The Frequency of Chromosomal Abnormalities and Y Chromosome Microdeletions in Infertile Non-Obstructive Azoospermic and Severe Oligozoospermic Males

Original Article

Moheb M. Abdel-Razek[1], Ezzat S. Elsobky[2], Yousry M. Moustafa MD[1], Moustafa A. Elsaied[1], Shreif Refaat[1]

[1]Andrology Unit, Faculty of Medicine, Mansoura University, Mansoura, Egypt [2]Department of Pediatrics, Ain Shams University, Cairo, Egypt, [3]Medical Genetics Center, Cairo, Egypt

ABSTRACT

Purpose: To estimate the frequency and types of both chromosomal abnormalities and Azoospermia Factor (AZF) microdeletions among patients with non-obstructive azoospermia (NOA) and severe oligozoospermia (SOZ) with sperm count less than 5 million/ml.

Methods: Karyotyping was performed for all 1127 patients, whereas AZF microdeletions assay was done for 811 patients including 653 NOA and 158 SOZ by multiplex polymerase chain reaction (PCR). All patients were subjected to clinical examination, scrotal duplex ultrasound and hormonal evaluations.

Results: The frequency of chromosomal abnormalities was 14.4%, higher in NOA than SOZ men (22.6% versus 3.7%). Numerical chromosomal abnormalities were higher than structural type (11.8% versus 2.4%). Klinefelter syndrome (KS) represented 11.2% of the total chromosomal and 94.1% of sex chromosomal abnormalities. AZF microdeletions were higher in NOA than SOZ (6.1% versus 3.16%). AZFc microdeletions represented the most frequent finding: 31/45 (68.9%), followed by AZFbc: 7/45(15.6%), AZFb: 4/45 (8.8%) and AZFa: 3/45 (6.7%). All patients with AZFa (3), AZFb (4) and AZFbc (7) deletions were NOA, while 26/31(83.87%) with isolated AZFc deletion were NOA and 5/31(16.13%) were SOZ.

Conclusion: In according to the results shown, we emphasize the importance of karyotyping and AZF microdeletions analysis in such groups. Counseling for such patients before ARTs is warranted to decrease the risk of transmitting genetic abnormalities to off spring.

Key Words: AZF, azoospermia, karyotyping, male infertility.

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Corresponding Author: Moustafa Ahmed Elsaied, **Tel.:** +201019509962, **E-mail:** maelsaied@mans.edu.eg

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INTRODUCTION

Approximately 15 percent of couples are infertile, and among these couples, male factor infertility accounts for approximately 50 percent of the causes[1].

Genetic abnormalities are found in about 5–10% of oligozoospermic and in 15–20% of azoospermic cases[2]. Including numerical and structural chromosomal abnormalities and AZF microdeletions[3,4].

Klinefelter syndrome (KS) is one of the most common chromosomal abnormalities in humans, occurring in 1:600 males[5].Y chromosome microdeletions are the second most frequent cause of genetic male infertility after KS, grouped in three main regions (AZFa, AZFb and AZFc) on the Yq arm. About 10% of men with non-obstructive azoospermia (NOA) and 5% of severely oligozoospermic men (SOZ) show Y chromosome microdeletions[6,7]. Few studies evaluated AZF deletions in Egyptian infertile males, however, most of them had few numbers with only azoospermic patients included[8 -10].

This work aimed to estimate the frequency of chromosomal abnormalities and AZF microdeletions among Egyptian infertile males with NOA and SOZ.

PATIENTS AND METHODS

In the period from July, 2011 to June, 2014, a total of 1127 infertile male patients admitted to Andrology-Outpatient Clinic, Mansoura University Hospital, Mansoura, Egypt, were enrolled in this research. They were classified as 653 NOA and 474 SO patients (sperm count less than 5 million/ml).

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Patients with possible etiological factors for male infertility including past or present history of testicular malignancy, radiotherapy or chemotherapy, varicocele, trauma, mumps orchitis, testicular maldescent and orchidectomy were excluded. Obstructive azoospermic patients diagnosed by signs of obstruction (e.g. enlarged or nodular epididymis; nodular, beaded or absent vas), semen analysis (e.g. low semen volume, acidic pH, absent coagulum and low level of semen markers including fructose, alpha-glucosidase and spermatogenic cells) were excluded. Trans-rectal ultrasound (TRU) was performed in case of doubt.

