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Cytogenetic Investigations and Y-Chromosome Microdeletion Screening in some Infertile Kurdish males In Erbil province/ Iraq

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Abstract:

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Worldwide, infertility affects approximately 15% of all couples trying to conceive. Male infertility is responsible for about 50% of the infertility cases. Chromosomal abnormalities and Y-chromosome microdeletions are the most common genetic causes of male infertility. Klinefelter syndrome (KS) is the most prevalent factor of the chromosomal abnormality in the infertile male. Azoospermia Factor (AZF) microdeletions located on the Y chromosome are one of the recurrent genetic cause of male infertility. This study aims to investigate the prevalence of chromosomal anomalies and AZF microdeletions in 296 infertile Kurdish men in Erbil province, 289 patients diagnosed as azoospermia (97.6%) and 7 patients as severe oligozoospermia (2.4%) and 50 healthy men as control group. Twenty nine patients (9.8%) had various chromosomal abnormalities. The most common chromosomal abnormalities were found in sex chromosomes (93.1%; 29/27), among these abnormalities 20 patients (69%) had Klinefelter syndrome 47,XXY karyotype, 4 patients (13.8%) had 45X0/46, Xder(Y), 2 patients (6.9%) had XXY t(11;22)(q25;q13) and 1 patients (3.4%) had Mosaic Turner syndrome 46XY/45X0. The autosomal chromosomal abnormalities (6.9%; 2/29) detected in 2 patients 45, XY, rob (13;14) (q10;q10). Y chromosome microdeletions were found in 10 of 289 patients with azoospermia (3.5%), three of them (30%) had microdeletions in the AZFc region, 3 of them (30%) had microdeletions in the AZFb region, also other 3 patients had microdeletions in the b and c of AZF (AZF b,c) region, and the final one patient (10%) had microdeletions in the all a, b and c (AZF a,b,c) region. Combined Y chromosome microdeletions and chromosomal abnormalities were detected in 3 patients.

Keywords: AZF microdeletions, Human karyotyping, Klinefelter syndrome, Male infertility, Real Time PCR.

Introduction:

Infertility is the inability of a sexually active, non-contraception couple to achieve spontaneous pregnancy in one year. Worldwide infertility is affecting approximately 15% of all the couples attempting pregnancy. Male infertility refers to a male's inability to make pregnancy in a normal fertile female¹⁻³ and generally it is responsible for near 50% of the all infertility cases⁴.

Generally male infertility is divided into Azoospermia and Coital infertility^{4,5}. Azoospermia is complete absence of spermatozoa in the ejaculation and it is diagnosed in 15% infertile men. The Azoospermia itself is also classified into two

parts: obstructive infertility and non-obstructive infertility⁵.

In obstructive infertility the ejaculate is empty of spermatozoa while the spermatogenesis is normal and may be caused by one of the following: obstruction in the ejaculatory duct or epididymal obstruction or vasectomy or vassal obstruction or some time congenital absence of vas deferens⁵.

Non-obstructive Infertility is characterized by abnormal spermatogenesis and the causes include: cryptorchidism (undescended testicle) or testicular torsion or testicular trauma or testicular cancer or varicocele or genetic factors or hormonal imbalance or immunologic infertility or exposure to

gonadotoxins^{6,7}. Coital infertility is characterized by normal observation in both sperm production and genital tract. Therefore, the disease is secondary to the patient's impotence that impact ejaculation and illness may be due to penile deformities or premature ejaculation or retrograde ejaculation or anejaculation or erectile dysfunction^{5,7}.

Genetic factor is the main factor causing infertility in males, which may affect hormonal balance, spermatogenesis processes and quality of the sperm⁸, and it is diagnosed in approximately 15–20% of severe male factor infertility (azoospermia or severe oligozoospermia)^{9,10}. Genetic causes of male infertility may include the followings: _ chromosomal abnormalities, autosomal gene mutation, polymorphism and epigenetic errors^{11,14}.

More than 50% of all infertile males diagnosed with azoospermia or severe oligozoospermia and genetic abnormalities are account for 15%–30% of factors that contributed to male infertility¹⁵. Chromosomal abnormalities are proved to be as one of the recurrent causes of male infertility, and it is present in about 20% in azoospermic males, and the sex chromosomes mostly include¹⁶.

Chromosomal abnormality is considered as one of the most genetic factors found in infertile males within the range of 2.4-16.4%¹⁷, while it is about (0.3- 0.4%) in general male population¹⁸.

In males with azoospermia the chromosomal abnormalities generally are high, the incidence range is about 13.1% to 23.6% and in men with severe oligospermia it is 10.6% but in males with oligospermia, the incidence is 2.1-6.6%^{19, 20}. Klinefelter's syndrome and its variants are the most common sex chromosome abnormality in infertile males²¹. After the klinefelter syndrome, Y chromosome microdeletions are the leading genetic causes of male infertility²².

Various studies have shown that 10% of the men suffering from azoospermia have microdeletions in their three types of azoospermia factor genes. These deletions remove number of genes responsible for germ cell development in male (spermatogenesis) and its maintenance^{23, 24}. The Y chromosome in human is important for sex determination and male germ cell progress and maintenance²⁵.

