

Prevalence of Y-chromosomal microdeletions and karyotype abnormalities in a cohort of Lebanese infertile men

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Abstract

Background: Male infertility is the main issue that accounts for 50% of infertility in couples. There are about 25% of men suffering from nonobstructive infertility with chromosomal abnormalities and/or microdeletions of the long arm of the Y-chromosome.

Materials and Methods: A retrospective chart review was performed on 241 men who performed Y-chromosome microdeletions and karyotype testing.

Results: Six patients had microdeletions. Three patients had AZFc microdeletion, of which one had both AZFc/d microdeletions. Three patients had AZFb/c microdeletion. There was no AZFa microdeletion. One out of the six patients had abnormal karyotype (mos, X[17]/46, XY[13]). Four patients were azoospermic, two had severe oligospermia, with sperm count <5 million/ml, and two patients had small size testicles on ultrasound. All were advised microsurgical testicular sperm extraction. Three were done, and one was successful resulting in sperm retrieval. The most common karyotype abnormalities were 47, XXY (Klinefelter syndrome) in 27% of cases.

Conclusion: Laboratory genetic testing is advised for males with nonobstructive infertility. Any abnormal finding can yield substantial consequences to assisted reproductive techniques or fertility treatment. It can offer a stable diagnosis for those with infertility issues. It is important to conduct counseling and routine genetic testing before assisted reproductive techniques.

Keywords: Azoospermia, karyotype, male infertility, microdeletion, Y-chromosome

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INTRODUCTION

Male infertility is a crucial concern for the couple, families, and society.^[1] Men with infertility are prone to exhibit psychological issues due to stigma, causing divorce, remarriage, and resorting to alternative medicine that may carry dire consequences.^[1] Infertility is referred to as the incapability of conceiving after a year of engaging

in unprotected intercourse.^[2] Male infertility is caused by numerous elements such as endocrine disorders, infection, varicose veins, spermatic duct obstruction, presence of antisperm antibodies, cryptorchidism, retrograde ejaculation, systemic diseases, testicular trauma and testicular cancer, and other factors.^[3] There is also the idiopathic cause of male infertility.^[4]

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The human Y-chromosome determines sex and is essential for spermatogenesis. The long arm comprises the azoospermia factor (AZF) area. Microdeletions in this region account for male infertility.^[5] These deletions are the most common genetic cause of male infertility following Klinefelter syndrome.^[6] Comprehensive and rational treatment is absent in most idiopathic cases.^[1] There is dearth of studies about the occurrence and the reasons of male infertility. There is scarce information about the prevalence of Y-chromosome microdeletion and other genetic causes of infertility in the Lebanese population. The following study aims to determine the frequency and characteristics of Y-chromosome microdeletions and karyotype abnormalities in a cohort of Lebanese infertile men.

METHODS

A retrospective chart review was performed on 241 men who performed Y-chromosome microdeletions and >1200 patients who performed karyotype testing, as part of infertility workup, at the American University of Beirut Medical Center (AUBMC), between January 2005 and March 2020. This study was approved by the Institutional Review Board at AUBMC (IRB# BIO-2019-0512). Patients' demographics, karyotype profile, Y-chromosome microdeletion status, along with their hormonal profiles, semen analysis, and testicular ultrasound findings were all recorded. Literature review, of similar studies from Levant and Middle East countries, was obtained through a PubMed and Google Scholar search using the keywords: AZF and Middle East, karyotype, male infertility, and Y-microdeletion.

All semen analyses were performed in the same laboratory of our institute. Semen samples were obtained 5 days after ejaculatory abstinence. Semen analysis was repeated on two separate occasions, and azoospermia diagnosis is made after centrifugation of the semen sample for 15 min at room temperature with a high-power microscopic examination of the pellet.^[7]

Cytogenetic evaluation was carried out by the Department of Pathology and Laboratory Medicine at our institution. Chromosomal preparation was performed using the conventional method for karyotype analysis on phytohemagglutinin-stimulated lymphocyte cultures. Twenty-five metaphases were examined for each patient and karyograms were prepared at a resolution of 550 BPHS, using the applied spectral imaging systems software for karyotyping. The final results were written according to the international system for chromosome nomenclature guidelines.^[8]

Identification of the Y-chromosome microdeletions was performed using a polymerase chain reaction (PCR)-based

technique using multiple sequence-tagged sites (STSs). The PCR analysis was performed in accordance with the recommendations of the European Academy of Andrology and the European Molecular Genetics Quality Network. The following STSs were analyzed: SY84, SY86, SY127, SY134, SY143, SY152, SY157, SY254, and SY255. Internal controls including SRY (SY14) and ZFY were used.^[9]

The reference ranges for the hormones of interest, according to our laboratory, are as follow: testosterone from 249 to 836 ng/dl, estradiol from 11.3 to 43.2 pg/ml, follicle-stimulating hormone (FSH) from 1.5 to 12.4 mIU/ml, luteinizing hormone from 1.7 to 8.6 mIU/ml, and prolactin from 4.10 to 18.40 ng/ml.

