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Research Article

Detection Y Chromosome Microdeletions Among Iraq Population in Infertile Patients with Azoospermia and Severe Oligospermia

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ABSTRACT

Objective: To detection of microdeletions of Y chromosome and study the frequency of microdeletions in infertile men with non-obstructive azoospermia or severe oligozoospermia(Middle Euphrates center)in Iraq population. Material and methods: 153 males were included in the study, the caseweredivided into groups according to the infertility etiology and semen analysis according to Word health organization, the frequencies and the characteristicsof Y chromosome microdeletions were investigated in groups. Multiplex PCR was applied to detect the microdeletions. Results:Y chromosome microdeletion was detected in 42 (40.7%) of 153 cases ,Microdeletions in azoospermia showed more frequently detected 28 (52.8%), followed by severe oligospermia 14 (28 %),Microdeletions in the AZFc region were the most common 12 (22.64%), followed by AZFb 11(20.75%) and AZFa 5(9.43%) in azoospermia compared to severe oligospermisAZFc 6 (12%) AZFb 4 (8 %) and AZFa 4 (8%). Conclusion: Y chromosome microdeletions were detected quite frequently in certain infertility subgroups. Therefore, detailed evaluation of an infertile man by physical examination, semen analysis, hormonal evaluationsand when required, karyotype analysis may predict the patients for whom Y chromosome microdeletionanalysis is necessary and also prevent cost increases. Recommendation: This study emphasizes that analysis of microdeletions should be carried out for all patients with idiopathic azoospermia and severe oligospermia who are candidates for intracytoplasmic sperm injection.

Keywords: Y chromosomeMicrodeletions, Infertility, Azoospermia, Severe oligozoospermia.

INTRODUCTION

Infertility is a distracting major health problem that affects the human beings especially when the cause of infertility is the male partner[1]. It is defined as inability of a sexually active couple not using contraception, to achieve pregnancy within one year of regular intercourse attributed to deficiencies in the semen quality[2]. It accounts for about 30-40 % of overall cases of infertility and it affects approximately 7% of all males worldwide[3].

Azoospermia is defined as the total absence of spermatozoa in the ejaculate, classification of can be based on obstructive and nonobstructive forms[4]. In many instances non-obstructive azoospermia was related to the history of clinical unilateral or bilateral varicocele[5].

The role of Y chromosome in male infertility was first elucidated in 1976 when[6] have proposed the existence of a gene factor which controls spermatogenesis that is localized within the euchromatic region of the Y chromosome long arm (Yq11), which was called the Azoospermia Factor. (*AZF*), because the first six men observed with microscopic terminal deletions in Yq, by routine karyotyping, were azoospermic.

Many genes controlling spermatogenesis were mapped within these *AZF* regions[7]. Interstitial and terminal deletions in *AZFa*, or *AZFb*, or *AZFc*alone or in any combination of the Yq are all associated with dramatic non-obstructive spermatogenic failure, therefore, there is a clear cause-effect relationship between *AZF* loci deletion/s and male infertility[8]. The two main genes located in the AZFa region are USP9Yand DBY (also called DDX3Y)[9]. Deletions in the AZFa region that remove both of these genes cause Sertoli cell–only syndrome, a condition characterized by the presence of complete Sertoli cells in the testes but a lack of spermatozoa in the ejaculate[10]. Deletions of the AZFb region cause arrest of spermatogenesis at the primary spermatocyte stage[11], indicating that the region is essential for fertility (Nuti and Krausz,2008). The main gene in the AZFb region is RBMY, and there are six copies of the gene located on the Y chromosome[12]. While Deletions in the AZFc region produce a wide range of phenotypes, many of which are associated with low sperm concentration due to reduced spermatogenesis[13]. AZFc deletions cause approximately 12% of non obstructiveazoospermia and 6% of severe oligozoospermia[14]. The AZFc region contains DAZ genes involved in spermatogenesis, this gene has four copies on the Y chromosome which are thought to serve a variety of roles throughout the spermatogenic process because they are expressed in all stages of germ cell development[15].

