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Molecular Analysis for Azoospermia Factor Microdeletions in the Y chromosome for Azoospermic and Severe Oligospermic Infertile Iraqi Patients

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ABSTRACT

Defined infertility as incapability of a married couple to have children for the one-year of unprotected intercourse. Y chromosomal microdeletions are the second greatest common genetic reason of men sterility. This research aims to find the prevalence of AZF Y chromosome microdeletions in azoospermic and severe oligospermia patients. Further, to evaluate the prevalence types of AZF/c sub-region microdeletions in patients with AZF/c deletion in Iraq. A total of 75 infertile Iraqi males and 25 control were included in this study. The DNA samples were extracted, and they were analysed for AZF microdeletions by utilizing eight sequence-tagged sites through a q/real-time PCR system. After that, partial AZF/c deletion sub-region was investigated using four specific primers. These markers were chosen according to the EAA/ EMGQ recommendations. Out of 75 infertile patients, 46 patients (61.33%) revealed AZF microdeletions in the Y chromosomal with at least one STS deletion for one or more AZF regions. (32.6%) of patients with microdeletions observed in three regions AZFabc. Out of 24 patients who have AZF/c microdeletions (37.5%) were exhibited b2/b4 deletion (complete AZF/c deletions), (58.3%) were showed gr/gr microdeletion (partial AZF/c deletions. and (4.1%) with b2/b4 deletion continues the terminal heterochromatin region. The incidence of classical AZF microdeletions in our study subjects is high. In our study population, gr/gr partial AZF/c microdeletions were higher than b2/b4 complete AZF/c deletion. The mean levels of sex hormones in azoospermic sterile patients with AZF microdeletion were higher than the mean of azoospermic sterile men without deletion of AZF.

Keywords: AZF / microdeletion, male infertility, non-obstructive azoospermia and severe oligospermia, Y Chromosome / microdeletions.

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INTRODUCTION

The Y chromosome in human is important for sex determination and male germ cell progress and maintenance[1]. The /azoospermia factor (AZF) area located on the long arm of the Y chromosome Y/q and show a main role in the genetics/of male sterility and is dividing into three sections: AZF a, AZF b, and AZF c. These sections contain genes that are involved in spermatogenesis and the development of tests. Their failure or loss is commonly associated with spermatogenetic defects and male infertility. However, Microdeletions at AZF are the most common structural chromosomal defects and the leading induce of male infertility[2].

The microdeletion of AZF a results in sertoli cell only syndrome, and whole removal of AZFb[3] is associating with spermatogenic arrest. Also, the microdeletion of AZF c induces variable clinical and histologic phenotypes, ranging from Sertoli Cell only syndrome to oligospermia[4]. While Partial deletions of AZF c classify as 1.6Mb gr/gr, 1.8Mb b2/b3, 1.6Mb b1/b3, and 3.5Mb b2/b4 (complete AZF c deletions) were also known to influence spermatogenesis[5]. Among these, gr/gr sub-deletions with occurrence varying from 2.1% to 12.5% in infertile people and zero to 10.2% infertile males are frequently reported[6]. The gr/gr deletion, the common partial deletion of AZF/c, deletes about half of the b2/b4 area and can be described by different phenotypes depending on the ethnic and geopolitical origin[7].

AZF microdeletions are causing by mistakes in the intrachromosomal recombination route[8]. While intracytoplasmic sperm injection (ICSI) assists infertile males with Y chromosome microdeletions to become fathers, the risk of infertility that may be transmitting from infertile fathers to their offspring should be a note[9].

There are few data on the occurrence of testicular tumors in males with Y q micro-deletions, especially males born to fathers with deletion. The recent documents on the higher occurrence of neurological problems in infertile males with Y q deletions are reported[10].

AZF microdeletions are too small to be noticed by karyotyping. but, with the advance of molecular biology technology, now AZF microdeletions can be recognized by multiplex polymerase chain reaction (PCR) technique within a short time[11]. According to the European Academy of Andrology /European Molecular Genetics guidelines, Six sites were selected for preliminary screening of AZF absence, including SY84/SY86 (AZFa), SY127/SY134 (AZFb), and SY254/SY255 (AZF c for the identification of microdeletions in the Y chromosome)[12].

Our study has been undertaken for analyzing the prevalence and types of microdeletions in the AZF region of a Y chromosome in patients with azoospermia and severe oligospermia from Al-Anbar Governorate west of Iraq by using six sequence-tagged sites (STSs) as follow: sY84 and sY86 for AZF a; sY127 and sY134 for AZF b; sY254 and sY255 for AZF c according to the recommendations by European Academy of Andrology/ European Molecular Genetics Quality Network EAA/ EMQN. Also, to determine the types of partial AZF c sub-region deletion. and to determine the association between hormonal imbalance levels in the Luteinizing hormone,

Follicle stimulating hormone, Prolactin hormone in addition to testosterone, and the occurrence of Y chromosome microdeletions.