RESULTS

A total of 241 Y-microdeletion tests were performed on Lebanese men presenting with infertility to our men's health clinic at the AUBMC. Those infertile men exhibited azoospermia or severe oligospermia (count <5 million/mL). Genetic testing included karyotype and Y-chromosomal microdeletions status.

A total of 6 (2.5%) patients had microdeletions, with a mean age of 30 years. Three patients had AZFc microdeletion; one of which had both AZFc/d microdeletions too. Since the importance of AZFd microdeletion is still unknown, this deletion was not taken into consideration in our further analysis. Three other patients had AZFb/c microdeletions. No AZFa microdeletion was noted in our series. Of those six patients with microdeletions, one had an abnormal karyotype (mos, X[17]/46, XY[13]), and the others had a normal karyotype of 46, XY.

Four (67%) patients were azoospermic, and two (33%) had severe oligospermia, with sperm count <5 million/ml. Two (33%) patients had small size testicles on ultrasound, measuring almost 3.6 cm × 1.4 cm × 2.1 cm, each. The hormonal profile of Y-microdeletion patients is listed in Table 1. Two of those patients had an abnormally elevated FSH level of 13.3 and 16 mIU/ml, with their respective testosterone levels slightly on the low side.

The six patients who had microdeletions were advised microsurgical testicular sperm extraction (m-TESE). These patients were already maintained on hormonal treatment after being diagnosed with oligo/azoospermia. Three of those subsequently underwent m-TESE at our medical center; one had successful sperm retrieval intraoperative but failed embryo transfer. The microdeletions in those six patients were as follows: three in AZFc region (SY254, SY255, and

Table 1: Genetic and hormonal profile of patients with Y-chromosomal microdeletions

Patient number	Age (years)	Y-microdeletion	Karyotype	Sperm count (million/ml)	Testosterone (ng/dl)	FSH (mIU/ml)	LH (mIU/ml)	Prolactin (ng/ml)	Estradiol (pg/ml)
1	30	AZFb/c	mos 45, X[17]/46, XY[13]	AZO	192.3	13.3	8	12.58	16.81
2	30	AZFb/c	46, XY	AZO	531	6.52	9.51	22.81	NA
3	29	AZFb/c	46, XY	1.0	500	3.5	NA	NA	NA
4	27	AZFc	46, XY	AZO	156.1	16	9	17.76	NA
5	36	AZFc	46, XY	2.2	NA	NA	NA	7.2	NA
6	30	AZFc and AZFc/d	46, XY	AZO	937	8.9	3.9	NA	NA

AZF: Azoospermic factor, mos: Mosaic, AZO: Azoospermic, NA: Not available, FSH: follicle-stimulating hormone, LH: Luteinizing hormone

SY277) and deletion in AZFc/d region (SY152). The two patients with oligospermia [patients 3 and 5 from Table 1] attempted *in vitro* fertilization previously from semen ejaculate but were unsuccessful.

We listed all karyotype results, from January 2000 to December 2018, and performed several etiologies including mostly recurrent or repetitive abortions, abnormal sperm parameters, and difficult conception, among others. A total of 1272 karyotypes were found. Among those, 165 patients exhibited a karyotype different from the normal karyotype (i.e., 46, XY). A list of all abnormal karyotypes is listed in Table 2.

The most common karyotype abnormalities in this cohort of patients were 47, XXY (Klinefelter syndrome) in 45 (27.3%) cases, 46, XY,9qh+ and 46, XY,21pstk+ in 15 (9%) cases, respectively, and finally, 46, XY,15pstk+ in 10 (6%) cases.

