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Chromosomal evaluation in a group of Tunisian patients with non-obstructive azoospermia and severe oligozoospermia attending a Tunisian cytogenetic department



Anomalies chromosomiques et infertilité masculine : étude rétrospective de 476 hommes tunisiens azoospermiques ou oligozoospermiques sévères

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ABSTRACT

Male infertility is the cause in half of all childless partnerships. Numerous factors contribute to male infertility, including chromosomal aberrations and gene defects. Few data exist regarding the association of these chromosomal aberrations with male infertility in Arab and North African populations. We therefore aimed to evaluate the frequency of chromosomal aberrations in a sample of 476 infertile men with non-obstructive azoospermia ($n = 328$) or severe oligozoospermia ($n = 148$) referred for routine cytogenetic analysis to the department of cytogenetics of the Pasteur Institute of Tunis. The overall incidence of chromosomal abnormalities was about 10.9%. Out of the 52 patients with abnormal cytogenetic findings, sex chromosome abnormalities were observed in 42 (80.7%) including Klinefelter syndrome in 37 (71%). Structural chromosome abnormalities involving autosomes (19.2%) and sex chromosomes were detected in 11 infertile men. Abnormal findings were more prevalent in the azoospermia group (14.02%) than in the severe oligozoospermia group (4.05%). The high frequency of chromosomal alterations in our series highlights the need for efficient genetic testing in infertile men, as results may help to determine the prognosis, as well as the choice of an assisted reproduction technique. Moreover, a genetic investigation could minimize the risk of transmitting genetic abnormalities to future generations.

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R É S U M É

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On estime à près de 10 % la fréquence de l'infertilité masculine dans la population humaine. Les causes de cette infertilité sont multiples, notamment génétiques, et plus particulièrement chromosomiques. Dans ce travail, nous avons exploré 476 patients tunisiens présentant des troubles non obstructifs de la spermatogenèse (328 azoospermiques et 148 ayant une oligospermie sévère). Une étude du caryotype constitutionnel est réalisée chez l'ensemble de ces patients, avec marquage chromosomique en bandes RHG. Nous avons pu révéler ainsi 52 anomalies chromosomiques (fréquence 10,9 %), réparties en 46 anomalies gonosomales et 6 anomalies autosomiques. La fréquence de ces anomalies est plus élevée dans le groupe des azoospermies, chez qui la constitution 47,XXY prédominait. Les autres anomalies étaient autosomales, correspondant à des translocations, une inversion et des chromosomes marqueurs surnuméraires. Nos résultats confirment la forte prévalence des anomalies chromosomiques chez les hommes atteints de troubles sévères de la spermatogenèse. Nos chiffres sont comparables à ceux décrits dans la littérature, incitant à la considération de l'étude cytogénétique au cours du bilan d'infertilité masculine et justifiant la pratique systématique du caryotype avant toute tentative de procréation médicalement assistée.
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1. Introduction

Infertility is a major health problem affecting up to 15% of couples of reproductive age [1]. For many years, it was assumed that most reproductive problems could be attributed to the female partner, but research in recent years has demonstrated that 30–50% of infertility is caused by a male factor [2].

The term “male infertility” does not constitute a defined clinical syndrome, but rather a collection of different conditions exhibiting a variety of etiologies. Genetic factors accounts for 10–15% of severe male infertility [3,4]. Among the genetic abnormalities found in infertile men, those involving chromosome anomalies amount to about 8%, the most frequent one being the 47,XXY karyotype that characterizes the Klinefelter Syndrome [5]. Because modern artificial reproduction techniques like intracytoplasmic sperm injection (ICSI) can help couples to overcome infertility, it is imperative to analyze the underlying genetic causes of male infertility.

The aim of this study was to determine the frequency of chromosomal abnormalities in a group of Tunisian infertile men attending the Pasteur Institute of Tunis.

2. Materials and methods

2.1. Patients

A total of 476 Tunisian infertile patients with idiopathic oligozoospermia or azoospermia were enrolled in the study. These infertile men with sperm disorders were referred for karyotyping to the department of histology and cytogenetics at the Pasteur Institute of Tunis between 2006 and 2012. Patients were checked for the history of relevant medical disorders, e.g., diabetes, renal, liver disease, radiation, endocrine abnormality (e.g., hypogonadotropic hypogonadism), exposure to toxins and/or medication affecting spermatogenesis, acquired and congenital structural defects of urogenital system; history of surgical

intervention of genital tract. All cases of azoospermia or severe oligozoospermia resulting from endocrine or obstructive cases were excluded from our study. Upon verifying that sperm density was lower than $5 \times 10^6/\text{mL}$, patients were asked to sign and informed consent form for genetic analysis.