Patients and methods

Study patients and ethics approval

This study has been approved by the Medical Ethics Committee at the University of Anbar, College of Medicine, according to the Scientific Research Ethics Committee, Book No. 113 validated on 4/11/2019. We have studied 75 Iraqi infertile patients with impairment of spermatogenesis, 40 non-obstructive azoospermia/35 severe oligozoospermia men who have been seeking consultation for infertility at the private infertility clinics. The study included 25 Health controls with normal spermatogenesis, and their fertility status was assumed by the reports that they were fathers for a child or more.

Semen analysis

Semen analysis was done according to normal standard criteria laid down by World Health Organization[13]. The identification of azoospermia depended on the lack of sperm in at least two separate semen analyses and after centrifugation. The chosen infertile males were categorised as azoospermic or severe oligospermic patients with less than five million/mL of sperm count. Moreover, the volume of semen, the concentration of sperms, the sperms morphology, and the movement type of the sperms, whether it was progressive, sluggish or immobile were evaluated.

Hormonal study

All participants have submitted for evaluation for hormonal studies including LH, FSH and prolactin in addition to testosterone hormone, in human serum on TOSOH Automated Immunoassay System (TOSOH Bioscience, Japan).

Molecular part

DNA extraction

Genomic DNA was extracted from 400-µL blood samples with the SaMag-12Automated Nucleic Acids Extraction System (Sacace biotechnologies/Italy), according to the Al-Ouqaili, and Al-Qaysi protocols respectively[14,15]. The quantity of nucleic acid has been detected by using the Quantus™ Fluorometer with QuantiFluor® dsDNA System (promega/ USA) provides a fast, easy and sensitive method for determining DNA concentration. DNA purity was also estimated by OD260/OD280 UV spectrophotometer (Unico/ USA).

Molecular investigation using qReal-Time PCR technique

This part was achieved using two reactions in RT-PCR (Sacace biotechnologies, Italy). Reaction A consisted of sY86, sY127, sY254, and ZFY/X STSs, and the other reaction, B consisted of sY84, sY134, sY255, and SRY STSs. Hydrolysis probes within a reaction were labelled using channels of FAM, HEX, ROX, and CY5, and the STSs information were listed in the table 1.

Table 1. The information about the study sequence tag site (STS)

locus	STS Sequence (5′to 3′)	Size Base pair
	MIX A	
ZFX/Y	**ZFX/Y-F 5′- ACC RCT GTA CTG ACT GTG ATT ACA C-3′** **ZFX/Y-R 5′- GCA CYT CTT TGG TAT CYG AGA AAG T-3′**	**495**
AZF a	**sY86-F 5′- GTG ACA CAC AGA CTA TGC TTC-3′** **sY86-R 5′ - ACA CAC AGA GGG ACA ACC CT - 3′**	**318**
AZF b	**sY127-F 5′- GGC TCA CAA ACG AAA AGA AA-3′** **sY127-R 5′- CTG CAG GCA GTA ATA AGG GA-3′**	**274**
AZF c	**sY254-F 5′- GGG TGT TAC CAG AAG GCA AA-3′** **sY254-R 5′ - GAA CCG TAT CTA CCA AAG CAG C-3′**	**380**
	MIX B	
SRY	**sY14-F 5′ - GAA TAT TCC CGC TCT CCG GA-3′** **sY14-R 5′ - GCT GGT GCT CCA TTC TTG AG-3′**	**472**
AZF a	**sY84-F 5′- AGA AGG GTC CTG AAA GCA GGT-3′** **sY84-R 5′ - GCC TAC TAC CTG GAG GCT TC-3′**	**326**
AZF b	**sY134-F 5′- GTC TGC CTC ACC ATC AAC CG-3′** **sY134-R 5′- ACC ACT GCC AAA ACT TTC AA-3′**	**301**
AZF c	**sY255-F 5′- GTT ACA GGA TTC GGC GTG AT-3′** **sY255-R 5′- CTC GTC ATG TGC AGC CAC-3′**	**123**

The whole reaction amount was 35 µl; the amount of DNA sample was seven µl. All obtained DNA samples were examined in two tubes: one with PCR-mix-A, and the other with PCR-mix-B. The mix-A and mix-B consisted of 24.5 µl of diluent, 3.5 µl of mix-A or B and 0.3 µl of Taq Polymerase. The cycling circumstances were 94 C° for 90 second, followed by 45 cycles of 94 C° for 15 seconds, 64C° for 40 seconds, and 72 C° for 40 seconds.