The azoospermia factor (AZF) is located on the long arm of the Y chromosome Y/q and shows a main role in the genetics/of male sterility and is divided 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 spermatogenic defects and male infertility. However, Microdeletions at AZF are the most

common structural chromosomal defects and the leading induce of male infertility²⁶.

Both cytogenetic analysis (karyotype) and Y-chromosome microdeletion tests are important and useful for patient and physician to specify appropriate assisted reproductive technology (ART) like the intra-cytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF) techniques²⁷.

The aim of this study is to determine the type of chromosomal abnormalities and it is frequency in infertile Kurdish males having primary infertility, also for analyzing the prevalence and types of microdeletions in the AZF region of a Y chromosome: SY84 (AZF-a), SY86 (AZF-a), SY127 (AZF-b), SY134 (AZF-b), SY254 (AZF-c/DAZ) and SY255 (AZF-c/DAZ) in infertile Kurdish males with azoospermia and severe oligospermia in Erbil province northern of Iraq.

Materials and Methods:

Patients and control

The study was approved by the Research Ethics Committee at College of Science, Salahaddin University-Erbil, Iraq, No. 4S/345, 24/12/2019. This study was conducted on 296 Iraqi Kurdish infertile males in Erbil province between December 2019 and December 2020. The selection of patients who participated in this study is based on their medical history recorded in government and private hospitals and infertility centers. All cases were diagnosed with primary infertility, and they did not have obstructive azoospermia. The majority of patients had azoospermia and only seven of them were with severe oligozoospermia (sperm count $<5 \times 10^6/\text{ml}$) with median age 35 year. Semen analysis was done according to WHO guidelines²⁸. The control group in this study is 50 healthy males with proved paternity without ART with the median age 36 year. All patients were interviewed with male infertility questionnaire form prepared in this study; the questionnaire form involved many fields starting from Fertility History, Sexual History, Environmental Exposures, Past Medical and Surgical History and Family infertility History. General urine examination was also performed for all patients for microbial infections.

Karyotyping Analysis

Chromosomal analysis was performed according to Benn, P. and Delach, J. protocol²⁹. Three ml of peripheral blood was drawn from each patient and collected in vacuum tubes containing anticoagulant lithium heparin (VACUTEST KIMA S.r.l -Italy), 0.5 ml of this blood sample was mixed with 4.5ml of ready to use chromosomal medium P (Euroclone S.P.A. Italy). Samples were incubated for 65-70 h. at 37 °C. About 1.5-2 h. before the end of

the culture 100µl of colchicine (gibco, USA) was added then centrifuged and the recovered pellet was treated with a hypotonic solution (0.075 M KCl) and incubated at 37°C for 10minutes. The samples were centrifuged then fixed by fixative consisting of acetic acid/methanol (1:3 v/v). About 2-3 drops of cell suspension were dropped on the height of three feet on to the clean tilted slide at 45° angle then holding edge of slide on the bench. These dried slides were immersed in the trypsin solution which consists of 2.5ml of trypsin solution (Trypsin-EDTA 1X in PBS, Euroclone S.P.A. Italy) with 50ml of normal saline for one minute at 37 °C. Then they underwent a Giemsa staining, and finally reading slides by G-banding technique using a cytovision version 7.5 system. About 20 metaphases were analyzed for each sample, whereas if karyotype is abnormal more than 30 metaphase were tested for confirmation the result. Karyotype at 550 band resolution was considered good enough.

DNA extraction

From each patient, two ml of peripheral blood was drawn using sterile syringe and collected in EDTA tube. Genomic DNA was isolated from blood samples according to (Genomic DNA Mini Kit, Geneaid, Taiwan) kit, the sequential procedures were done according to a manufacture protocol, the average yield of genomic DNA was 10-50 ng/µL, with purity about (1.36-1.75). These results (quality and quantity of each DNA samples) were determined by Thermo scientific Nanodrop1000 spectrophotometer.

Real Time PCR testing:

Y chromosome microdeletion screening was performed for each patient and controls according to the European academy of andrology (EAA) and the European molecular genetics quality network (EMQN) protocol²². Real Time PCR technique was used to investigate Y chromosome microdeletions for both patients and controls to diagnose deferent sequence tagged sites (STS) of AZF microdeletions on the Y chromosome, the STS were screened are SY84 (AZFa), SY86 (AZFa), SY127 (AZFb), SY134 (AZFb), SY254 (AZFc/DAZ) and SY255 (AZFc/DAZ) whereas genes that responsible or encoding for human zinc-finger protein (ZFX/Y) that present on the X and Y chromosomes, and sex determining region (genes) located on the Y chromosome (SRY, i.e., STS SY14) used as Control. Y chromosome Microdeletion Real Time PCR Kit

(Cat. No: 15R-10-08, SNP Biotechnology, TURKEY) was used, eight RT PCR mixtures provided by the kit, each mixture contains sequence specific primers and probes. The fluorescence of AZF's analysis was FAM. Also, each RT PCR mixture contains an internal control labeled with HEX or JOE dye. For each region 20 µL of RT PCR mixtures was mixed with 5 µL (10-100ng) of DNA sample, then running with RT PCR machines (Prime Pro 48 Real-Time PCR System-Techne) with the condition program ; 95c for 5 Min. one cycle. 95c for 15sec. 60C for 1Min. 40 cycles according to manufactures protocol.