MATERIAL AND METHOD

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Table 1: List of primers used to detection AZF microdelations.

Region	STS	Primer	Product size (bp)
SRY(Yp)	SRY-F	5'GAA TAT TCC CGC TCT CCG GA -3'	472
	SRY-R	5'GCT GGT GCT CCA TTC TTG AG -3'	
AZFa	sY86-F	5'- GTG ACA CAC AGA CTA TGC TTC -3'	326
	sY86-R	5'- ACA CAC AGA GGG ACA ACC CT -3'	
AZFb	sY127-F	5'- GGC TCA CAA ACG AAA AGA AA -3'	274
	sY127-R	5'- CTG CAG GCA GTA ATA AGG GA -3'	
AZFb	sY134-F	5'-GTC TGC CTC ACC ATA AAA CG -3'	301
	sY134-R	5'-ACC ACT GCC AAA ACT TTC AA -3'	
AZFc	sY255-F	5'- GTT ACA GGA TTC GGC GTG AT -3'	123
	sY255-R	5'- CTC GTC ATG TGC AGC CAC -3'	
AZFc	sY254-F	5'-GAA CCG TAT CTA CCA A GC AGC-3'	380
	sY254-R	5'-GGG TGT TAC CAG AAG GCA AA -3'	
AZFc	sY150-F	5'-GGG AGA GTC ACA TCA CT T GG -3'	158
	sY150-R	5'-TTG AAT TAT CTG CCT GAG TGC -3'	

F- Forward primer, R- Reverse primer, SRY- Sex-determiningthe region of Ychromosome, STS- sequence- tagged site, bp- base pair.

Table 2: Y chromosome microdeletion rates according to the results of semen analysis.

Sperm count groups	Microdeletion n, (%)		
Azoospermia	Absent	Present	Total
	25(71%)	28(52%)	53
0-5 million/mL	36(72%)	14(28%)	50
>20 million/mL	0(0%)	0 (0%)	50
Total	61(39.8%)	42 (40.7%)	153

This study was conducted in the laboratories of Faculty of Medicine, and in the laboratory of the Fertility Center's in AL-Sader Medical city in the Province of Najaf, AL-Najaf Health Directorate / Ministry of Health /Iraq during the period from 1 April/2018 to 30 August/2018.

The mean and stander deviation of age of infertile patients was (33± 1.24) years, the semen samples collected are 153 samples which divided after analysis according to the procedure described by the WorldHealth Organization (16) to 53 azoospermia, 50 samples from sever oligospermia infertile patients and control group (fertile) were 50 samples (Normozoospermia)**.**

The blood samples were obtained from persons through drag 3ml of blood by using of medical sterile syringes from brachial vein, and placed in ethylene diamine tetra acetic acid (EDTA) tube for DNA extractionkit(Promega-U.S.A) according to the manufacturerûsinstructions. PCR was carried out using master mixfrom Promega PCR kit (Promega,U.S.A) and STS primers (Bioneer ,Korea) for the regionsused were: for AZFa sY86 , for AZFb sY127 and sY134 for AZFc sY254,sY255 and sY150(table-1). This primer set was suggested by[17] and is prescribed by the European academy of andrology (EAA) and European molecular genetics quality network (`EMQN)[18]**.** The internal control was STS primer sY14 for sex determining region of the Y (SRY). Thermo cycling for PCR was carried out at 95 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 56°C for 1 min, extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. PCR products were separated on agarose gel electrophoresis, stained with ethidium bromide, and visualized using UV light.

RESULTS

Y chromosome microdeletion was detected in 42 (40.7%) of 153 cases. Microdeletions in azoospermia showed more frequently detected 28 (52.8%), followed by severe oligospermia 14 (28 %), No Y chromosome microdeletions were detected in cases with control group. The results are shown in Table (2).