All subjects underwent a comprehensive examination, including a detailed history taking and physical examination. Semen analysis was performed according to WHO laboratory manual for the examination of human semen (1999)[11] on two occasions at 3 weeks interval, following 3- 5 days of sexual abstinence. Azoospermia was verified in at least 3 ejaculates from each patient by pellet analysis after semen centrifugation (3000 rpm for 15 minutes).

All patients underwent a scrotal color duplex examination to evaluate testicular volume, scrotal contents and varicocele. Testicular volume was determined using 7.5-MHz transducers (Medison Sonoace X8, Samsung Medison Co, Ltd, Seoul, Korea). The ultrasonographic testicular volumes were calculated as the length × width × depth × 0.71[12].

All patients underwent hormonal evaluation of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone (T) and estradiol (E2) using a chemiluminescent immunoassay system (Immulite 1000; Siemens Medical Solutions and Diagnostics, Los Angeles, California). Assessment of free testosterone was done for KS patients (127) by (Stat Fax303 Plus, USA) ELISA reader, using Diagnostics Biochem Canada (DBC) Kit. Normal ranges were as follows; FSH = 0.7 -11.1 mIU/ml; LH= 0.8- 7.6 mIU/ml; Prolactin =2.5 -17 ng/ml; Testosterone (total) = 2451600- ng/dl; Testosterone (free) = 9.3 -26.5 pg/ml; Estradiol = 0.0 -56 pg/ml.

Chromosomal analysis was performed in all 1127 cases. All samples were subjected to phytohemagglutinin-stimulated whole blood culture in RPMI-1640 media according to standard procedures. Twenty G-banded metaphases were analyzed from each patient. However, in case of mosaicism or presence of any abnormal cell among previous 20 metaphases was suspected, the number was increased to 30 then up to 50 metaphases to confirm the abnormality. Chromosomes were arranged and prepared for analysis according to the international system for human cytogenetic Nomenclature (ISCN)[13].

AZF microdeletions were done for 811 patients (653 NOA and 158 SOZ patients). Genomic DNA was obtained from peripheral blood leukocytes. Microdeletions of the Y chromosome were screened by multiplex polymerase chain reaction (PCR) using Wizard genomic DNA purification kit (#A1120, Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. The European Academy of andrology and the European Molecular Genetics Quality Network (EAA/EMQN) guidelines recommend the use of six STS loci (sY84, sY86, sY127, sY134, sY254, sY255) to detect up to 95% of all reported Y-chromosome microdeletions in the AZF regions. Additional STS loci can then be used to define the deletion breakpoints further[14].

Multiple PCR amplifications of 25 loci covering AZFa, AZFb, AZFc regions on Y-chromosome were used. The following loci were screened: ZFY in the PAR region; SY81, SY86, SY84, DFFRY, SY182 in the AZFa region; SY1235, SY113, SY118, SY127, SY130, SY134, SY143 in the AZFb region; SY1161, SY145, SY148, SY1291, SY152, SY153, SY254, SY255, SY157, SY158 in the AZFc region; and SY160 in the Yq12 heterochromatic region. The SRY gene (testis-determining factor on the short arm of the Y chromosome) was included in the analysis as an internal control for the presence of Y-specific AZF sequences.

Multiplex fluorescent PCR primer mix included PCR mix with buffer, dNTP and MgCl2, Taq polymerase, 30 ng DNA sample, and H2O in a final volume of 25 µl. The PCR program was set at 95°C for 10 min, followed by 35 cycles at 94°C for 60 sec, 60°C for 60 sec, and 72°C for 60 sec, thereafter one cycle of 45 min at 65°C for final extension. In each reaction, one sample of fertile male genomic DNA as positive, one sample of female DNA and one sample of distilled water without DNA were used as controls for each set of primers. After amplification, PCR products were subjected to capillary electrophoresis on genetic analyzer (thermal cycler). A STS was considered absent only after at least two amplifications failure in the presence of successful amplification of control (SRY-sY14). The study was approved by the Research Ethics Committee (REC) for experimental and clinical studies at Faculty of Medicine, Mansoura University, Mansoura, Egypt. The importance of the study was explained to all participants and an informed written consent was obtained from all subjects before performing the study. The study was conducted according to the Declaration of Helsinki Principles.

