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RESEARCH ARTICLE

Investigation of Azoospermia Factor Microdeletions in Infertile Men in Diyala Governorate

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Abstract

Background:Y chromosomes microdeletions (YCM) are the most frequently observed structure abnormalities in the male specific region (MSY), 15% of total primary spermatogenic failure cases are related to at least 6 known major YCM patterns. These microdeletions present in 5-10% of infertile men and they are very rare in fertile and normospermia men.

Objectives: This study was conducted to investigate the prevalence of Azoospermia Factor (AZF)region microdeletions in a sample of Diyala governorate infertile men.

Subjects and Methods: An included 80 infertile men who had been referred to private clinics of New Baquba as a test group and 20 proven fertile men as a control group. Tests included seminal fluid analysis, sex hormones profile and polymerase chain reaction using specific tagged sites.

Results: Showed that eleven infertile men (13.75%) had AZF microdeletions, while no microdeletion were found in control group. Seven (63.6%) microdeletions were in AZFc sub-region while 2(18.1%) microdeletions were in AZFbc and AZFb each. This study showed that men with sperm count below <5 millions/ml should be evaluated for Yq11 microdeletions before attempting any surgical or hormonal treatment or attending assisted reproductive techniques.

Conclusion:AZFc is the dominance pattern of microdeletions in Diyala governorate infertile males and AZFa pattern of microdeletions is the rarest pattern, also genetic screening for long arm of Y chromosome microdeletions should be listed in the routine tests for men who suffer from azoospermia prior to providing ART service or surgical treatments.

Keywords: Factor (AZF)region microdeletions,infertile men,Diyala governorate.

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Introduction:

Infertility is a major public health problem. It affects 10-15% of total couples who seek to have children(1). Affected couples suffer from enormous emotional and physiological trauma. Male and female infertility contributes to 40% each of total cases, while the other 20% are common infertility were both male and female contributes (2), (3). Y chromosomes microdeletions (YCM) are the most frequently observed structure abnormalities in the male specific region (MSY), 15% of total primary spermatogenic failure cases are related to at least 6 known major YCM patterns (4). These microdeletions present in 5-10% of infertile men and they are very rare in fertile and normospermia men (5).

Most AZF microdeletions in Y chromosome are de novo (6). However several cases reported transmission of these microdeletions(7-12). Since when they discovered by Tiepolo and Zuffardi (13) , a lot of studies done to understand the mechanism of development and characteristics of these microdeletions. Vogt and colleagues recognized three recurrently deleted sub-regions in Yq11 (14), they termed them as AZFa, AZFb and AZFc. The microdeletions in AZFa sub-region were linked with the lack of germ cells or Sertoli cells only syndrome, microdeletions of AZFb sub-region have been linked to spermatogenic arrest, while failure of maturation process of post-mitotic germ cells were linked to AZFc pattern microdeletions, Currently, we know that there is at least 14 protein encoding genes as a part of azoospermia factor locus (15), (16).

Subjects and Methods

Current Study included eighty infertile males 40 non-obstructive azoospermic (sperm count=0) and 40 oligospermic (sperm count ≤ 20 millions)(17)residing in Diyala governorate who attempt infertility unit of Al-Batool hospital and private clinics between June 2016 and December 2017, while control group consisted of 20 proven fertile males defined as conceiving one child at least without any medical assistance.

Semen samples were collected according to procedure described by the world health organization(17). in case of zero sperm count fructose test was done to differentiate between obstructive and non-obstructive azoospermia.Approximately 5ml venous peripheral blood samples were collected and divided into two tubes one contains EDTA to prevent blood form clotting and used later for DNA extraction, and the other one was plane tube used to separate blood serum for hormonal analysis.

Chemical analyzer and tumor markers Cobas e-411 was used to measure serum concentration of total testosterone, FSH, LH and prolactin in both study and control groups.

Genomic DNA extracted from whole blood using geneaid® DNA extraction KIT provided from Taiwan and according to manufacturer instructions. The quality of extracted DNA was assessed using nanodrop device at 260/280nm wavelength. Value of 1.6-1.9 of purity considered as good quality.