Results:

Karyotyping Analysis:

Karyotyping was performed for 296 Kurdish infertile men among them 289 patients with azoospermia (97.6%) and 7 patients with severe oligozoospermia (2.4%). No chromosomal abnormalities were detected in severe oligozoospermia group and in control group. Of the 289 infertile patients, 29 patients (9.8%) had various Chromosomal abnormalities (Table 1). The most common chromosomal abnormalities were found in sex chromosomes (93.1%; 29/27), among these abnormalities 20 patients (69%) had Klinefelter syndrome 47,XXY karyotype (Fig. 1) , 4 patients (13.8%) had 45,X0/46, XY,der(Y),t(18,Y)(q13; q25) (Fig. 2), 2 patients (6.9%) had XXY t(11;22)(q25;q13) (Fig. 3)and 1 patients (3.4%) had Mosaic Turner syndrome 46XY\ 45X0 (Fig. 4). The autosomal chromosomal abnormalities (6.9%; 2/29) detected in 2 patients 45,XY,rob(13;14) (q10;q10).

Table 1. Chromosomal abnormalities in Kurdish infertile males with primary infertility.

Abnormal Karyotype	Number	Total n (%)
Sex Chromosomal		
47,XXY	20	69%
45,X0/46, XY,der(Y),t(18,Y)(q13; q25)	4	13.8%
47, XXY, t(11;22) (q25;q13)	2	6.9%
46, XY/45, X0	1	3.4%
autosomal chromosomal		
45, XY, rob(13;14) (q10;q10)	2	6.9%
Total	29	

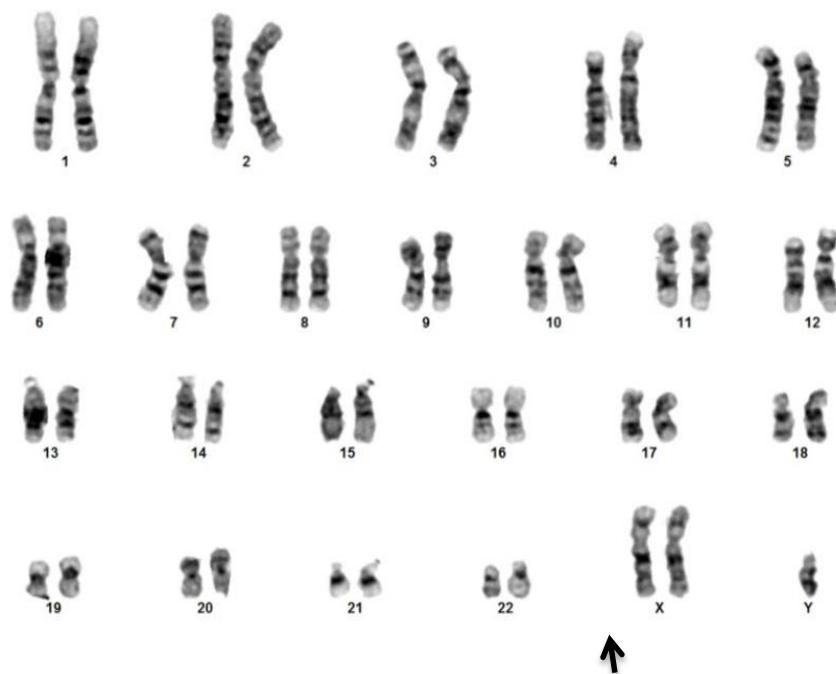


Figure 1. Karyotype of Klinefelter's syndrome patient showing 47,XXY.

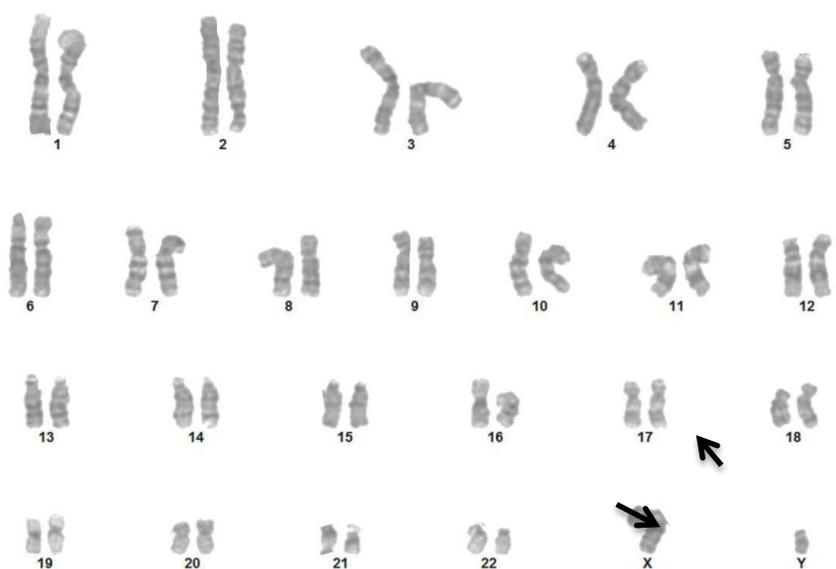


Figure 2. Karyotype of a patient showing 46,XY,der(Y),t(18,Y)(q13;q25)