The screening for AZF/c sub-region microdeletions by Conventional PCR

All the patients who showed AZF c microdeletion by classical RT-PCR technique were undergone additional primers by Conventional PCR (ESCO, Canada) to determine the type of partial AZF c microdeletion.

Two primers (forward and reverse) were requested and designed by Alpha DNA Company, Canada in a lyophilised form which was dissolved with sterile D.W to provide the

final concentration of each primer in 100 pmol /µl as revealed in the table 2.

Table 2. The characters and preparation of finale concentrations of study PCR primers

Primer name	Sequences	Conc. P moles	Required sterile D.W (µl)	Final Conc. P moles /µl	Size
sY14-F	5′-GAA TAT TCC CGC TCT CCG GA -3′	66096	661	100	472
sY14-R	5′-GCT GGT GCT CCA TTC TTG AG -3′	101680	1017	100	472
sY1291-F	5′-TAA AAG GCA GAA CTG CCA GG -3′	59281	593	100	527
sY1291-R	5′-GGG AGA AAA GTT CTG CAA CG -3′	79888	799	100	527
sY1191-F	5′-CCA GAC GTT CTA CCC TTT CG -3′	100955	1010	100	385
sY1191-R	5′-GAG/ CCG AGA TCC AGT TAC CA -3′	67978	680	100	385
sY160-F	5′-TAC GGG TCT CGA ATG GAA TA -3′	66895	669	100	236
sY160-R	5′-TCA TTG CAT TCC TTT CCA TT -3′	99001	990	100	236

The DNA was amplified by conventional PCR/Thermal cycler (ESCO, Canada), and the reaction settings for the markers were at first denaturation 95 C° for15 minutes for (SY14 Internal control), 95 C° for 3 minutes for (SY160 Terminal AZF c) and 94 C° for 3 minutes for (SY1291, SY1191) then, 35 cycles for (SY14, SY160) and 30 cycles for (SY1291, SY1191) at denaturation 94 C° for 30 sec for all markers, annealing 57 C° for 90 sec for (SY14), 56 C° for 30 sec for (SY160), and 60 C° for 30 seconds for (SY1291, SY1191), then extension 72 C° for 60 seconds for (SY14), 72 C° for 45 sec for (SY160), and 72 C° for 30 seconds for (SY1291, SY1191) with finall extension at 72C° for 10 min for (SY14), 72 C° for 7 minutes for (SY160), and 72 C° for 5 minutes for (SY1291, SY1191)[16].

Preparation of agarose gel with ethidium bromide

The PCR results were noticed by garose gel electrophoresis. Then, the identification of the band-special in ultraviolet light. Tris Boric Acid EDTA (TBE) buffer (10X) were diluted ten times to be (1X) by added 100 ml of TBE buffer (10X) with 900 ml of distilled water, (2 %) of agarose gel was prepared through dissolving 2gm of agarose powder in (1X) 100 ml TBE buffer awaiting the solution became apparent, the solution was cooled to under 50 C° and added one µl of ethidium bromide staining solution (red DNA dye). Mixed it well and poured into the gel pouring chamber. The /comb was Placed in the chamber and let it cool for about 30 minutes. The DNA ladder 100 base pairs kit designed by BIONEER company/Korea is perfect for the identification of the dual-stranded DNA size from l00-2000bp, The ladder consist of 13 double-stranded DNA segments with the size of 100, 200, 300, 400, 500,600,700, 800, 900, 1000, 1200,1600 and 2000. After polymerisation the agarose gel (at room temp about 30 minutes), they were removed the tape and comb. The gel was sited inside the gel tank filled with a (1X) TBE buffer. The gel must be wholly lined with buffer.

Firstly, five µl of l00 bp DNA ladder placed inside the first well. Ten µl of the amplified DNA products were placed to the designed wells of the gel pocket, The tray with gel/ was laid with (1X) TBE inside the chamber, and ensured the gel was enveloped entirely with TBE, The protection cover was carefully placed on the chamber to ensure the two plugs were protected and joined with power source. The electrophoresis was then done for about 75 minutes with the next conditions (5volt/cm, l00 watt, and 75 minutes), Once the electrophoresis was finished, the gel was placed on a UV/ transilluminator. Finally, a digital image was made for the estimation and record of the results.