DISCUSSION

Genetics has a chief role in male infertility with abnormal semen parameters.^[10] The Y-chromosome microdeletion screening is a crucial test to provide appropriate genetic counseling and to determine appropriate assisted reproductive technology (ART) in males having azoospermia or severe oligozoospermia.^[11]

Very few studies from the Middle East and the Levant countries have shed light on the cytogenetic causes of male infertility, including karyotype abnormalities and Y-chromosome microdeletions. Ours is the largest cohort of infertile men exclusively from Lebanon. Table 3 is a summary of all cases of Y-chromosome microdeletion cohorts from the Middle East and North African (MENA) region. In a study among Saudi infertile men with severe oligozoospermia or azoospermia, there were 49 out of 76 patients with complete semen analysis who had azoospermia and 27 had sperm concentrations between 0.1 and 5.0 million/ml.^[11] Testicular biopsy was done after testicular sperm extraction in 25 patients and 1 had seminoma. Genetic abnormalities pertinent to fertility were

seen in 11 patients (complete semen data available for nine patients). AZFb, c deletions were seen in two men both azoospermic with slightly elevated FSH levels. Five patients revealed Klinefelter syndrome with a 47, XXY karyotype on chromosome analyses: one patient with mosaic karyotype 48, XXXY (3)/47, XXY (47); two patients with supernumerary Y-chromosomes with a 47, XYY (2)/46, XY (48) mosaic karyotype and a 47, XYY (34)/46, XY (16) mosaic karyotype, respectively; and one patient with 46, XY constellation with a Y-deletion of q11.2.^[11]

In a study that gathered information about 880 patients from the Middle East and Levant countries, including Lebanon, 28.41% of all nonobstructive azoospermic men showed genetic abnormalities.^[6] These include 184 patients with karyotype abnormalities and 66 patients with Y-chromosomal microdeletions. The most predominant karyotype abnormality was Klinefelter syndrome (18.3%); 143 patients were nonmosaic, and 18 (2.05%) patients were mosaic. The overall frequency of AZF microdeletions was 7.5% in nonobstructive azoospermic men, divided as microdeletions in the AZFb region in 60.61% of cases, followed by the AZFc, AZFd 13.64%, and then AZFa in 48.48%, 13.64%, and 9.09% of cases, respectively.^[6]

Another study from Algeria including 84 infertile men (mean age, 39.3 years ± 5.6) showed that 11.1% of azoospermic patients had chromosome abnormalities and 6.7% from the severe oligozoospermic group. Y-chromosome microdeletions were 9.5%. The percentage of infertile patients with microdeletions in the AZFc region was 7.14% (four azoospermic and two severe oligozoospermic males), one azoospermic male (1.19%) in the AZFbc regions, and one in AZFb (1.19%).^[12] Another study in Iraq showed that the prevalence of Y-chromosome microdeletions was 40.7%. Around 53% of azoospermic patients had microdeletions, and those with severe oligospermia exhibited 28% microdeletions. Microdeletions for azoospermia in the AZFc region=22.64%, AZFb=20.75%, and AZFa=9.43%. For severe oligospermia: AZFc=12%, AZFb = 8%, and AZFa = 8%.^[13]

Table 2: List of all abnormal karyotypes in a separate cohort of infertile men, with their respective frequencies

Karyotype (s)	Frequency
46, XY	1107
46, XX	2
46, X, der (Y)	1
46, XY,1qh+	5
46, XY,9qh+	15
46, XY,9qh-	2
46, XY,9qh+,16qh+	1
46, XY,13pstk+	1
46, XY,14pstk+	7
46, XY,14pstk+,22pstk+	1
46, XY,15pstk+	10
46, XY,16qh+	8
46, XY,21pstk+	15
46, XY,22pstk+	3
46, XY, del (15)(q11q13)	1
46, XY, inv (4)(p14q12)	1
46, XY, fra (16)(q22)	1
46, XY, inv (1)(q42.2q44)	1
46, XY, add (17)(p13.3)	1
46, XY, fra (16)(q22.1)	1
46, XY, inv (9)	1
46, XY, inv (9)(p11q12)	1
46, XY, inv (9)(p11q13)	4
46, XY, t (6;1)(q25.2;p15.3)	1
46, XY, t (1;6)(p36;q23)	1
46, XY, t (4;7)(q28;q34)	1
46, XY, t (5;13;16)	1
46, XY, t (9;22)(q34;q11.2)	1
46, XY, t (13;18)(q14.3;q22)	1
46, XY, t (4;7)(p14;p13)	1
46, XY, t (14;15)(q32.1;q24)	1
46, XY, t (1;13)(q25;q22)	1
46, XY, t (13;18)(q14.1, q21.31)	1
46, XY, t (5;13)(q34;q21.3)	1
46, XY, t (4;12)(q26;p12.3)	1
46, XY, t (4;15)	1
46, XY, t (2;9)(q11.2;q32)	1
46, XY, t (4;7)(q25;q31.1)	1
46, XY, t (3;7)(p21;p24)	1
46, XY, t (16;17)(p13.3;q11.2)	1
46, X, idic (Y)(p10)	1
46, X, inv (Y)(q11.22p11.2)	1
47, XXY	45
47, XYY	1
47, XY+21	3
47, XXY,16qh+	1
47, XY,+21[39]/46, XY[11]	1
47, XXY[48]/46, XY[2]	1
47, XY,+mar[4]/46, XY[46]	1
45, XY, rob (13;13)(q10;q10)	1
45, XY, rob (13;22)(q10;q10)	1
45, XY, rob (22;22)(q10;q10)	1
45, XY, dic (18;21)(p11.1;p11.1)	1
45, X[10]/46, XY[40]	1
45, X[3]/46, XY[47]	1
45, XY, t (14;21)(q10;q10)	1
mos 45, X,1dmin[13]/46, X, iso (Yq)[12]	1
mos 45, X[17]/46, XY[13]	1
49, XXXXY	1