Microdeletions in the AZFc region were the mostcommon 12 (22.64%), followed by AZFb 11(20.75%) andAZFa 5(9.43%) in azoospermia showed figure (1) and figure (2) compared to severe oligospermisAZFc 6 (12%) AZFb 4 (8 %)and AZFa 4 (8%) showed figure (3) and figure (4). The results are shown in Table (3).

The results showed rates of STS had AZFb sY134 and AZFc sY255 deleted more frequantily in azoospermia patients followed by AZFa sY86 , AZFbsY 127, AZFc sY150 and AZFc sY254 respectively shows table (4) .

The results showed rates of STS had AZFcsY254, sY255 and AZFc sY150,deleted more frequantily in severe oligospermia patients followed by, AZFb sY134 ,AZFb sY127, andAZFasY86 showed table (5).

DISCUSSION

Y chromosome deletions are one of the most common genetic causes of male infertility, the prevalence of AZF microdeletionsdifferent worldwide among both infertileazospermic and severeoligospermic males; it generally ranges from 1-13% worldwide but may reach up to 55%[19].

In this study, the frequency of AZF microdeletion was 40.7% in 153 cases, this is fluctuate than that reported in some previous studies such the study by[20]. This difference between the results may be due to some factors such as ethnic differences, patient selection criteria, methodological aspects, and even the type and number of markers used in the studies. Moreover, the frequency of microdeletions detected in the present study was leftover

Table 3: AZF deletion patterns in cases with Y chromosome microdeletions.

Deleted SRY		Deleted *AZFa*	Deleted *AZF*b	Deleted *AZF*c	Deleted *AZFb+c*	Deleted *AZFa+b+c*
0	Azoo	5	11	12	23	28
(0%)		9.43%	20.75%	22.64%	43.3%	52.8%
0	Severe	4	4	6	10	14
(0%)		8%	8%	12%	20%	28%

Table 4: Y chromosome microdeletion rates according STS in *Yq11*analysed.

Region	STS	Percentage of*Yq11* deletions
AZFa	sY 86	5(9.43%)
AZFb	sY 127	5(9.43%)
	sY134	6(11.32%)
AZFc	sY255	6(11.32%)
	sY254	2(3.77%)
	sY150	4(7.54%)

Table 5: Y chromosome Microdeletion rates according STS in *Yq11*analysed.

Region	STS	Percentage of*Yq11* deletions
AZFa	sY 86	4(8%)
AZFb	sY 127	2(4%)
	sY134	2(4%)
AZFc	sY255	2(4%)
	sY254	2(4%)
	sY150	2(4%)

the range reported by previous studies from Turkey (1.3-9.1%)[21], However, those studies consisted of limited number of cases and generally cases with azoospermia and severe oligozoospermia were evaluated.

Y chromosome microdeletion frequencies were reported in a wide range from 0.1% to 25% based on the sample size in the studies, ethic variations and the type and number of primers used[22]. Most of these reports were observed the correlation between azoospermia ,severeoligospermia and AZF microdeletions. Such deletions have been also detected in oligoasthenospermia , teratoasthenooligospermia[23], Sertoli cell-only syndrome[24] and normal fertile men[25].

Microdeletions in azoospermia showed more frequently detected (52.8%), followed by severe oligospermia (28 %), the wide range of frequencies reflection of several factors that include varying selection criteria, accuracy in testing and social restrictions that limit sample collection.This agreement with another study in There is variation in the frequency of Y chromosome microdeletions for different patients reported in various investigations including the ones performed in different parts of Iran[26]. In West Azarbaijan, 15.4% and 30% Y chromosome microdeletions were observed in infertile male with severe oligospermia and azoospermia, respectively, was reported in[27]. However, 2.13% and 1.8% Y chromosome microdeletions were detected in azoospermic and oligospermic cases in[28,29], respectively. Different patients' selection criteria and composition of the study population, various diagnostic protocols and inaccurate or wrong diagnostic, may result in frequency variations in different reports, there is also heterogeneity in selecting PCR markers both in type and number in different methods[18].