2.2. Karyotyping

Cytogenetic analysis was performed from phytohemagglutinin-stimulated lymphocyte cultures by routine laboratory protocol. For microscopic analysis, R-banded metaphase spreads were analyzed and abnormalities recorded according to the current International System for Human Cytogenetic Nomenclature [6]. A resolution of 550 to 700 bands per haploid karyotype was used for the routine analysis.

For each patient, at least 20 well-spread metaphases were analyzed and two to five metaphases were karyotyped. When at least one of the 20 showed a loss or gain of a chromosome, especially X or Y chromosome, the number of analyzed metaphases was increased to 30. If a second abnormal cell was observed, the analysis was considered complete; otherwise, the number of metaphases was increased to 50. Sex chromosome mosaics occurring at a level of less 5% were not considered as well as pericentric inversions of chromosome 9 or other structural chromosome variants and polymorphisms that were considered as normal cytogenetic events.

The statistical analysis was performed using the χ^2 test. A P -value < 0.05 was considered to indicate statistical significance.

3. Results

The present study only entailed 476 men with non-obstructive subfertility. They included men with azoospermia ($n = 328$; 68.91%) and severe oligozoospermia with sperm counts lower than 5 million/mL ($n = 148$; 31.09%).

Table 1

Type and frequency of chromosomal anomalies in 476 Tunisian infertile men with non-obstructive azoospermia and severe oligozoospermia.

	% (No. of men/total No.)		
	Non-obstructive azoospermia	Severe oligozoospermia	Overall those with counts < 5 million/mL
Sex chromosome aberrations			
47,XXY	9.4 (31/328)	0.67 (1/148)	6.7 (32/476)
Mosaic 47,XXY:	1.2 (4/328)	0.67 (1/148)	1 (5/476)
*46,XY/47,XXY	3	1	4
*47,XXY(85%)/48,XXXY(15%)	1	—	1
47,XXY	0.3 (1/328)	0 (0/148)	0.2 (1/476)
45,X(15%)/46,XY(85%)	0.3 (1/328)	0 (0/148)	0.2 (1/476)
46,X,del(Y)(q11.2 qter)	0.3 (1/328)	0 (0/148)	0.2 (1/476)
XX males (46,XX)	0.6 (2/328)	0 (0/148)	0.4 (2/476)
Subtotal	12.19 (40/328)	1.35 (2/148)	8.8 (42/476)
Autosomal chromosome abnormalities			
Reciprocal translocation	0.9 (3/328)	0.67 (1/148)	0.8 (4/476)
*46,XY,t(4;6)(p12;p22)	(1)	—	1
*46,XY,t(7;16)(p11;p13)	1	—	1
*46,XY,t(4;17)(q11;p11)	1	—	1
*46,XY,t(1;17)(q21;p12)	—	1	1
Robertsonian translocation	0.3 (1/328)	0.67 (1/148)	0.4 (2/476)
45,XY,der(13;14)(q10;q10)	—	—	—
Inversion: 46,XY,inv(7)(q22;q35)	0.3 (1/328)	0 (0/148)	0.2 (1/476)
Supernumerary marker chromosomes	0.3 (1/328)	1.35 (2/148)	0.6 (3/476)
*47,XY,+mar	—	(1)	(1)
*46,XY/47,XY,+mar	(1)	(1)	(2)
Subtotal	1.8 (6/328)	2.7 (4/148)	2.1 (10/476)
Total	14.02 (46/328)	4.05 (6/148)	10.92 (52/476)

No.: number.

The average age was 37.94 ± 3.00 . The average duration of infertility was 5.05 ± 4.03 .

As summarized in Table 1, 52 infertile patients tested to rule out cytogenetic causes of male infertility had an abnormal karyotype including: (37) 47,XXY and variants consistent with Klinefelter syndrome (KS), (1) 47,XXY, (1) Yq deletion, (2) 46,XX males, (7) balanced autosomal rearrangements, and (4) unbalanced rearrangements. Sex chromosome abnormalities accounted for 42 (or 80.7%) of all abnormal karyotypes detected (Table 1). The most frequent sex chromosome abnormality observed was 47,XXY or variant karyotype (mosaic 47,XXY/46,XY, 47,XXY/48,XXXY) consistent with KS. Of autosomal abnormalities, balanced rearrangements are identified in 7 males including 4 with balanced reciprocal translocations, 2 balanced Robertsonian translocations between chromosome 13 and chromosome 14 and 1 with pericentric inversion of chromosome 7. An unbalanced rearrangement was identified in the five last patients (a supernumerary marker chromosome in a low level mosaic [3 out of 20 metaphases] in two patients whether another oligozoospermic patient have this marker chromosome in all cells, one man had three cells with a 45,X constitution and the last have a deletion of the long arm of the Y chromosome). The remaining 424 infertile patients showed normal 46,XY karyotype.