A total of 165 abnormal cases were detected

Y-chromosome microdeletion analysis should regularly be given to patients with nonobstructive azoospermia. There are some deliberations that support a regular evaluation of Y-chromosome microdeletions. A positive test would

offer a diagnosis of the patient's concern. The knowledge of the type of Y-microdeletion may support the physician in identifying the appropriate technique for assisted reproduction. It is necessary to convey this information to

Table 3: List of Y-chromosomal microdeletions cohorts published from the Middle East and North African region

Author list	Date	Materials	Results
Beg <i>et al.</i> , ^[1] Western KSA	2019	88 patients (age not mentioned)	Six patients with Klinefelter syndrome, two with 47, XYY syndrome and two with Y-chromosome microdeletions AZFb, c. Azoospermia seen in 49/76 patients
Chabchoub <i>et al.</i> , ^[12] Tunisia	2019	84 infertile Patients (mean age, 39.3 years±5.6)	Frequency of chromosome abnormalities in azoospermic patients=11.1% and 6.7% in the severe oligozoospermic group. Y-chromosome microdeletions=9.5%. The percentage of infertile patients who had microdeletions in the AZFc region=7.14% (four azoospermic and two severe oligozoospermic males), one azoospermic male (1.19%) in the AZFbc regions and one in AZFb (1.19%)
Chellat <i>et al.</i> , ^[2] Algeria	2013	80 patients 35.46±4.97 years (24-48 years)	Y-chromosome microdeletions=1.3%
Hammood <i>et al.</i> , ^[13] Iraq	2019	103 patients (33±1.24 years)	Y-chromosome microdeletions in azoospermia=2% Y-chromosome microdeletions=40.7% Microdeletions in azoospermia=52.8% and severe oligospermia=28% Microdeletions for azoospermia in the AZFc region=22.64%, AZFb=20.75%, and AZFa=9.43%. For severe oligospermia: AZFc=12%, AZFb=8% and AZFa=8%
Alhalabi <i>et al.</i> , ^[6] Middle East, Syria, Iraq, Palestine, Lebanon, Jordan, Saudi Arabia, Qatar, and the United Arab Emirates	2013	880 patients (32.6±6.8 years)	Y-chromosome microdeletions=7.5% Microdeletions in the AZFb region=60.61%, AZFc=48.48%, AZFd=13.64%, and AZFa=9.09%

AZF: Azoospermic factor

patients, so they learn that their male child will be subfertile and necessitate reproductive monitoring starting from sexual maturity.^[14]

CONCLUSION

Male infertility is a serious health concern across the globe. This descriptive study represents one of the largest series of Y-chromosomal microdeletions in the MENA region, and the first in Lebanon. It did not include any follow-up or other interventions to the cohort of patients of whom the karyotype was performed. The percentage of Y-chromosomal microdeletions in Lebanon is like that of the world prevalence, i.e., around 2.5%. The subgroup of infertile patients, with chromosomal abnormalities, usually benefits from m-TESE, to assist in sperm retrieval and reproduction techniques. There must also be the need for a neonatal screening program, as such microdeletions are vertically transmitted to male progenies during pregnancy. Genetic testing and counseling, before ART, are essential. Nevertheless, there is still more to go with research to better explain the reasons of male infertility and to advance applicable treatment methods.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Conflicts of interest

There are no conflicts of interest.

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