Statistical analyses:

The SPSS statistical system (Social Science Statistical Package) version 22.0 Statistical significance was taken with P-value < 0.05 and 0.001 to analyze all data. The significant differences were identified by using the goodness fit test in non-parametric statics, including test of Chi-square. Each the study charts (bar chart, scatter diagram or dot chart) were prepared through using Microsoft Excel 2010.

Ethical considerations

There was obtained from all human adult participants' patients who have been seeking consultation for infertility at the private infertility clinics. This study has been accepted by the Ethics Committee.

RESULTS

Age distribution of the study samples

The total of 100 clinical specimens were obtained through the period of study (October 2019- March 2020) and divided in two groups: 75 Sterile patients (including 40 Non-obstructive Azoospermia 33.3 ±7.8, 35 Severe Oligozoospermia 32.4 ±8.4), and 25 normal male controls 32.1 ±5.3. The age of all the study subject 32.7 ±7.4, with minimum 20, and maximum 52 years

The effect of smoking according to the study group

Distribution the effect of smoking according to the study groups as in the table 3.

Table 3. The effect of smoking according to the study group

			Smoking		
			no	yes	Total
group	Azoospermia	Count	19	21	40
		% in group	47.5%	52.5%	100.0%
	Sever oligospermia	Count	14	21	35
		% in group	40.0 %	60.0 %	100.0 %
	Control	Count	20	5	25
		% in group	80.0 %	20.0%	100.0%
Total		Count	53	47	100
		% in group	53.0%	47.0%	100.0%

The classification of the exposure to radiation or uranium according to the study group
Distribution of the exposure to radiation or uranium according to the study groups as in table 4.

Table 4. The classification of the exposure to radiation or uranium according to the study group

			Exposure to radiation or uranium		
			no	yes	Total
group	Azoospermia	Count	29	11	40
		% in group	72.5%	27.5%	100.0%
	Sever oligospermia	Count	21	14	35
		% in group	60.0 %	40.0 %	100.0 %
	Control	Count	25	0	25
		% in group	100.0%	0.0%	100.0%
	Total	Count	75	25	100
		% in group	75.0%	25.0%	100.0%

Molecular part of study

A total of 40 cases of azoospermia, 35 men with severe oligospermia and 25 healthy control were screened for AZF microdeletions. The subjects' mean age was 32.7 ±7.4 years range of (20-52) years. While a history of family infertility was reported by 29 men, lifestyle habits including smoking and alcohol consumption were also observed in 47 and 8 men respectively. The mean ± SD of extracted DNA concentrations was 78.61±20.35 ng/µl, while the purity was 1.67 ±0.09.

a) Quadruplex Real-Time PCR

Out of 75 infertile males, 46 patients (61.33) revealed with at least one STS deletion for one or more AZF regions in the y chromosome, (32.6%) of microdeletions noticed in AZFa,b,c, (23.9 %) of microdeletions appeared AZFab, (8.6 %) of microdeletions appeared in AZFac, (4.3 %) of microdeletions appeared in AZFbc, (15.2 %) of microdeletions noticed in AZFa, (8.6 %) of microdeletions noticed in AZFb, (6.5 %) of microdeletions showed in AZFc. The proportion of microdeletions appeared high in azoospermic men (33.33%) while (28%) in severe oligospermic men. One healthy control male revealed with AZF microdeletion presented in one STS (SY86) only at AZFa region (4%).

The results of the polymerase reaction were labelled with FAM, HEX, ROX, and CY5 canals in the qRT-PCR system. Figure 1.

