

Male infertility is a worldwide problem. It affects about 15% of those who are trying to be fathers [1]. Of the many factors causing it, genetic factors alone constitutes 10 – 15% of this problem [1]. However, chromosomal anomalies whether numerical or structural was placed as the first of these factors as in case of klinefelter syndrome[2]. Previously, Tiepolo and Zuffardi considered microdeletion of the Y chromosome as the most frequent genetic cause resulting in azoospermia and hence leading to male infertility [3].

In Sudan, despite of availability of advanced fertility centres providing assisted reproduction services, no investigations was carried out to explore genetic causes of this major problem. The present study is aimed to find out whether or not occurrance of microdeletion on Y chromosome is causative factor of azoospermia and oligozoospermia among Sudanese infertile men.

MATERIALS AND METHODS

Subjects and place of study

The study was conducted at Abu Alhassan fertility centre in Khartoum. The centre receives its clients from all over Sudan. Thirty two male subjects from those enrolled at the centre were chosen for this study. These subjects had failed to conceive or produce progeny after one year of unprotected sexual intercourse and seeking in vitro fertilization (IVF) as a treatment option. The subjects aged between 25 and 49 years. All participants were ensured to be medically fit, free of any congenital disorders, and enjoying normal sexual life. Prior to commencement, ethical clearance was guaranteed to conduct the study, and written consent was obtained from each participant.

Examination of the subjects

Semen analysis

Semen ejaculate from each subject was obtained by masturbation at the fertility centre after three days of abstinence. 10 µL of each semen sample was then examined for the number of sperms under phase contrast microscope using special counting chamber. Those with number of sperms less than 20×10^6/ mL were denoted as oligozoospermic while those with no sperms in their ejaculates as azoospermic. Absence of sperms was confirmed even further by

centrifugation of each sample at 12000 rpm for fifteen minutes. This analysis was performed according to WHO criteria[4].

Testicular sperm extraction (TESE)

On the day of oocyte retrieval, scrotal exploration was performed under general anaesthesia by professional surgeon. Two to three millimetre piece of epididymal testicular tissue was removed and dissected by fine needle under sterilized condition. Then the dissected tissue was examined under light microscope (20X lens) to check for sperms. Those with sperms were denoted as (+ve TESE) and those without them were denoted as (-ve TESE).

Determination of microdeletion on Y chromosome

Loci of microdeletion on Y chromosome q arm were located by using sequence tagged sites (STSs). This process was described by Vollrath *et al.* [5]. For this purpose, three ml of blood was taken in EDTA vacutainer tube from each participant and preserved for DNA extraction. DNA was extracted according to the modified salting-out method [6]. For each sample, DNA was quantitatively and qualitatively measured using NANO DROP spectrophotometer (ND-1000). Three sequence tagged sites at AZFb region and three sequence tagged sites at AZFc region on chromosome Y at *q* arm (long arm) lying within intervals 5 and 6 were selected (table-1). Internal control known as sex-determining region on Y chromosome (SRY) at *p* arm (short arm) were also selected according to Vogt *et al*. [7] and Mohammed *et al*. [8]. Two multiplexes were applied at every single polymerase chain reaction (PCR), and the individual multiplex determined considering the length of the PCR product. The PCR profile was performed followed Mirfakhraie *et al*. [9]. All PCR products were allowed to run in 2% agarose gel. Electrophoresis was performed in 1X TBE buffer at voltage of 86 V and ampere of 11 mA for 30 to 40 minutes. The documentation performed using WISD documentation system. Each sample was scored using 100 base pair DNA ladder.

Data entry and analysis

Data were entered in excel sheet and then analysed by SPSS version 17.

RESULTS

Among 32 subjects, AZF microdeletion was found in 12 participants. The frequency of each STS Microdeletion among azoospermic and oligozoospermic is also demonstrated in table-2.

Among 10 subjects who perform TESE, microdeletion were not detected in any of study participant those showed negative TESE. However, 4 study participants with positive were found to have microdeletion table 3.

Table-1: sequence tagged sites (STSs) selected for the multiplex PCR

STS	REGION	SEQUENCE	PRODUCT SIZE
sY 14	SRY	F-5'GAATATTCCCGCTCTCCGGA3' R-5'GCTGCTGCTCCATTCTTGAG3'	472
sY127	AZFb	F-5'GGCTCACAAACGAAAAGAAA3' R-5'CTG CAG GCA GTA ATA AGG GA3'	274
sY128		F-5'GGATGAGACATTTTTGTGGG3' R-5'GCCCAATGTAAAACTGGACA3'	228
sY134		F-5'GTCTGCCTCACCATAAAACG3' R-5'ACCACTGCCAAAACTTTCAA3'	301
sY239	AZFc	F-5'CATTCATCTTCCCTTTTGAAGG3' R-5'ATGCAAGTCGCAGGAAATCT3'	201
sY254		F-5'GGGTGTTACCAGAAGGCAAA3' R- 5'GAACCGTATCTACCAAAGCAGC3'	370
sY255		F-5'GTTACAGGATTCGGCGTGAT3' R-5'CTCGTCATGTGCAGCCAC3'	126

.**Table-2: Number, percentage, and type of microdeletion among the study subjects**

Diagnosis	No.	%	Microdeletion presence in (%)	Microdeletion sub-region			Avg age (years)
				AZFb (%)	AZFc (%)	Both (%)	
All	32	100	12 (37.5)	4 (33.3)	6 (50)	2 (16.7)	37.25
Azoospermic	18	56.3	8 (44.4)	2 (25)	4 (50)	2 (25)	38.17
oligozoospermic	14	43.7	4 (28.5)	2 (50)	2 (50)	-	36.07