Primer set used to detect the presence or absence of AZF sub-regions was obtained from (18) two sets of primerswere used, each set contains three primers, one for each sub-region (AZFa,b,c) plus another primer SRY as an indicator for successful PCR reaction as shown in table 1.

PCR reaction was conducted in 20µl of reaction mixture containing 5µl of template DNA and 2µl of each primer(1µl forward and 1µl reversed) and 7µl of free RNase-free water. Amplification was conducted using a master cycler Eppendorf programmed with 35 cycles of Initial denaturation 95ºC for 15 minutes, Denaturation 94ºC for 30 seconds, annealing 57ºC 30 seconds, Extension 72ºC for 1 minute, final Extension 72ºC for 10 minutes as shown in table 2.

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Table 1. STS primers used for AZF microdeletions analysis (18)

STS	region	Sequence	Product (bp)size	Group
sY14-F sY14-R	SRY	5′-GAA TAT TCC CGC TCT CCG GA-3′ 5′-GCT GGT GCT CCA TTC TTG AG-3′	472	Both sets
sY86-F sY86-R	AZFa	5′-GTG ACA CAC AGA CTA TGC TTC-3 5′ - ACA CAC AGA GGG ACA ACC CT - 3	318	Set A
sY127-F sY127-R	AZFb	5′-GGC TCA CAA ACG AAA AGA AA-3′ 5′-CTG CAG GCA GTA ATA AGG GA-3′	274	
sY254-F sY254-R	AZFC	5′-GGG TGT TAC CAG AAG GCA AA-3′ 5′-GAA CCG TAT CTA CCA AAG CAG C-3′	380	
sY84-F sY84-R	AZFa	5′-AGA AGG GTC CTG AAA GCA GGT-3′ 5′-GCC TAC TAC CTG GAG GCT TC-3′	326	Set B
sY134-F sY134-R	AZFb	5′-GTC TGC CTC ACC ATA AAA CG-3′ 5′-ACC ACT GCC AAA ACT TTC AA-3′	301	
sY255-F sY255-R	AZFc	5′-GTT ACA GGA TTC GGC GTG AT-3′ 5′-CTC GTC ATG TGC AGC CAC-3′	123	

Table 2.PCR program for amplification fragment

No.	Steps	Temperature(C)	Time(minutes)	No. of cycles
1-	Initial denaturation	95°	15	1
2-	Denaturation	94°	0.5	35
3-	Annealing	57°	1.5	
4-	Extension	72°	1	
5-	Final Extension	72°	10	1

PCR products were separated on 1% agarose and visualized by UV gel documentation system.

SPSS software for Windows (SPSS,V.23,SPSS Inc.,Chicago,IL,USA) was used to calculate mean and Mean±SD. Statistical differences between two means were obtained using unpaired t test. Data were considered statistically significant when $P<0.05$.

Results:

The results of sequenced tagged sites (STS PCR) showed that there is AZF microdeletions in 11 individuals which accounts for 13.75% of the total 100 individuals included in this study as shown n figure 1 and 2. No microdeletions found in control group. AZFc region microdeletions was the most frequent microdeletion, it was detected in 7 cases which accounts 63.6%, followed by AZFbc, AZFb region microdeletions that were found in 2 cases each forming 36.5% of total microdeletions as shown in table 3.

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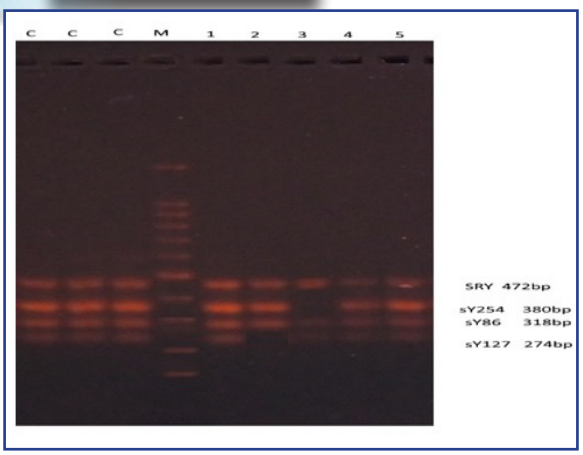