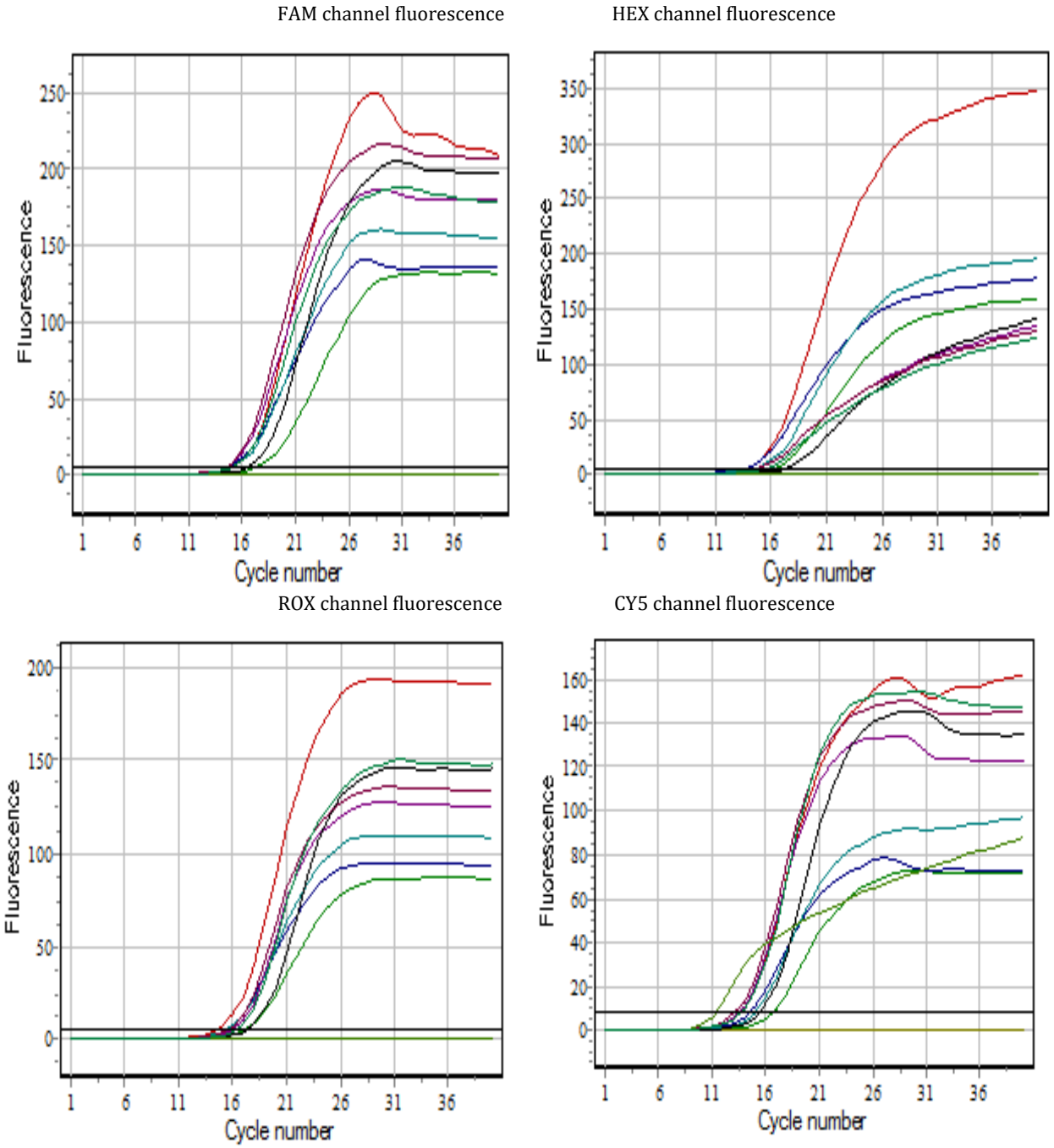

Figure 1. The amplification curve of AZF Y chromosome-DNA genome in quadruplex assay Fluorescent canals detected by qReal-Time Polymerase Chain Reaction.

Conventional PCR

The screening of the types of partial micro-deletions in the AZF c sub-area by using conventional PCR. This part of the study was involved 24 patients of both azoospermic and severely oligospermic men who previously screened by classical real-time PCR and revealed AZF c microdeletions. In this part, we use additional primers included sY160 at heterochromatin region for terminal AZF c deletion, sY1291at gr/gr AZF c sub-region, sY1191 at b2/b3 AZFc sub-region and SRY14 as an internal control. Out of these 24, nine patients (37.5%) with b2/b4 deletion (complete AZFc deletions): 5 azoospermic/ 4 severe oligospermic, 14 patients (58.3%) were showed gr/gr microdeletion (partial AZFc deletions): 4 azoospermic/ 10 severe oligospermic, and one severe oligospermic patient only was with b2/b4 continues terminal heterochromatin region deletion (4.1%).

After amplification of SY160, and SY1191 primers by a PCR and electrophoresed by 2 % agarose gel, the bands of amplified targets within AZF/c sub-region were offered in figures 2, and 3 respectively.