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, Chicago, Illinois) for Windows 17.00. The parametric data were expressed as mean, and standard deviation. The nonparametric data were expressed as median, and range. Mann-Whitney test was used as a test of significance for comparison of two groups. The qualitative data were presented in the form of number and percentage. Spearman rank correlation

coefficient was used for evaluation of correlations between variables. Multiple stepwise logistic regression was used to determine the most significant predictors for AZF microdeletions. Differences were considered statistically significant at *P* value < 0.05.

RESULTS

This study included 1127 infertile male; 653 (57.9%) NOA and 474 (42.1%) SOZ patients. Left and right testicular volume were significantly lower in NOA than SOZ. NOA patients showed significantly higher FSH and LH and significantly lower total testosterone than SOZ patients (Table 1).

The frequency of chromosomal abnormalities was 162/ 1127 (14.4%), higher in NOA than in SOZ men (22.6% versus 3.7% respectively). Numerical chromosomal abnormalities were higher than structural type (11.8%) versus 2.4%). KS represented 11.2% (127 /1127) of the total chromosomal and 94.1% (127 /135) of sex chromosomal abnormalities. Chromosomal translocations were found in 20/ 1127 (1.8%), chromosomal inversions 646 ,(0.5%) 1127/XX male 547 ,(0.4%) 1127/XYY 2/ 1127 (0.2%) and marker chromosome 2/ 1127 (0.2%).

The frequency of sex chromosomal abnormalities was higher than autosomal type (11.9% versus 1.8%), higher in NOA than SOZ (20.5% versus 0.2%), while the autosomal abnormalities were higher in SOZ than NOA (2.9% versus 1.1%, respectively). Chromosomal translocation was the most frequent autosomal abnormality, 16/ 21(76.1%) (Table 2).

Left and right testicular volumes were significantly higher in patients with normal karyotype, 46, XY (965) than those with abnormal karyotype (162).

Patients with abnormal karyotype showed lower total T and higher FSH and LH than those with normal karyotype (Table 3).

AZF microdeletions were the most common genetic abnormality after KS (5.5% versus 11.2%, respectively). AZF microdeletions were higher in NOA than in SOZ (6.1% versus 3.16%, respectively). AZFc microdeletions represented the most frequent finding, 31/ 45 (68.9%), followed by AZFbc, 7/ 45(15.6%), AZFb, 4 /45 (8.8%) and AZFa, 3 /45 (6.7%). All patients with AZFa (3), AZFb (4) and AZFbc (7) deletions were NOA, while 26 /31(83.87%) with isolated AZFc deletion were NOA and 5/ 31(16.13%) were SOZ (Table 4).

Testicular volume was lower but not statistically significant in patients with AZF microdeletions than those without. Patients with AZF microdeletions showed statistically significant higher serum FSH and LH than those without microdeletions (Table 5).

Using multiple stepwise logistic regression, age, duration of infertility, sperm count, serum FSH, LH, PRL, T and E2, left and right testicular volumes could not predict either karyotype abnormality or AZF microdeletion (Table 6 and 7 respectively).

Table 1: Patients' characteristics and the results of testicular volume and hormonal analyses.