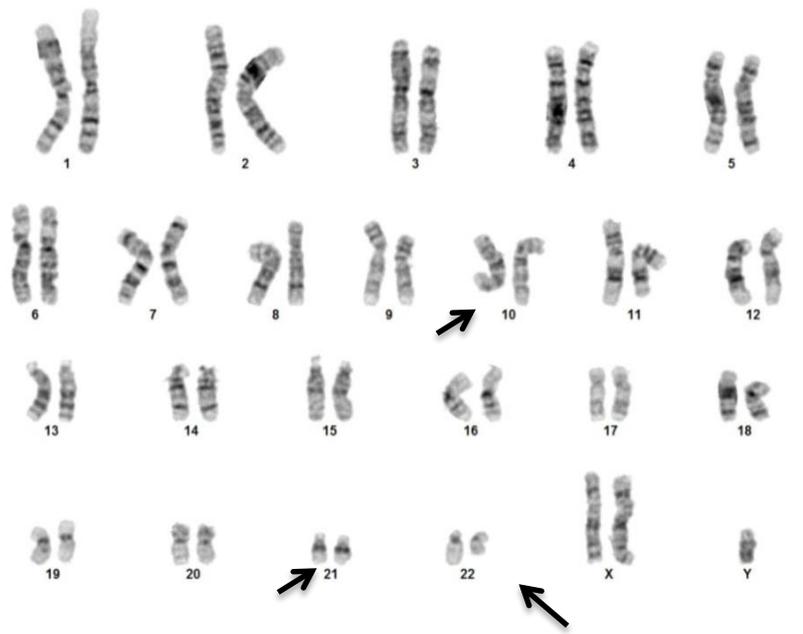


Figure 3. Karyotype 46,XXY,t(11;22)(q25;q13), patient with two abnormality: Klinefelter's syndrome 47,XXY and translocation between chromosomes 11 and 22..

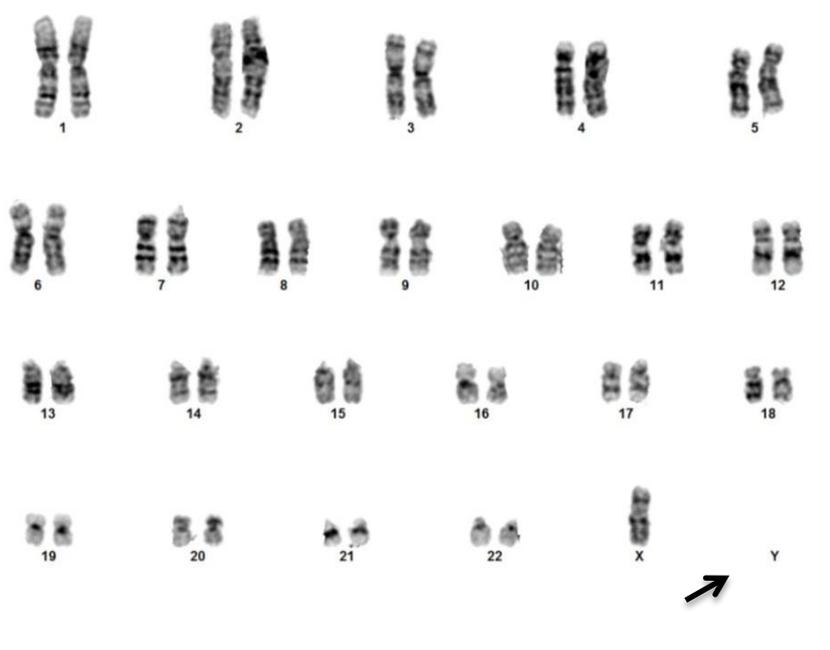


Figure 4. Karyotype showing Turner syndrome 45,X0 .

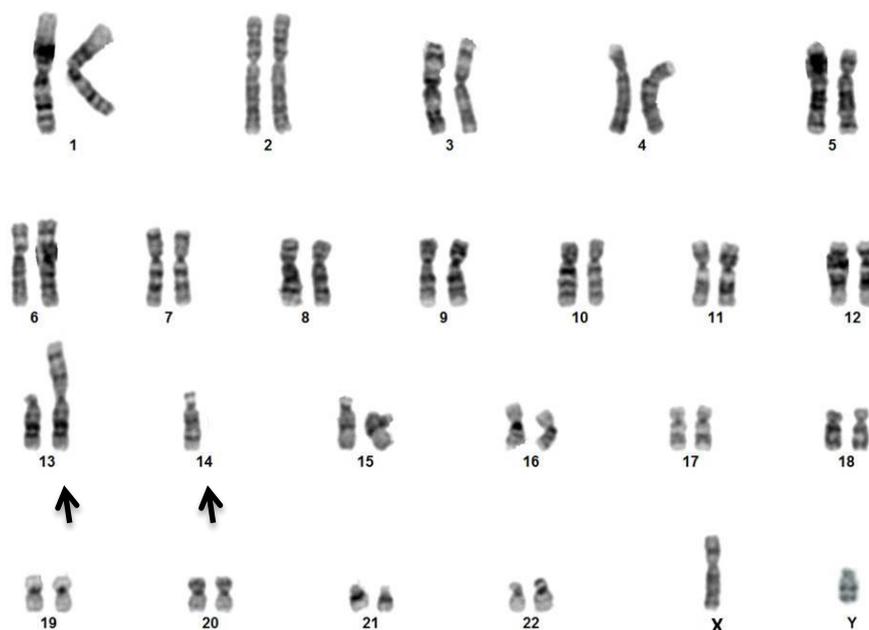


Figure 5. Karyotype showing 45,XY,rob(13;14)(q10;q10)