Figure (1): Gel electrophoresis results for PCR product of group A primers set.
C=control , M= size ladder. 1,4,5=DNA of normal male. 2=DNA of AZFb deleted patient
3=DNA of AZFc deleted patient

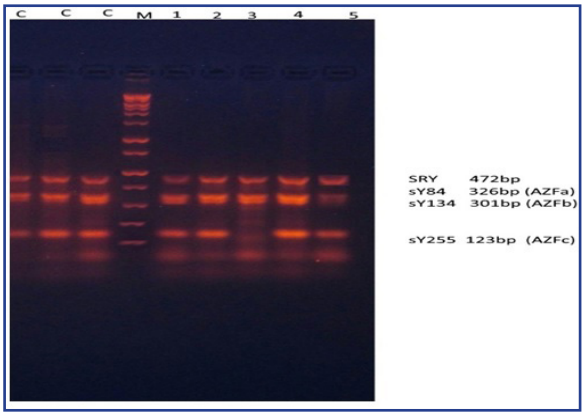

Figure (2):Gel electrophoresis results for PCR product of group A primers set.

C=control. M= size ladder. 1,2,4= DNA of normal male. 3= DNA of AZFc deleted patient.
5= DNA of AZFb deleted patient.

Table 3. STS PCR results

Character	Test group		Control group
	Azoospermia	Oligospermia	
N	40	40	20
Number of deletions	9	2	0
AZFc	7	0	0
AZFbc	2	0	0
AZFb	0	2	0

There was FSH elevation in significant levels in azoospermic males group compared to other two groups as shown in table 4.

Table 4. sex hormones profile for experiment group

Hormone	Control	Oligospermia	Azoospermia
FSH	7.5±4.33	12.01±8.21	16.69±10.58
LH	3.9±2.2	10.62±4.59	8.84±4.81
Prolactin	7.57±1.5	15.6±5.91	11.78±5.49
Total Testosterone	5.7±0.04	3.83±0.14	2.51±0.51

There was no significant relationship between sex hormones levels and AZF microdeletions occurrence as shown in table 5.

Table 5.compression of sex hormones level between normal males and males who suffer from microdeletions

Hormone	Without AZF microdeletions	With AZF microdeletions
FSH	15.2±7.4	11.3±9.7
LH	9.5±5.2	8.2±2.12
Prolactin	14.2±6.1	13.7±3.5
T.testosterone	2.9±2.01	2.5±1.2

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Discussion:

Y chromosome microdeletions are considered nowadays a potential genetic cause of infertility(19). The frequent of AZF microdeletions observed in this study was about 13.75%, whichconsidered low when compared to the frequencies in the previous Iraqi studies (20-23). While it considered close or relatively higher when compared to studies done in other Arabic countries (24-27).

AZFc microdeletions being the most frequent abnormality in this study because it's extremely fragile compared to other AZF regions, AZFc region contains the DAZ family genes which encode proteins with RNA-binding motive and involve in the regulation of RNA metabolism during testicular development (28), (29).This result is consistent with most of the literature that indicate that the prevalence of microdeletions in AZFc is high comparted to AZFb and AZFa regions(1), (30-34).

Nine of the total eleven microdeletions were observed in azoospermic men , the other two microdeletions were observed in a severe oligospermic men, while no microdeletions were observed in oligospermic and fertile men,which indicates a strong relationship between sperm count decrease and having AZF microdeletions. There was no distinguishable relationship between sex hormones profile results and having AZF microdeletions. also, the etiology of male infertility may differ between ethnic population, and the AZF microdeletions are not always detected (35).

Conclusion:

AZFc is the dominance pattern of microdeletions in Diyala governorate infertile males and AZFa pattern of microdeletions is the rarest pattern, also genetic screening for long arm of Y chromosome microdeletions should be listed in the routine tests for men who suffer from azoospermia prior to providing ART service or surgical treatments.

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