Group (Number)	NOA (653)	SO (474)	P
Age (years) mean ±SD range	31.44±6.43 (20.00- 55.00)	29.97±4.95 (20.00-50.00)	<0.001
Duration of infertility (years)	4.26±4.50 (1.00-25.00)	2.86±2.44 (1.00-15.00)	<0.001
Lt. testis volume (cm3)	7.42 ±4.74 (0.25- 20.00)	12.94±3.01 (4.00-19.00)	<0.001
Rt. testis volume (cm3)	8.13 ±4.88 (0.50- 21.00)	13.76±3.01 (5.00-20.00)	<0.001

FSH (mIU/ml)	18.41±14.46 (1.00-77.00)	7.62 ±6.42 (0.90-39.00)	<0.001
LH (mIU/ml)	9.85 ±6.91 (0.60-49.00)	5.86 ±3.21 (1.00-32.00)	<0.001
PRL(ng/ml)	10.58±6.75 (2.00-82.00)	11.13 ±5.96 (2.00-29.00)	0.155
Total T(ng/dl)	387.64 ±196.87 (17.00-987.00)	500.22 ±211.06 (137.00-996.00)	<0.001
E2 (pg/ml)	25.99 ±23.01 (1.00-480.00)	26.38 ±14.20 (4.60-93.00)	0.740

Table 2: Numerical and structural chromosomal results of the studied patients

Chromosomal findings		NOA (653)	SOZ (474)
Normal	Normal 46,XY	495/653 (75.8%)	456/474 (96.2%)
Numerical abnormalities	Klinefelter (KS) 47,XXY	118/653 (18.1%)	0/474 (0.0%)
	Mosaic(KS) 47,XXY/46,XY	9/653 (1.3%)	0/474 (0.0%)
	47,XYY	1/653 (0.1%)	1/474 (0.2%)
	Total	128/653 (19.6 %)	1/474 (0.2%)
Structural abnormalities	46,XX	5/653 (0.7%)	0 / 474 (0.0%)
	Inversion	4/653 (0.6%)	2/474 (0.4%)
	Translocation	7/653 (1.1%)	13/474 (2.7%)
	Marker chromosome	0/653 (0.0%)	2/474 (0.4%)
	Total	16/653 (2.5%)	17/474 (3.5%)
Total		144/653 (22.1 %)	18/474 (3.7 %)

Table 3: Comparison of patients according to the karyotyping

Group	Normal karyotype (965) Mean ± SD Range	Abnormal karyotype (162) Mean ± SD Range	P
Lt. testis volume (cm3)	10.91±4.14 (2.00-20.00)	2.90±3.66 (0.25-13.00)	<0.001*
Rt. testis volume (cm3)	11.70±4.21 (2.00-21.00)	3.47±3.80 (0.50-15.00)	<0.001*
FSH (mIU/ml)	10.91 ±9.970 (0.90-59.00)	28.27±15.66 (2.00-77.00)	<0.001*
LH (mIU/ml)	6.79 ±4.42 (1.00-47.00)	16.44±8.62 (2.00-49.00)	<0.001*
Prolactin(ng/ml)	10.94±6.30 (2.00-82.00)	9.96± 6.98 (2.00-44.00)	0.066
Total T (ng/dl)	460.88 ±204.04 (17.00-996.00)	277.17±154.35 (41.00-968.00)	<0.001*
E2 (pg/ml)	25.84 ±19.669 (3.00-480.00)	27.11±17.44 (1.00-139.00)	0.510

Table 4: AZF microdeletions among studied patients

Type	NOA (653)	SOZ (158)	Total (811)
Normal AZF	613/653 (93.8%)	153/158 (96.9%)	766/811 (94.4%)
AZF a deletions	3/653 (0.4%)	3/653 (0.4%)	3/811 (0.4%)
AZF b deletions	4/653 (0.6%)	0/158 (0.0%)	4/811 (0.5%)
AZF c deletions	26/653 (3.9%)	5/158 (3.16%)	31/811 (3.8%)
AZF bc deletions	7/653 (1.07%)	0/158 (0.0%)	7/811 (0.9%)
Total AZF deletions	40/653 (6.1 %)	5/158 (3.16 %)	45/811 (5.5 %)