Real Time PCR Testing for Y chromosome microdeletion

A total of 296 infertile Kurdish males were tested for microdeletion in the Y chromosome in addition to 50 fertile males as a control group. The microdeletion was not detected in both severe oligospermia and in the normal group. Y chromosome microdeletions were found in 10 of 289 patients with azoospermia (3.5%) (Table 2). Among the 10 patients that had Y chromosome microdeletions, three of them (30%) had microdeletions in the AZFc region, three of them (30%) had microdeletions in the AZFb region, also other three patients had microdeletions in the both b and c of AZF (AZF b,c) region, and the final one patient (10%) had microdeletions in the a, b and c (AZF a,b,c) region.

Among the 10 patients that had Y chromosome microdeletions, three patients also had Chromosomal abnormalities. Two of them with AZFb,c deletion also had 45,X/46, x,der(Y) karyotype and the other patient with AZFa,b,c deletion had Mosaic Turner syndrome 46,XY\ 45,X. The remaining patients had a normal karyotype.

Combined Y chromosome microdeletions and chromosomal abnormalities were detected in three patients in which two patients with karyotype 45X0/46,X,der(Y) had microdeletions in both AZF b and AZF c and one patient with karyotype mosaic Turner syndrome 46XY\ 45X0 had microdeletions in all AZF a, AZF b and AZF c, the overall genetic causes of male infertility in this study were found in 36 patients 12.16% (Table 3).

Table 2. Frequency and types of AZF microdeletions on Y chromosome in Kurdish infertile males

Yq microdeletions	AZF	Non-obstructive azoospermia (n)	Total (%)	n
AZFb		3	(30%)	
AZFc		3	(30%)	
AZFbc		3	(30%)	
AZFabc		1	(10%)	

Table 3. Genetic causes and association between types of chromosome abnormalities and AZF microdeletions in Kurdish infertile males.

No	Age	Karyotype	AZF region
1	33	47,XXY Klinefelter	Present
2	35	45,XY rob(13;14) (q10;q10)	Present
3	30	47,XXY t(11;22)(q25;q13)	Present
4	22	45,X0/46, XY,der(Y),t(18,Y)(q13; q25)	AZF bc deletion
5	35	47,XXY Klinefelter s.	Present
6	29	45,X0/46, XY,der(Y),t(18,Y)(q13; q25)	Present
7	35	47,XXY Klinefelter	Present
8	38	47,XXY Klinefelter	Present
9	40	47,XXY Klinefelter	Present
10	31	47,XXY Klinefelter	Present
11	32	47,XXY Klinefelter	Present
12	42	47,XXY Klinefelter	Present
13	28	47,XXY t(11;22)(q25;q13)	Present
14	38	47,XXY Klinefelter	Present
15	36	45,X0/46, XY,der(Y),t(18,Y)(q13; q25)	AZF bc deletion
16	31	47,XXY Klinefelter	Present
17	26	45,X0/46, XY,der(Y),t(18,Y)(q13; q25)	Present
18	35	47,XXY Klinefelter	Present
19	38	47,XXY Klinefelter	Present
20	30	45 XY rob(13;14) (q10;q10)	Present
21	20	47,XXY Klinefelter	Present
22	31	47,XXY Klinefelter	Present
23	31	XXY Klinefelter	Present
24	41	47,47,XXY Klinefelter	Present
25	32	Mosaic Turner syndrome 46XY\ 45X0	AZF abc deletion
26	46	47,XXY Klinefelter	Present
27	32	47,XXY Klinefelter	Present
28	34	47,XXY Klinefelter	Present
29	35	47,XXY Klinefelter	Present
30	29	46,XY Normal	AZF b deletion
31	30	46,XY Normal	AZF b deletion
32	33	46,XY Normal	AZF b deletion
33	45	46,XY Normal	AZF c deletion
34	31	46,XY Normal	AZF c deletion
35	32	46,XY Normal	AZF c deletion
36	36	46,XY Normal	AZF bc deletion

Discussion:

Chromosomal abnormalities are one of the main genetic factors that contribute to male infertility. In the current study, the recurrence rate of major chromosomal abnormalities was 9.8% (29/289) in infertile males with primary infertility, with an incidence of sex chromosome abnormalities which was 93.1% and autosomal chromosome abnormalities which was 6.9% among both azoospermia and severe oligozoospermia patients. That was within the range of the several previous

published studies were reported a wide range (2% to 16%) of chromosomal abnormalities in infertile males⁴.