In the present study, microdeletions in the AZFc region were the most common (22.64%), AZFb (20.75%) and AZFa (9.43%) regions. The frequent appearance of AZFcmicrodeletions was consistent with previous studies and the distribution rate of other microdeletions was similar[30]. It is well known that the deletions of the AZF regions cause spermatogenic impairment and that the complete deletion of any of them is usually associated with the total depletion of spermatogenic cells[31]. The AZFa deletion was found to associate with complete absence of germ cells and presence of Sertoli cells in the seminiferous tubules[32] while AZFb and AZFc deletions are associated with developmental arrest of germ cells at pachytene stage or at spermatid stage respectively[33]. Also AZFc was found to associate with hypospermatogenesis, maturation arrest and a variety of testicular phenotypes[34].

When the cases were analyzed according to semen analysis, microdeletion was most frequently (52%) found in the azoospermic group as expected. Furthermore, no microdeletions were detected in cases with sperm counts above 20 million/mL. Microdeletions occur in about one in 4000 men in the general population but their frequency is significantly increased among infertile men[18]. Study also agreement with a similar study in which the cases were assessed according to semen analysis, AZF microdeletions were detected in the moderate oligozoospermic group, even if its frequency was quite low[8].

The importance of genetic studies of male infertility in Iraq has been demonstrated by[35] was conducted to investigate the AZF and SRY regions microdeletions and hormonal disturbance in 43 azoospermic and 20 healthy and fertile menTwelve (27.9%) of azoospermic men have shown deletions with undetected chromosomal abnormalities, Five of these deletions have been detected in men with a history of post pubertal mumps also agreed with result in showed that AZFc is recorded as the most frequent deleted region in azoospermic men and Follicle stimulating hormone and prolactin hormone elevation levels were also detected in patients but with undistinguishable correlation parallel to the detected microdeletions.

The high percentage of the AZFc distributed among azoospermia in our study suggesting that it is possible that AZFc sY255 AZFb sY134 is predominant in Iraqi azoospermic population. The azoospermiaeffects by disseses infection, hormone disturbance, protein deficiency reflects or suggesting that sY255 of the AZFc and sY134 of the AZFb in azoospermia patients fragile sites toward imbalance factors of male infertility.The

causes of azoospermia such as failure of spermatogenesis and obstruction of the ductal system particularly the vas deferens have been investigated[36]. It was reported that