Table 5: Comparison of patients according to AZF microdeletions

Group	Normal (766)	AZF microdeletion (45)	P
Age (year)	31.15±6.25 (20.00-55.00)	30.16±5.54 (23.00-47.00)	0.385
Lt. testis volume (cm3)	8.65±1.68 (7.00-13.00)	8.44±5.03 (0.25-20.00)	0.495
Rt. Testis volume (cm3)	9.58±1.82 (7.00-14.00)	9.18±5.17 (0.50-21.00)	0.246
Semen volume(mlL)	2.74±0.96 (2.00-5.00)	1.68±0.71 (0.10-7.00)	0.730
Sperm count (million/ml)	0.34±0-.74 (0.00-4.00)	0.23±0.55 (0.00-1.90)	0.429
FSH (mIU/ml)	13.13±6.97 (1.00-29.00)	16.71±14.11 (1.00-77.00)	0.011*
LH (mIU/ml)	7.29±3.61 (1.00-19.00)	9.22±6.65 (0.60-49.00)	0.004*
Prolactin (ng/ml)	10.69±6.58 (2.00-82.00)	10.42±5.71 (3.00-22.00)	0.842
Total T (ng/dl)	433.48±211.49 (146.0- 985.0)	418.37±210.31 (17.00-996.00)	0.659
E2 (pg/ml)	26.45±22.16 (1.00-480.00)	24.39±11.64 (11.00-59.00)	0.605

Table 6: Multiple stepwise logistic regression analysis of different variables to predict karyotype abnormality.

Predictor	B	Exp(B)	95.0% CI for EXP(B)		P
			Lower	Upper	
Age (year)	-0.022	0.979	0.925	1.035	0.448
Duration of marriage (year)	-0.010	0.990	0.916	1.070	0.794
Semen volume (mlL)	-0.024	0.966	0.897	1.051	0.561
Semen count (million/mlL)	-0.438	0.684	0.314	1.032	0.163
FSH (mIU/ml)	-0.002	0.992	0.871	1.013	0.745
LH (mIU/ml)	-0.115	0.871	0.659	1.307	0.431

PRL (ng/ml)	-0.010	0.990	0.951	1.030	0.612
Total T (ng/dl)	-0.029	0.972	0.871	1.108	0.760
E2 (pg/ml)	0.006	1.006	0.997	1.014	0.182
Lt. Testis volume (cm^3)	-0.310	0.733	0.461	1.167	0.191
Rt. Testis volume (cm^3)	-0.216	0.805	0.514	1.262	0.345

B: B coefficient; Exp (B): exponential B; CI =Confident interval

Table 7: Multiple stepwise logistic regression analysis of different variables to predict AZF microdeletion.

Predictor	B	Exp(B)	95.0% CI for EXP(B)		P
			Lower	Upper	
Age (year)	-0.025	0.976	0.898	1.060	0.562
Duration of marriage (year)	-0.019	0.981	0.860	1.118	0.771
Semen volume (mlL)	-0.127	0.880	0.637	1.217	0.440
Semen count (million/mlL)	-0.236	0.789	0.415	1.501	0.471
FSH (mIU/ml)	-0.033	0.967	0.911	1.026	0.269
LH (mIU/ml)	-0.002	0.998	0.893	1.115	0.969
PRL (ng/ml)	-0.013	0.987	0.929	1.049	0.669
Total T (ng/dl)	0.000	1.000	0.998	1.002	0.910
E2 (pg/ml)	-0.003	0.997	0.972	1.024	0.846
Lt. Testis volume (cm^3)	-0.538	0.584	0.324	1.051	0.173
Rt. Testis volume (cm^3)	0.395	1.485	0.839	2.627	0.175

B: B coefficient; Exp (B): exponential B; CI =Confident interval

DISCUSSION

The frequency of chromosomal abnormalities, in our study, was 14.4%, higher in NOA than in SOZ men (22.1% versus 3.8%, respectively). These results were comparable to the previously reported range of 2.02–22.2% for infertile men[2,4,15 -18,19- 36].

The lower frequency (2.02%) reported by Clementini *et al*.[19] was due to the heterogeneity of their studied patients and inclusion of mild oligozoospermic with sperm count ranging from five to 20 million/ml. while Dada *et al*.[2] reported higher frequency (22.2%) by including only azoospermic and severe oligozoospermic (sperm count less than 5 million/ml). The higher frequency of chromosomal abnormalities in our NOA than SOZ men (22.6% versus 3.7%, respectively) was in agreement with the results of other studies suggesting that the prevalence of chromosomal abnormalities increases as the sperm count decreases[18,20,21,23,25,32,35,37].