This study revealed that the frequency of sex chromosome abnormalities was the most common abnormalities among the infertile patients 93.1% (27 of 29 cases) while the autosomal chromosome abnormalities were detected in just two patients 6.9%. All abnormalities in this study have been within the azoospermia group, no chromosome abnormalities have been found in the severe oligozoospermia patients. Within chromosome abnormalities, sex chromosome abnormalities are the most common cause of chromosome related infertility^{16,30}, klinefelter syndrome 47, XXY is the most common sex chromosomal abnormality, it was detected in 20 of 29 (69%) of the general detected chromosomal abnormalities. Klinefelter syndrome is the most common one and is associated with severe spermatogenic failure causing a marked reduction in testicular size and azoospermia resulting in childlessness³¹.

Interestingly, out of the 29 patients with sex chromosomal abnormalities, four (13.8%) had a rare condition mosaic karyotype 45,X0/46, XY, der(Y),t(18,Y)(q13; q25), and two patients with Klinefelter karyotype also had autosomal translocation 45,XY rob(13;14) (q10;q10) between chromosome 11 and 22.

The autosomal chromosomal abnormalities were detected as Robertsonian translocations in two cases of twenty nine cases (6.9%) among chromosomal abnormalities. Robertsonian translocation involving chromosomes 13 and 14 occur with a prevalence of 0.97 in 1,000 in general population, but it is most frequent in infertile men³². Balanced autosomal translocation carriers generally have a normal phenotype, but the failure of spermatogenesis is frequently seen because translocations can damage the structure of important genes related to spermatogenesis³¹, and have a variable influence on the carrier's sperm counts, which can range from normal to low, or even to total aspermia leading to fertility problems, such as infertility, repeated miscarriages, or birth of a child affected with congenital abnormalities^{34,35}.

The results of this study are similar to the studies conducted in countries bordering Iraq, for example, in the comparing the results of studies regarding the percentage of Klinefelter Syndrome, it has been found that the percentage of Klinefelter syndrome in this study (93.1%) is higher than any other studies around the Iraq, for example in Iran the study by Akbari, M. T. 2012 was conducted on 212 infertile patients, The chromosome abnormality rate was 13.96%, 27 had azoospermia among them 21

patients had Klinefelter syndrome (77.7%), followed by other numerical and structural abnormalities³⁶.

In Turkey, it was found that the percentage of Klinefelter is the most common abnormalities in the sex chromosomes, in a study by Özdemir TR, et al²⁷, conducted on 1696 patients, chromosomal abnormalities were detected in 140 (8.3%), the most common chromosomal abnormalities were found in sex chromosomes (76.4%; 107/140) and the Klinefelter syndrome (47,XXY) was found as in the 75 of 140 cases.

Also, in the State of Syria, in a study conducted on 162 infertile males, chromosomal abnormalities were found in 20 patients (12.34%), sex chromosome abnormalities were detected in 17 of them (11 of 17 infertile males in the azoospermic group had a klinefelter syndrome of (64.7%)³⁷.

As in Saudi Arabia, in a study conducted on a group of 88 male infertile (azoospermia and severe oligospermia), chromosomal abnormalities were found in 10 patients (12.5%) with structural and numerical abnormalities, all changes or abnormalities happened in sex chromosome, Klinefelter syndrome and its variants were detected in six patients (55.4%), followed by abnormalities related to Y chromosomal³⁸.

While in Jordan, it seems to be a much lower percentage of chromosomal abnormalities comparing with the neighboring countries, in a study conducted on 1100 infertile males, 30 of them suffering from azoospermia, chromosomal abnormalities were found in 7 out of 30 azoospermic patients; six subjects with Klinefelter's syndrome 47XXY confirming giving a prevalence of 20% and one case of structural chromosomal abnormality in the form of 46XY,inv(9)P giving a prevalence of 3.3%; this constitutes a total prevalence of 23.3% of azoospermic group³⁹.

In Kuwait, in a study conducted on a group of 289 infertile males (azoospermia and severe oligospermia), chromosomal abnormalities were found in 23 patients (8.6%), from them 22 patients (95%) had sex chromosomal abnormalities and the Klinefelter syndrome and its variants detected in 16 patients ((74%)⁴⁰. Reasons for this disparity may be differences in race or the general criteria used to select a patient.

The molecular study was performed by using Real Time PCR technique to detect Y chromosome microdeletions in 289 kurdish infertile males. No microdeletions were identified in any of the severe oligozoospermia patients. According to our results the occurrence of AZFc, AZFb and AZFbc are the same (30 %) in the azoospermia patients.

In the present study, the prevalence of Y microdeletions in the (non-obstructive azoospermic)

infertile men was 3.5% (10/289). This result is considered one of the lowest ratios in the world. The rate of AZF deletion in infertile men in global surveys ranges from 5 to 20%⁴¹. Y microdeletions are found almost exclusively in patients with azoospermia or severe oligozoospermia⁴².

The findings of this study revealed that deletions in both three regions AZFa, AZFb, and AZFbc are detected in same proportions 30%, followed by deletions of AZFabc (10%), thus this result slightly differ from other studies.

The results of this study are near to the results of a study carried out in Saudi Arabia by Beg, et al³⁸ the study included 88 infertile males with with azoospermia and severe oligozoospermia, they detected AZFbc microdeletion only in two patients and frequency of Y chromosome microdeletions were about 2.27% and they didn't find any other types of AZF microdeletions.