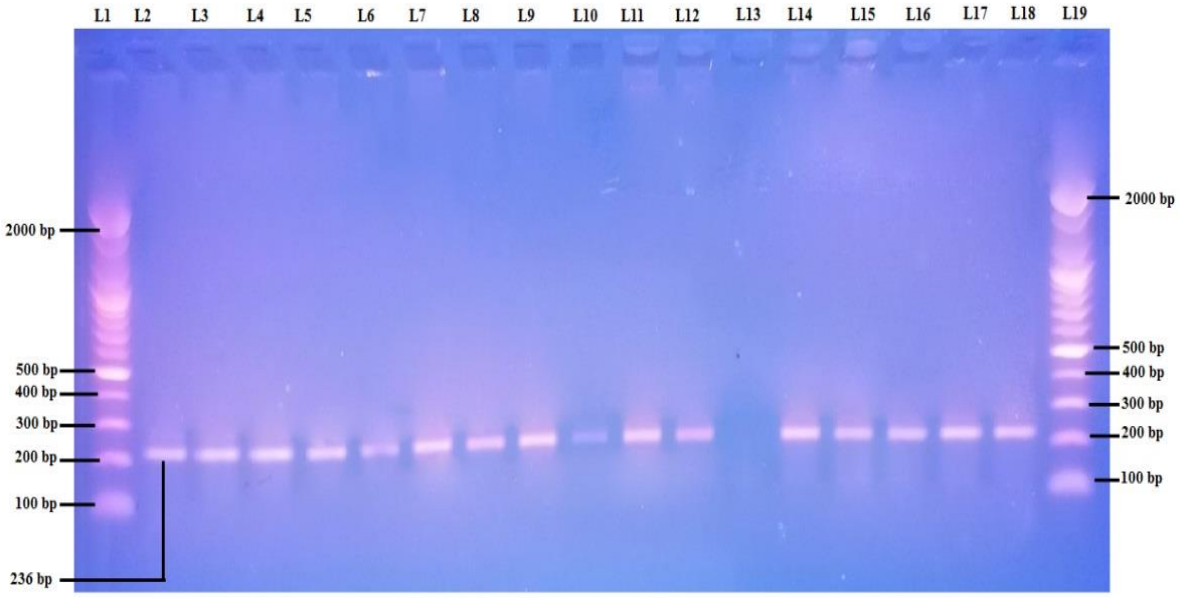

Figure 2. The agarose gel electrophoresis (2 %) with the ethidium bromide stain reveals the amplified bands the Terminal AZFc region sY160 marker with 236 bp of the Y chromosome in azoospermia and severe oligospermia cases. (L2-L12): cases with no deletion. (L13) severe oligospermic patient (case no 36) with sY160 deletions, (L14-L18): cases with no deletion, DNA ladder with (2000-100bp) on the left (L1) and right (L19) were used as DNA molecular weight marker.

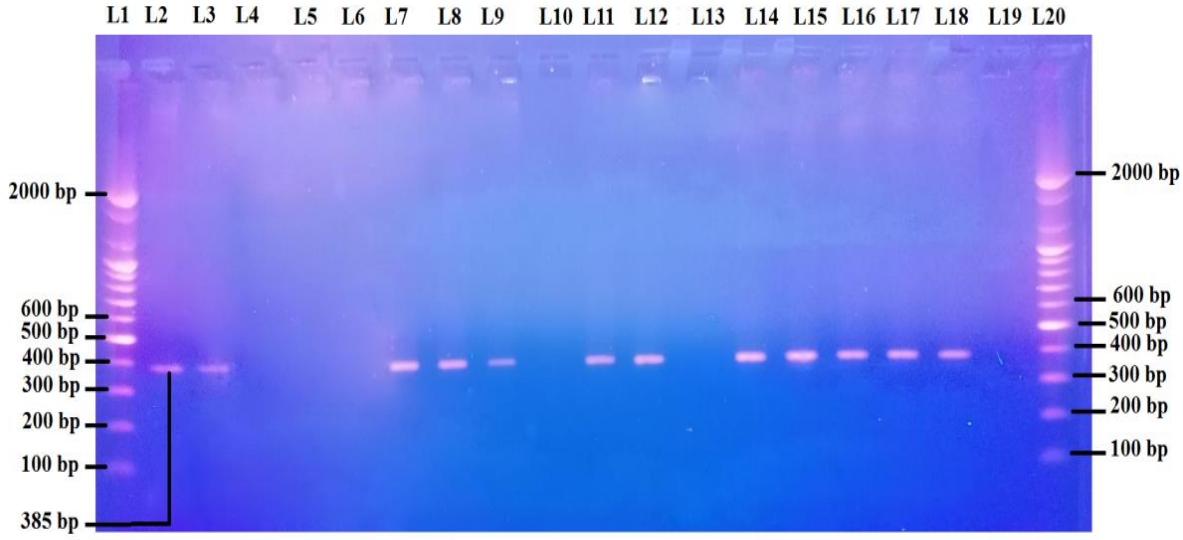

Fig 3. The agarose gel electrophoresis (2 %) with ethidium bromide stain reveals the amplified bands of the microdeletions in AZFc region marker sY1191 with 385 bp of the Y chromosome in azoospermia and severe oligospermia cases. (L2, L3): cases with no deletions. (L4, L5, L6): cases with sY1191 deletions. (L7, L8, L9): cases with no deletions. (L 10): a case with sY1191 deletion. (L11, L12): cases with no deletions. (L13): a case with sY1191 deletion. (L14, L15, L16, L17, L18): cases with no deletions. (L19): a case with deletion. DNA ladder with (2000-100bp) on the left (L1) and right (L20) was used as a molecular weight DNA marker.