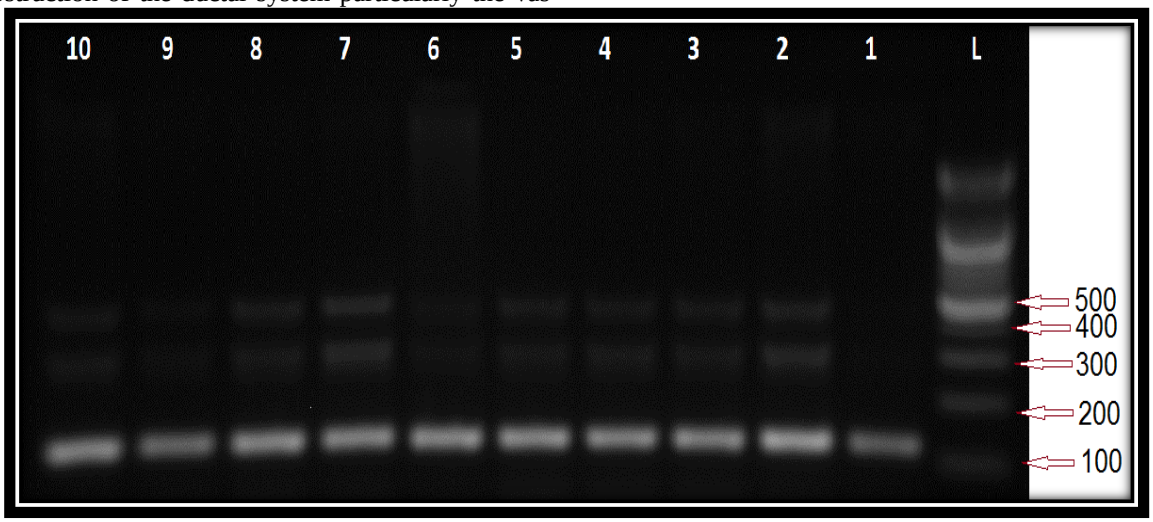

Figure 1: Multiplex PCR Product for azoospermia SRY (472 bp) as internal control , AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp) ,lane (L symbolizes the 100 bp DNA ladder).

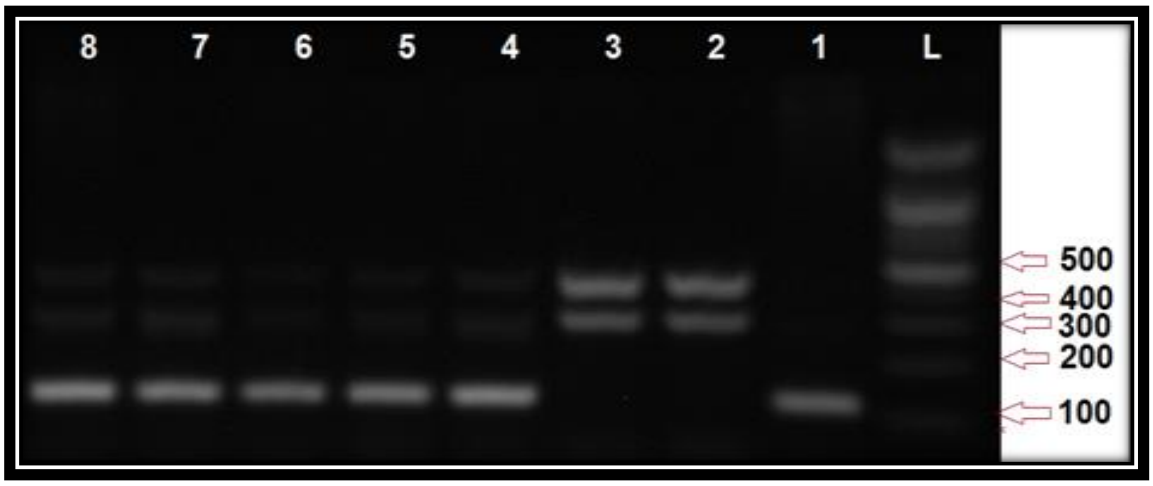

Figure 2: Multiplex PCR for azoospermia SRY (472 bp) as internal control ,AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp),lane (L symbolizes the 100 bp DNA ladder).