In Kuwait the ratio of Y chromosome microdeletions was about (2.6%) according to a study by Mohammed F, et al⁴⁰ that carried out on 286 infertile males with azoospermia and severe oligozoospermia, the most frequent AZF type was AZFbc 71% and AZFc 29%, while other AZF microdeletion types not detected.

In Jordan, in a study done by Khabour O, et al⁴³ conducted on 100 infertile male with 36 azoospermia and oligozoospermia male, they didn't find microdeletions in oligozoospermic group. In Azoospermia group three patient had AZF microdeletions on Y-chromosome (8.6%), among them two patients had AZFc microdeletions (66.7%), and the other patient had a microdeletion that involved AZFb region and part of AZFa and AZFc regions (33.3%).

In Iran, several studies were carried out to determine the frequency of AZF microdeletions among infertile men, Yousefi-Razin E, et al⁴⁴ reviewed 13 relevant studies as a Meta-analysis; on the ratio Y chromosome microdeletions between infertile males that covered all Iranian population with ethnic and territorial differences over the country. They reached a conclusion that frequency of Y chromosome microdeletions was about 12.1% (95% CI, 6.5-21.5) of Iranian infertile men with azoospermia and severe oligozoospermia, and the AZFc was most frequent followed by AZFb and AZFa.

In Turkey also low frequency of AZF microdeletion has been detected (2.6%) in a study performed by Özdemir TR, et al²⁷, in which conducted on 1696 infertile Turkish male, the study is one of the few were a large number of cases was studied in Turkey. Y-chromosome microdeletions were detected in 45 of 1696 cases (2.6%). The AZFc

region was found as the most affected site (44.4%), followed by AZFb+c (31.1%), AZFa+b+c (11.1%), AZFa (8.9%), and AZFb (4.5%) regions.

The most prevalent Y chromosome microdeletions among infertile males in countries bordering the Iraq were recorded in Syria. In a study conducted on 162 infertile males including 97 azoospermia, 49 oligozoospermia and 16 severe oligozoospermia patients, and 100 normal males as a control by Al-achkar, et al³⁷, they detect 46 microdeletions on patients Y chromosome (28.4%) but not in both normal males. AZFc microdeletion was the most prevalent type (34.8%), followed by the AZF b, c (15.21%), AZFa (13.04%) and AZFa, c (8.7%). Accordingly, the frequency of microdeletions was 33% (32/97) in the azoospermic group compared to 22.44% (11/49) in the oligozoospermic and 18.8% (3/16) in the severely oligozoospermic group.

In Anbar, in western central Iraq, a study carried out by Al-Qaisi, et al⁴⁵, including 75 infertile males and 25 healthy control, according to their study; 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, they noticed (32.6%) of microdeletions in AZFabc, (23.9 %) of microdeletions in AZFab, (8.6 %) of microdeletions in AZFac, (4.3 %) of microdeletions in AZFbc, (15.2 %) of microdeletions noticed in AZFa, (8.6 %) of microdeletions noticed in AZFb, (6.5 %) of microdeletions showed in AZFc. They found high proportion of microdeletions 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%).

In this study combined abnormal karyotype and AZF microdeletion has been detected in three patients, in which two patients with karyotype 45X0/46, X, der (Y) had deletions in AZFbc and one patient with karyotype mosaic turner syndrome 46XY/45X0 had deletions in AZFabc. While the SRY gene has been detected in all patients, SRY region is responsible for determining sex identity towards male development⁴⁶.

Genetic counseling is important for the persons who carry translocation especially Robertsonian translocation between chromosomes 13 and 14. Pre-implantation genetic diagnosis (PGD) is an effective strategy for carriers of this chromosomal rearrangement. In order to avoid abnormal pregnancy, normal or balanced embryo should be selected for transfer by PGD analysis of translocation chromosomes, because these patients are at increased risk of conceiving chromosomally

abnormal embryos, resulting in implantation failure, miscarriage or delivery of affected offspring^{33, 35}.

The Y-chromosome microdeletion screening is an important test for providing appropriate genetic counseling and to determine appropriate ART in male infertility with azoospermia and severe oligozoospermia. Hence, it should be performed routinely. Generally, the AZFa or AZFb microdeletion has more severe effects than AZFc. ART treatments (TESE, ICSI) are not successful in cases with a complete deletion of AZFa or AZFb regions. Therefore, TESE should not be recommended in these cases. On the other hand, the cases with AZFc microdeletion have a variable histological and clinical phenotype. In general, residual spermatogenesis is present in AZFc microdeletions. These cases have approximately a 50% chance of sperm retrieval by TESE, and children can be conceived by ICSI^{4, 22}.

The microdeletions of the Y chromosome are transmitted from father to the male offspring, and therefore genetic counselling is compulsory. The men who carry complete AZFc deletion produce large proportion of sperms that lack the sex chromosomes, indicating the potential risk for offspring to developing 45, X0 Turner syndrome and other phenotypic abnormalities associated with mosaicisms of sex chromosomes, most likely ambiguous genitalia. When using ICSI with males that have AZF microdeletion, Long-term follow-up is needed for any male child regarding his fertility status. Also, in men with Klinefelter's syndrome the sperm recovery rates can be achieved through TESE or micro-TESE especially when it done at a younger age. Hence sperm can be preserved through cryopreservation procedure at an early (young) age⁴.