Table-3. TESE for some azoospermic participants

TESE status	No.	%	Microdeletion frequency (%)	AZFb (%)	AZFc (%)	Both (%)
All	10	10	4 (40)	-	-	-
-ve TESE	4	40	0 (0)	-	-	-
+ve TESE	6	60	4 (66.7)	1 (25)	2 (50)	1 (25)

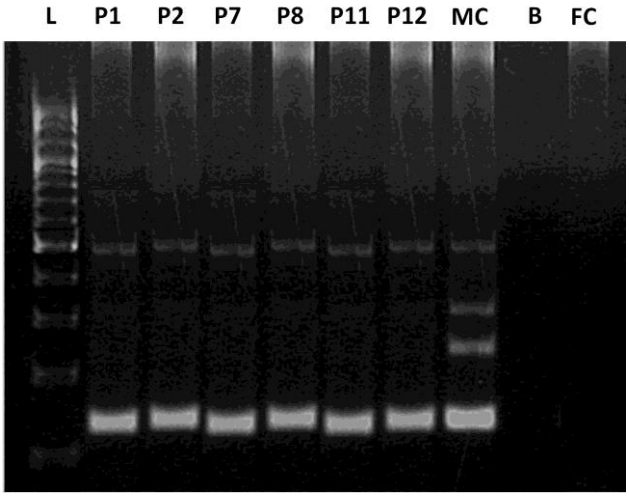

Fig-1: showing microdeletion of AZFb sub-region 4 azoospermic and 2 oligozoospemic.

P= Participant, L= ladder, MC= male control, FC= female control, B= blank
P1, P2, P7, and P8 are Azoospermic, P11, and P12 are Oligozoospermia

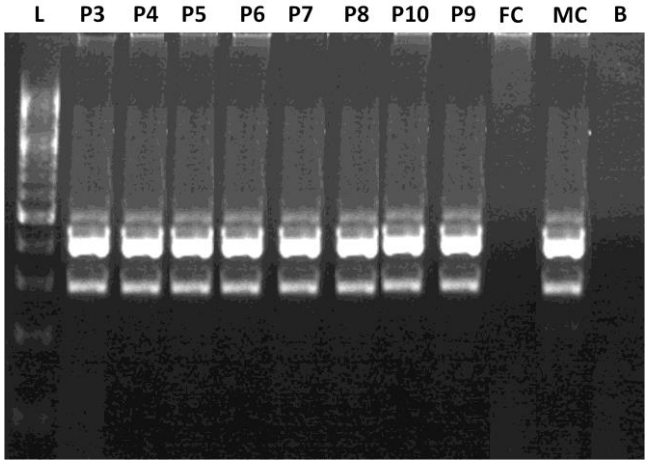

Fig-2: Showing microdeletion of AZFc sub-region 6 azoospermic and 2 oligozoospemic.

P= Participant, L= ladder, MC= male control, FC= female control, B= blank
P3, P4, P5, P6, P7, P8, are Azoospermic. P9 and P10 are Oligozoospermic

DISCUSSION

In the present study, infertile males, classified as azoospermic or oligospermic were investigated in relation to microdeletion that reported on chromosome Y [3].

Infertility attributed to azoospermia or severe oligozoospermia among male subjects of this study could be judge to be linked to microdeletion on chromosome Y as subjects had showed no signs of congenital defects and was physically fit, enjoying normal sexual life and the age average was below 40 years. Similar to previous reports [10, 4], the results of semen analysis and TESE adopted in this study had also confirmed quantitatively the occurrance of azoospermia

and severe oligozoospermia among the participants of this study.

However, the process of sequence tagged sites (STSs) as depicted by electrophoresis revealed that the microdeletion was confined to sub-regions AZFb and AZFc in both azoospermic as well as oligozoospermic. In this context, of 12 participants STSs, 50 % of 8 azoospermic males had AZFc deletion, 25 % in AZFb where as 25 % at both sub-regions. This in contrast to those oligozoospermic where microdeletion of AZF was confined equally to positions in the two sub-regions.

On the other hand, the prevalence of azoospermia and oligozoospermia of this study seemed to be more likely to match with the percentage ranges reported worldwide [11-18].

It is clear that the frequency of microdeletions in azoospermic patients is variable (possible ethnic or geographic factor), but the most frequent place of deletions is the AZFc sub-region [19].

Viewing the overall results together, it could be judged that, microdeletion detected in this study was main causative factor leading to male infertility among those seeking assisted reproduction services via in vitro fertilization technique particularly at the fertility centre of the present study.

The detection of a deletion in an infertile man provides a proper diagnosis of the phenomenon, allows the clinician to avoid empirical, needless, and often costly treatments to improve fertility, and has important ethical consequences if the patient is a candidate for assisted reproduction techniques [20].

There are many techniques for treatment like ICSI, IUI, and IVF. They are all common techniques in fertility clinics. Although the efficacy of these therapeutic methods; they all pass the de novo AZF microdeletion through sperm DNA[20-25].

The male couple with Y microdeletions should be advised to undergo genetic counselling before attempting pregnancy [26].

CONCLUSION

In conclusion, high incidence of Y chromosome microdeletions was found in the present study participants who attend fertility clinic. These microdeletions will be transfer to the offspring if ART used to achieve pregnancy, so genetic consulting is very important when ART is preferred [26].

The identification of the actual role played by the AZF candidate genes in spermatogenesis will provide significant advances to our understanding of the biology of spermatogenesis, as well as the analysis of novel Y-chromosomal genes with a potential role in male germ cell development will clarify other important features of this important chromosome [20].

Introducing the PCR technique to fertility clinics is very important in order to screen for microdeletion presence in males complaining from delay production of progeny [27-29].

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