In our research, Tthe frequency of numerical chromosomal abnormalities in our study iwas higher than the structural type (11.8% versus 2.4%). Several studies[4,20,28,31,33,38,39] reported similar result[4,20,28,31,33,38,39]. However, in other studies, the frequency of structural abnormalities was higher than numerical ones[18, 32]. These differences might be due to ethnic differences, different patient selection criteria and methodological aspects.

The sex chromosome abnormalities are higher in men with NOA than SOZ (20.5% versus 0.2%, respectively), while the autosomal abnormalities are higher in men with SOZ than NOA (2.9% versus 1.1%, respectively) as shown in (Table 2). These results supports the previous notion that sex chromosome abnormalities are more frequent in men with azoospermia, while autosomal abnormalities are more frequent in oligozoospermic men[25,32,35,37,40 -42]. KS is the most frequent sex chromosome abnormality among our patients, 127/ 135(94.1%), and this is in accordance with other studies[4,18,23,33,34,43,44].

Chromosomal translocations are the most frequent autosomal abnormality, 16/ 21(76.1%), which is in agreement with many studies[34,37,45].

The autosomal abnormalities were 3.5% compared with 0.42% of people within the general population. In addition, infertile males were reported to have rearrangements of sex chromosomes by karyotyping of 1.7%, compared with 0.10% among the general male population[46].

After KS, AZF microdeletions in this study is are the most common genetic abnormality (5.5%) among our study groups after KS (11.2%), (Table 4). This result is within the previously reported range of 0.98% to 55.5% for infertile men in several studies[4,14,24,25,27,28,31,34,47- 61].

The low frequency of AZF microdeletions (0.98%) reported by Van der Ven *et al*.[47] was due to the heterogeneity of the studied patients and together with inclusion of mild oligozoospermic (sperm count from 5 to 20 million/ml) and severe teratozoospermic patients with sperm counts >20 million/ml. The higher frequency (55.5%) reported by Foresta *et al*.[62] might be attributed to inclusion of only azoospermic patients with Sertoli cell only in their testicular histology and the low number of studied patients.

In this study, the frequencies of AZF microdeletions were 6.1% (40/ 653) and 3.16% (5158/) in NOA and SOZ, respectively shown in , (Ttable 4). These results are within the previously reported in several studies, whereas the range of 2.13- 15.00% reported for men with NOA and 0.00- 13.1% for SOZ in several studies[24,25,27,31,49,52,54,55,59,61,63]. and withBesides, the European Association of Urology Guidelines on Male Infertility (The 2012 update) who reported that azoospermic men had the highest frequency of classical Y microdeletions (8 -12%) followed by those with oligozoospermia (3- 7%)[64].

This wide range of deletion frequency might be due to ethnic differences, different patient selection criteria and methodological aspects including the type and number of markers used.

This study foundOur research revealed no significant difference in the frequency of AZF deletions between the NOA and the SOZ groups (6.1% versus 3.16 %, *P*=0.145) as in (Ttable 4, consistent with). This result is in agreement with Ng *et al*.[25] who reported no significant difference (8.5% versus 8.2%). HoweverOn the other hand, Kumtepe *et al*. [24] and Ambulkar *et al*.[61] reported a significant difference in the frequency of AZF deletions between the NOA and SOZ groups (9.51% versus 1.86%) and (13.1% versus 5.2%, respectively).

AZFc deletion in our study is the predominant type of Y-microdeletions (31/ 45=68.8%) in our research (Table 4). It represents 66% (2640/) of AZF deletions in NOA and 100% (55/) in SOZ. This result is comparable to the previously reported range (46.687.1%-) for AZFc deletion in several studies from different populations[9,24,25,51,53,55,57,65]. In contrast to these studiesHowever, Mafra *et al*.[28] reported no AZFc deletions in their Brazilian NOA men.