It is necessary that both karyotyping and the Y-chromosome microdeletion test should be performed to provide appropriate genetic counseling and to determine an appropriate treatment in males with primary infertility. Genetic counseling is obligatory to provide information about the risks of giving birth to a son with an abnormal spermatogenesis. In case of partial deletion AZFa, AZFb and AZFc, the counseling (with AZF testing) is relevant also for other male members of the family as transmission of these types of deletions has been reported in the literature. But in cases complete AZFa, AZFb, AZFbc, or AZFabc deletions, A Y-chromosome microdeletion test is not recommended because such deletions are generally incompatible with sperm production⁴.

Conclusion:

In conclusion, the present study shows the occurrence of chromosomal abnormality and Y-

chromosomal microdeletion in some infertile Kurdish male with azoospermia and severe oligozoospermia. Overall Chromosomal abnormalities among azoospermia and severe oligozoospermia males is 9.8% and the klinefelter syndrome 47,XXY is the most common sex chromosomal abnormality (69%) of the general detected chromosomal abnormalities. The Y chromosome microdeletions in the infertile men were 3.5%. Neither Chromosomal abnormalities nor Y microdeletions have been detected in normal and oligospermia group. This finding is consistent with previous studies conducted in different societies and ethnic groups around the Kurdish population. It is highly recommended that infertile males be screened for chromosomal abnormalities and Y chromosome microdeletions to determine the causes of infertility in order to choose suitable assisted reproductive technology, and also to diminish the risk of passing this genetic disorder to next generations.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Authors sign on ethical consideration's approval
- The study was authorized and approved by the Research Ethics Committee at College of Science, Salahaddin University-Erbil, Iraq, No. 4S/345, 24/12/2019.

Authors' contributions statement:

M J A, developed the theory and performed the computations, acquisition of data, analysis, interpretation, drafting the manuscript, revision and proofreading. M S A., conceived of the presented idea, design, revision and proofreading and supervised the findings of this work. R A M., acquisition of data, analysis, verified the analytical methods, revision and proofreading

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دراسة الوراثة الخلوية والكشف عن مناطق الحذف الدقيق لكروموسوم Y لبعض الذكور الكرد في منطقة أربيل-العراق

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الخلاصة:

العقم هو مرض يصيب الجهاز التناسلي يُعرف بالفشل في تحقيق الحمل السريري بعد 12 شهرًا أو أكثر من الجماع المنتظم غير المحمي. في جميع أنحاء العالم، يؤثر العقم على ما يقرب من 15% من جميع الأزواج الذين يحاولون الإنجاب. يعد العقم عند الرجال مسؤولاً عن حوالي 50% من حالات العقم عامة. تشوهات الكروموسومات وحذف الكروموسوم Y هي الأسباب الجينية الأكثر شيوعًا لعقم الذكور، إذ تعد متلازمة كلاينفلتر والحذف الصغير لعامل فقد النطاق على كروموسوم Y من أهمهما. هدف هذه الدراسة هو التحري عن ومدى انتشار كلاً من التشوهات الكروموسومية وعمليات الحذف الدقيقة على كروموسوم Y في 296 رجلاً كردياً يعانون من العقم في محافظة أربيل، من بينهم 289 مريضاً يعانون من نقص النطاق (97.6%) و 7 مرضى يعانون من قلة النطاق الشديدة (2.4%) و 50 من الرجال الأصحاء كمجموعة مقارنة. وجد ان 29 مريضاً (9.8%) لديهم تشوهات كروموسومية مختلفة. تم العثور على تشوهات الكروموسومات الأكثر شيوعاً في الكروموسومات الجنسية (93.1%؛ 27/29)، من بين هذه التشوهات 20 مريضاً (69%) لديهم متلازمة كلاينفلتر النمط النووي 47,XXY، 4 مرضى (13.8%) لديهم نمط 45X0/46, Xder(Y)، كان لدى مريضان نمط 45X0/46, Xder(Y) و كان لدى مريض واحد (3.4%) نمط متلازمة تيرنر Mosaic Turner 46XY/45X0. تم اكتشاف تشوهات الكروموسومات الجسمية (6.9%؛ 29/2) في مريضين 45 XY rob (q10;q13) (13;14). تم العثور على الحذف الصغير لكروموسوم Y في 10 من 289 مريضاً يعانون من فقد النطاق (3.5%)، ثلاثة منهم (30%) لديهم حذف دقيق في منطقة AZFc، 3 منهم (30%) لديهم حذف دقيق في منطقة AZFb و AZFc، كما كان لدى 3 مرضى آخرين الحذف الصغير في كل من المنطقة من AZFb و AZFc، والمرضى الأخير (10%) كان لديه عمليات حذف صغيرة في المنطقة a و b و AZFc، و AZFa و AZFb). وكشفت هذه الدراسة ان كلا من تشوهات الكروموسومات والحذف الصغير لكروموسوم Y وجد في 3 مرضى.

الكلمات المفتاحية: الحذف الدقيق لل AZF، النمط الوراثي البشري، متلازمة كلاينفلتر، عقم الذكور، تفاعل البوليميرز المتسلسل بالزمن الحقيقي.