4. Discussion

Although it has long been recognized that among numerous etiologic factors, chromosomal abnormalities play a prime role in male infertility with abnormal semen

parameters, reports regarding the prevalence of cytogenetic anomalies in Tunisian population are scarce [7,8].

Previous studies [9] from different populations have shown that the incidence of chromosomal abnormality in infertile males was between 2.2% and 19.6%. In the present study, the frequency of chromosomal aberrations (10.92%) among infertile men (non-obstructive azoospermic and severe oligozoospermic men) was found to lie within the previously reported range (4.34% [10] – 19.73% [8]).

The frequencies of chromosomal aberration in our patient sample are in agreement with other studies, where an increase in chromosomal abnormalities with decreasing sperm counts has been documented. It is well known that the sperm count is inversely related to the existence of chromosomal anomaly [11]. It is consistent with our study where, the proportion of chromosomal anomalies in non-obstructive azoospermic men (14.02%) was significantly ($P < 0.05$) higher than in severe oligozoospermic men (4.05%). Reported frequencies of chromosomal aberrations range from 3% to 19%: 3% in the cases of mild infertility and 19% in men with non-obstructive azoospermia [12]. Table 2 lists the results of studies of the frequency of chromosomal anomalies in infertile men in Tunisia and some regions of Europe, America and Asia. In order to ensure comparability, all of the articles included in this table were selected to contain the same study objects as the current study (i.e., non-obstructive azoospermia and severe oligozoospermia with a sperm count < 5×10^6 /mL) [13–19].

In the group of azoospermic patients, sex chromosome abnormalities such as 47,XXY and 46,XX were predominant. We found 10.6% (35/328) of patients with Klinefelter

Table 2
Comparison of chromosomal anomalies between this study and other similar studies.

Authors	Regions	No. of cases	Frequencies %	Prevalence of chromosomal aberration % (No. of men/total No.)	
				Non-obstructive azoospermia	Severe oligospermia counts < 5 million/mL
Tuerlings <i>et al.</i> , 1998 [13]	Netherlands	968	3.51	6.45% (4/62)	3.47% (30/865)
Nagvenkar <i>et al.</i> , 2005 [14]	India	88	10.22	14.29% (6/42)	6.52% (3/46)
Mohammed <i>et al.</i> , 2007 [15]	Kuwait	289	7.95	19.44% (21/108)	1.10% (2/181)
Ng <i>et al.</i> , 2009 [16]	Hong Kong	295	5.08	21.1% (5/71)	4.46% (10/224)
Kosar <i>et al.</i> , 2010 [10]	South of Turkey	115	4.34	5.43% (5/92)	0% (0/23)
Alkhalaf <i>et al.</i> , 2010 [17]	Kuwait	142	18.30	^a	^a
Mafra <i>et al.</i> , 2011 [5]	Brazil	143	6.29	11.62% (5/43)	4% (4/100)
Ghorbel <i>et al.</i> , 2012 [8]	Tunisia	76	19.73	22.22% (12/54)	13.63% (3/22)
Zhang <i>et al.</i> , 2012 [18]	Northeast China	135	14.07	17.28% (14/81)	9.26% (5/54)
Cavkaytar <i>et al.</i> , 2012 [19]	Turkey	332	7.23	11.22% (22/196)	1.47% (2/136)
Our study	Tunisia	476	10.92	14.10% (46/328)	4.05% (6/148)

No.: number.

^a Missing or not precise data.

syndrome among those with azoospermia, and 1.35% (2/148) among those with severe oligozoospermia. Ferlin *et al.* reported that the frequency of KS among infertile men is up to 10% in azoospermia group and 5% in severe oligozoospermia group, and occurs in approximately 0.1–0.2% newborn males [4]. The large majority of subjects affected by this syndrome is azospermic and would not be able to naturally conceive [20]. However, testicular sperm extraction (TESE) and especially microsurgical TESE (micro-TESE) followed by ICSI with an average of 30–50% of testicular sperm recovery rate, may allow Klinefelter patients to generate their own genetic children [21]. Nevertheless, Klinefelter syndrome patients risk producing offspring with chromosomal abnormalities [22]. This fear was substantiated by several studies that observed that KS patients have large numbers of aneuploid gametes. FISH analysis has demonstrated that the frequency of aneuploidy for the sex chromosomes varies from 1.5% [23] to 7% [24] in sperm from Klinefelter mosaics, and 2% [25] to 45% [26] in the sperm of men who appear to have a non-mosaic 47,XXY karyotype. The majority of babies born to 47,XXY men have been normal although chromosomally abnormal fetuses have been reported [22,27] studied embryos by preimplantation genetic diagnosis (PGD) and reported a significant fall in the rate of normal embryos for couples with KS in respect to controls (54% versus 77.2%). Even that there appears to be a small increased risk for these men, it is advised that PGD or prenatal diagnosis be performed before ICSI to ensure that the offspring is not aneuploid [28].