Out of 75 infertile males, the results showed there are 46 have Y chromosome microdeletion and 29 have not microdeletion. Out of 40 azoospermic infertile male (62.5%) with AZF microdeletions, while out of 35 severe oligospermic patients (60%) with AZF microdeletions. The mean ±SD levels of hormonal imbalance for infertile male with/without AZF microdeletion are represented in table 5.

Table 5. The compression of sex hormones level in *P* value between infertile males (azoospermia, severe oligospermia) with and/or without microdeletion

Hormone	Azoospermia with AZF microdeletion	Azoospermia without AZF microdeletion	P value
LH	11.98 ±8.16	8.37 ±4.15	0.024
FSH	23.53 ±18.20	18.41 ±12.67	0.160
Prolactin	13.22 ±8.19	13.27 ±6.23	0.977
Testosterone	255.30 ±113.42	266.70 ±143.69	0.755
Hormone	**Sever oligospermia with microdeletion**	**Sever oligospermia without microdeletion**	**P value**
LH	6.08 ±2.74	6.02 ±3.09	0.972
FSH	10.5 ±7.41	9.51 ±3.64	0.887
Prolactin	10.91 ±5.71	9.97 ±3.33	0.643
Testosterone	256.41 ±98.62	262.66 ±105.01	0.871

DISCUTION

The microdeletion of the Y chromosome in loci of azoospermia factor (AZF) is a prevalent genetic source of infertility in men. The microdeletions of the Y chromosome are often detected worldwide in a different proportion of infertile males[17]. The current study evaluated AZF microdeletions in the Y chromosome for the prevalence and determined the type of AZF microdeletions among infertile Iraqi men. We also evaluate the types of AZFc sub-region microdeletions prevalence. In the screening of infertile men with AZF microdeletions, we applied eight STS as recommended by EAA and EMQN[12]. In our study, 75 azoospermic/ severe oligospermic infertile men, the occurrence of the microdeletions of the Y chromosome was 61.33 % (46/75). In general, The findings of the study agree with some previously circulated studies as Malekasgar and Mombaini, who documented a frequency of 52% in 50 patients[18]. Also, Khalaf *et al*., who informed a incidence of (64%) in 100 patients[19], and Hanoon *et al*. recorded a frequency of 65% in 40 patients[20]. while, the occurrence of microdeletions found in this study dissimilarity with some previously circulated studies as, Khabour, *et al*. which tell a frequency of 8.3 % in 34 patients[21], Also ElNahass, and co-workers announced that a frequency of 12 % in 50 patients[22], and Al-Mahdawi, *et al*. who documented a frequency of 13.75 % in 80 patients[23]. The diversity of the results may be because of various STS marker numbers used in microdeletions diagnosis, a difference in the targeted criteria used for conscripted sterile patients, size of the study sample, environmental influences, and the reality that the studies were carried out on diverse ethnic populations (researchers).

The microdeletions at the AZF are caused by mistakes in the intra-chromosomal recombination route, either pre-fertilise or post-fertilise[8]. AZF microdeletions are created or occur as de novo events via the paternal germline. Many studies have reported that higher than 80 % of AZF's microdeletions arise from de-novo[24]. Deletions of AZF a result from homologous intra-chromosomal recombination of two human endogenous retroviral sequences HERV15yqI and HERV15yq2 found in the proximal Yq11 area, While complete deletions of AZF b are due to homologous recombination of Palindrome P5 and the proximal arm of Palindrome PI in the Y q arm, and deletions of AZF c are said to arise from the homologous recombination of the sub-amplicons b2 and b4 in P3 and P1 palindromes[25]. The molecular investigation of AZF loci allows the recognition of many genes that have a major role in spermatogenesis, hence describing the infertility of men carrying microdeletions of these sequences. The main gene of the AZF a region is (DBY), which has an expression in the testis and is participates in the progress of pre-meiotic germ cells telling its role in infertility[26]. The USP9Y gene also assists in sperm formation[27]. Generally, removals of the AZF a section that both genes deletion yielded in sertoli cell. only syndrome (SCOS), a state characterised by the occurrence of full sertoli cells in the tests but by the absence of spermatozoa and azoospermia[28]. Thus, the diagnosis of total deletion of the AZF a area means a near impossibility for intracytoplasmic sperm injection (ICSI) to obtain testicular sperm or the chance of TESE zero[12]. The genes in the locus of AZF b promote sperm development, and differentiation is considered essential to successful sperm development through meiosis into spermatogenesis. Patients with AZF b removals get a testicular phenotype of maturing arrest, often to the spermatocyte phase, with the lack of post-meiotic germ cells in most tubules[29]. The probability of discovery sperm cells in males with whole AZF b deletion is pointedly lower, or the chance of TESE zero[30]. While the candidate genes within the AZF c region include Four DAZ copies (Deleted in azoospermia), three BPY2 copies (Basic Protein on Y chromosome 2). Also, two copies of Y chromo domain, Y-linked CDY (CDY1a and CDY1b)[31]. DAZ is encoding an RNA- binding protein essential for spermatogenesis[3]. AZF c deletions may be less