In this study, the AZFc deletion frequency was represents 68.8% (31 /45) followed by the AZFbc

region 15.5% (7/ 45), AZFb 8.8% (4/ 45) and AZFa 6.6% (3/ 45) illustrated in (Table 4,). These results which were similar to other studies[8,9,30,55]. On the other hand, Mohamed *et al*.[20] found that the AZFbc combination regions was the most frequently deleted regions (5, 71.4%) followed by AZFc (2, 26.8%) with no separate microdeletions in either AZFb and/or AZFa in their Kuwaiti infertile males from Kuwait. In addition, Saeed *et al*.[10] found that AZFbc (22 /7 4, 28.37%) to beas the most frequently deleted regions followed by AZFb (16 /74, 21.63 %) and then AZFc constituted (11 /74, 16.21 %), AZFab (2/ 74, 2.7 %), one patient had AZFac (1/ 74, 1.35%) and four patients showed AZFabc (4 /74, 5.4 %). This difference may be due to ethnic background & and the low number of studied patients in their study.

It is still not clear why the AZFc deletion is so frequent, but repetitive gene clusters in this region could be a cause. Beside DAZ (Deleted in azoospermia) genes, chromodomain Y 1 (CDY1), basic protein Y 2 (BPY2), PTA-BL related Y (PRY), and testis transcript Y 2(TTY2) have repetitive sequences in the AZFc region. Intrachromosomal recombination events among these repetitive sequence blocks might lead to the abundant AZFc microdeletions[1].

In the current work, Aall patients with AZFa (3), AZFb (4) and AZFbc (7) deletions are azoospermic, while 83.3% (26/ 31) of men with isolated AZFc deletion are azoospermic and 16.2% (5/ 31) are severe oligozoospermic (Table 4). These results are iIn agreement with Hopps *et al*.[66] who reported thatwhereas all men with AZFa (3), AZFb (11) and AZFbc (16) deletions were azoospermic, while 26/ 42 (62 %) of men with isolated AZFc deletion were azoospermic. These results support that deletions in the AZFa, AZFb or AZFbc regions usually lead to azoospermia and AZFc deletions lead to azoospermia or severe oligozoospermia[67, 68]. We did not include a control group of normozoospermic fertile men in our study due to financial issues. However, in a study ofa previous research on Egyptian azoospermic patients, the normozoospermic fertile men had no detected AZF deletions using the same technique[9].

Testicular volume in patients with AZF microdeletions is lower but not statistically significant than those without AZF microdeletions and both were below normal (Table 5). Other groups [61,69,70] reported similar to the revealed in previous studies[61,69,70].results.

FSH level is significantly higher in patients with AZF microdeletions compared with those without (Table 5), consistent with. Mirfakhraie *et al*.[71]. reported the same result. In addition, other Other studies[61,69,72,73] reported higher but not statistically significant FSH concentration in men with AZF microdeletions compared with those without microdeletions[61,69,72,73]. However, FSH values did not indicate presence or absence of spermatogenesis in cases with microdeletions[74].

Testicular volume is significantly lower and FSH and LH are significantly higher in patients with chromosomal abnormalities than those without (Table 1). These results are in agreement with A like, Koşar *et al*.[3] who reported smaller testicles and higher serum FSH and LH levels in patients with chromosomal abnormalities[3].

Our patients with chromosomal abnormalities showed significantly lower testosterone than those without (Table 1). consistent with the findings of Zhang *et al*.[29]. This is The high percentage of KS (78.3%) with lower serum testosterone among our patients with chromosomal abnormalities could explain this result.

In spite of that serum FSH, LH levels and testicular volume might have prognostic implications on testicular function[75]., In the current study, multiple stepwise logistic regression in this study could not predict either chromosomal abnormalities or AZF deletions (Table 6, 7). This is in agreement with previous the results of other studies[3,29] who reportedreporting that FSH, LH and testicular volume did not predict any chromosomal abnormalities[3,29]. In addition, other studies[75,76] showed that no clinical parameter would help to identify patients with Y- chromosome microdeletions[75,76].

CONCLUSION

We Our results emphasize the importance of karyotyping and AZF microdeletions analysis in NOA and SOZ patients. Counseling for such patients before ICSI is warranted to decrease the risk of transmitting genetic abnormalities to their offspring.

CONFLICT OF INTEREST

There are no conflicts of interest.

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