In the current study, two patients had XX karyotype. This rare condition was initially named “XX male syndrome”; however, this was revised in 2005 to its current nomenclature of “46,XX testicular disorder of sex development” (DSD) [29]. It represents the most common condition in which testicular development occurs in the absence of a Y chromosome. Occurring at a frequency of 1/25,000 new-borns [30], this

disorder has been reported with an incidence of 0.9% in azoospermic males [31]. Phenotypically the adults are similar to patients with Klinefelter syndrome, with normal male external genitalia, microrchidia and sterility [32]. With 46,XX DSD, the fertility options are limited. The only option currently available is artificial insemination with sperm donation but donor technologies are banned, as in the rest of the Sunni Islamic world [33].

A deletion in the long arm of the Y chromosome was seen in one patient. Studies have indicated that deletions on the long arm of the Y chromosome involving a particular and consistent segment might lead to azoospermia [34] and sometimes to severe oligozoospermia [35]. The long arm of the Y chromosome plays a primary role in the regulation of different stages of spermatogenesis. Loss of genes controlling spermatogenesis (by Y chromosome deletions, rearrangements, microdeletions, or 45,X/46,XY mosaicism) is particularly dramatic in spermatozoa production [35].

The ratio between gonosome and autosome abnormalities differs considerably between the two groups. We note that sex chromosome abnormalities predominate in non-obstructive azospermic men (86.9%; 40/46), whereas autosomal abnormalities are more frequent in patients with severe oligozoospermia (4/6; 66.66%). This is similar to or even higher than the data of the literature [5,16,19].

Autosomal abnormalities were identified in 10 patients (2.1%) (Table 1) of whom, six had balanced autosomal translocations. In the remaining 4 infertile patients, a pericentric inversion of chromosome 7 was found in an azospermic man while a supernumerary marker chromosome was detected in the last 3 men.

A relationship between balanced autosomal translocations and infertility has been reported among severely oligozoospermic and azospermic men [14]. In our study, reciprocal translocations [t(4;6); t(7;16),t(4;17)] were seen in 3 azospermic men and the last [t(1;17)] was

found in one man with azoospermia. Otherwise, two patients had a Robertsonian translocation involving chromosomes 13 and 14 (Table 1).

The effects of chromosomal translocations on spermatogenesis are obvious [36]. Assisted reproductive techniques have given the chance of having a child to infertile males with poor semen quality and autosomal abnormalities. However, using the ICSI in this group may increase the inheritance of paternal genetic disorders to offspring [13] because the structural chromosomal abnormality predisposes to abnormal segregation in meiosis leading to unbalanced gametes, in vitro fertilization IVF/ICSI failure [37] or poor embryonic development after fertilization. Significant heterogeneity was observed in the rates of unbalanced gametes, varying from 2.7% to 26.5% according to the translocations [38]. The risk of meiotic imbalance is primarily determined by the characteristics of the chromosomes involved, and the break-point positions. In addition, Robertsonian translocations can result in offspring with Down syndrome or Patau's syndrome or in gestational loss of a conceptus with monosomy of chromosome 13, 14 or 21, or trisomy of chromosome 14, which are not compatible with life. Sperm karyotyping studies and FISH studies have demonstrated that the frequency of unbalanced sperm in men is lower than theoretically expected, ranging from 7% to 40% of unbalanced spermatozoa, with a mean of 15% unbalanced [39,40]. Because of the risk of passing the translocation to the offspring in unbalanced state, FISH is recommended to have an estimation of the risk for abnormal offspring and to adapt genetic counselling accordingly.

Moreover, it is important to document whether structural chromosomal aberrations in infertile males are 'de novo' or inherited. In case a structural chromosomal aberration is familial and co-segregates with male infertility, this might pinpoint a chromosomal region harbouring one or more genes involved in spermatogenesis. Attempts were made to obtain blood samples to karyotype other family members, but in most cases the patients do not concur. Except for the Robertsonian translocation find in an azoospermic man, which was inherited from his normally fertile mother, no further information was obtained for the other cases.

5. Conclusion

In conclusion, our results support the existence of a relationship between chromosomal aberrations and non-obstructive azoospermia and severe oligozoospermia. Our data show that the pattern and the prevalence of chromosomal abnormalities are comparable within infertile groups from other North African, Asian and western countries. These findings strongly suggest that such patients should at least be karyotyped and receive counselling before they are referred for assisted reproduction techniques. Such investigation is a pre-requisite to minimize the risk of transmitting genetic abnormalities to future generations, such as intellectual disability, genital ambiguity and/or birth defects. Furthermore, a screening of Y chromosome should be done in patients with a normal karyotype. This screening is being performed since some years (data not yet published).

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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