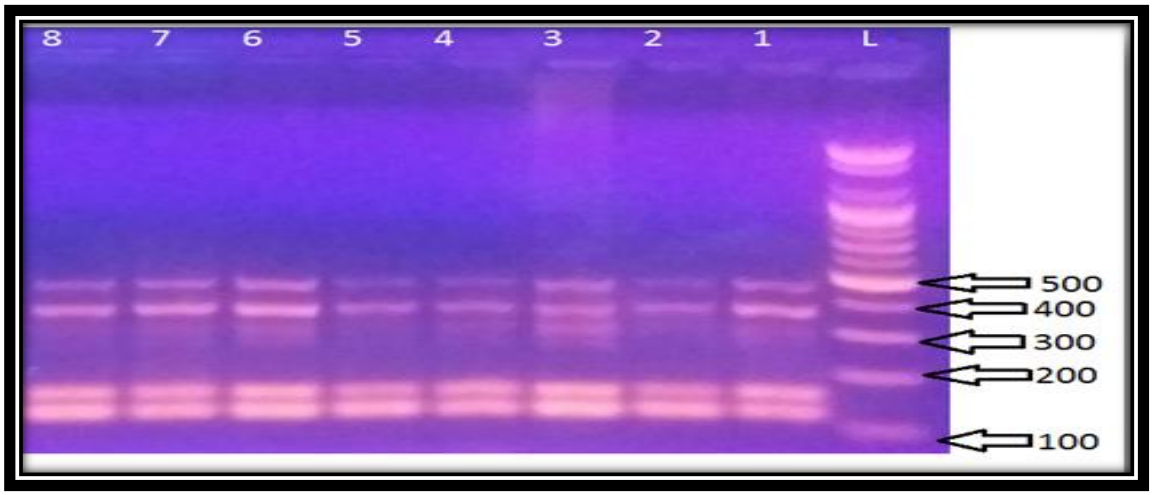

Figure 3:: Multiplex PCR for Severoligospermia ,SRY (472 bp) as internal control ,AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp) ,lane (L symbolizes the 100 bp DNA ladder).

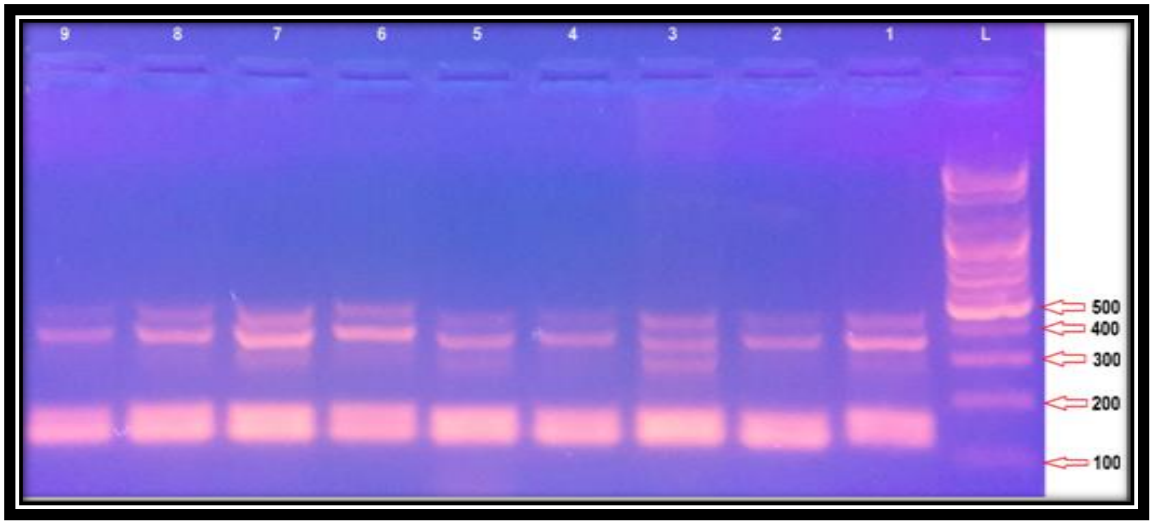

Figure 4: Multiplex PCR for Severoligospermia , SRY (472 bp) as internal control ,AZFa SY 86 (326bp), AZFb SY127,134 (274bp, 301bp) and AZFc SY 255,254,150 (123bp, 380bp,158bp) ,lane (L symbolizes the 100 bp DNA ladder).

obstruction of the vas deferens was not a major cause of azoospermia[37]. Infection of the seminal fluid has been implicated as the major cause of azoospermia in infertile males[38].

CONCLUSION AND RECOMMENDATION

Y chromosome microdeletions detection is very necessary for infertile male suffering from azoospermia and severeoligospermia before any decision for InvitroFertilization ,other solutions can be applied for infertile males with those deletionsAZFa or AZFbsperm retrieval is inevitably impossible in cases with, Moreover, there is always risk of transmission of the microdeletions mutation to any male child and genetic counseling is necessary prior to the treatment.

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