pathogenic, so the chance of TESE in the case of complete AZF c deletion is 50%[12].

In our study, the diverse types of AZF microdeletions among infertile Iraqi men were (32.6%) of microdeletions noticed in AZFa,b,c, (23.9 %) of microdeletions appeared AZFab, (8.6 %) of microdeletions appeared in AZFac, (4.3 %) of microdeletions appeared in AZFbc, (15.2 %) of microdeletions noticed in AZF a, (8.6 %) of microdeletions noticed in AZF b, (6.5 %) of microdeletions showed in AZF c. Regarding the diversity of microdeletions, our study results agree with what Zhang *et al*. tell[32]. The diverse kinds of AZF micro-deletions in patients reveal various severity of infertility. The deletions of AZF a and AZF b in the Y chromosome is reported to have an exceptionally poor sperm recovery prognosis, whereas most infertile males with AZF c deletion contain sperm in the semen or tests obtainable for use in IVF/ ICSI[30].

In our study, 24 patients with nine azoospermia/15 severe oligospermia, with AZFc microdeletions were screened by four additional primers to determine the types of AZFc sub-region microdeletions. The results were 14 with gr/gr AZFc sub-region partial microdeletion (58.3%), 4 in azoospermia, 10 in severe oligospermia and 9 with b2/b4 AZFc sub-region complete deletion(37.5%), 5 in azoospermia, 4 in severe oligospermia, while one patient with b2/b4 continues terminal heterochromatin region AZFc deletion. No microdeletion in the b2/b3 AZFc sub-region was reported. The results of the study agree with that observed by Choi *et al*. which told a rate of 54.2 % in 59 harm patients with gr/gr AZFc microdeletion[33]. The association between gr/gr AZFc sub-deletions and infertility varies according to ethnicity and geographic region[6].

In our study, The disorders of sex hormones for infertile males, especially in azoospermia male with AZF microdeletion in comparison with other groups. There is a statistically significant difference in LH values between azoospermia with AZF microdeletion and azoospermia without AZF microdeletion (P=0.024). This result was harmony with what noticed Kim, *et al*.[34]. Also, The FSH hormone revealed in this study, (23.58 ±18.20) which is elevated. This result was in agreement with those observed by Bahmanimehr and associates who revealed follicle stimulating hormone concentration was 28.45 ±22.2 in the infertile azoospermia group with YCD[35].

In our study, (25) infertile male (33.33%), (11) azoospermia and (14) severe oligospermia were exposed to the effect of weapons as a result of radiation in Al- Anbar governorate. There is a significant correlation among the exposure to radiation, uranium, pollutions, and the infertility groups, P-value 0.002 or > 0.05. The physical and psychological trauma that results from wars may be an increased hazard of sterility in males. The presence of reproductive organs pollutants from weapons, strenuous times of war, and direct harm for the reproductive system which may be impairs males fertility[36].

CONCLUTION

The present study concluded the AZF microdeletions in the Y- chromosome is the most significant genetic check for male infertility problems in Iraq. The incidence of classical AZF microdeletions in subjects is high; therefore, the incorporation of the classical AZF microdeletion test for male infertility in our population is useful. In our study population, gr/gr partial AZF c microdeletions were higher than b2/b4 complete AZF c deletion. Also, the mean levels of sex hormones in azoospermic sterile patients with AZF microdeletion were higher than the mean levels in azoospermic infertile males without AZF microdeletions. The quality and the amount of sperm in patients with AZF c microdeletion decrease with age. In fact, early detection of Y chromosome microdeletions can influence the successful of assisted reproductive techniques.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHORS' CONTRIBUTION

All authors listed have a substantial direct and intellectual contribution to the work and approved it for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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