Chromosomal defects in infertile men with poor semen quality

Myriam Ghorbel · Siwar Gargouri Baklouti ·
Fatma Ben Abdallah · Nacira Zribi · Mariem Cherif ·
Rim Keskes · Nozha Chakroun · Afifa Sellami ·
Neila Belguith · Hassen Kamoun · Faiza Fakhfakh ·
Leila Ammar-Keskes

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Abstract

Purpose To assess the incidence and the type of chromosomal aberrations in males with infertility we reviewed cytogenetic results in 76 Tunisian infertile men (54 non-obstructive azoospermia and 22 oligo-asthenospermia). **Methods** Karyotyping was performed on peripheral blood lymphocytes according to the standard methods. Molecular diagnosis of classical and partial Y-chromosomal microdeletions was performed by amplifying Y-specific STSs markers. **Results** Various numerical and structural chromosome abnormalities were identified in 15 patients (19.48%). The occurrence of chromosomal abnormality in the azoospermics and severe oligo-asthenospermic was 21.7% and 13.5%, respectively. The most common was Klinefelter syndrome, accounting for 10 of the 15 cytogenetic defects. The total frequency of Y chromosomal microdeletions was 17.1%, with respective frequencies in azoospermic and severe oligospermic groups, 11.1% and 31.8%. The most frequent of Y chromosomal deletions were the partial ones (11.1% in azoospermic and 27.2% in oligospermic). **Conclusion** The occurrence of chromosomal abnormalities among infertile males strongly suggests the need for routine

genetic testing and counseling prior to the employment of assisted reproduction techniques.

Keyword Male infertility · Chromosome abnormality · Klinefelter syndrome · Y microdeletions · Severe oligo-asthenospermia · Azoospermia

Introduction

Infertility is a major health problem today affecting about 10–20% of couples and genetic abnormalities are thought to account for 15%–30% of male factor infertility [4, 11, 26]. Genetics contribute to infertility by influencing a variety of physiological processes including hormonal homeostasis, spermatogenesis, and sperm quality [22]. The incidence of chromosome abnormalities is about ten times higher in infertile men than in the general population [4]. Numerical and structural chromosomal abnormalities are seen frequently in azoospermia and oligospermia cases with unknown etiology [6, 32]. Indeed, chromosomal abnormalities account for approximately 5% of infertility in males, and their prevalence increases up to 15% in the population of azoospermic males [11]. Sex chromosome aneuploidy, is the most common abnormality, especially in nonobstructive azoospermia [8, 30]. Klinefelter syndrome (KFS) is the most frequent sex chromosome aneuploidy, occurring in 1/600 cases of newborn males [27, 31] and in 3% of infertile men cases [1, 14]; it is more prevalent among men with severe oligozoospermia (5%) and among azoospermic men (10%) [14]. The syndrome usually causes the arrest of spermatogenesis at the primary spermatocyte stage, but occasionally subsequent stages of sperm development are observed [16]. In addition to KFS, the microdeletion of azoospermia factor (AZF) region in Y chromosome is also the most common

Capsule Cytogenetic results have been reviewed in 76 Tunisian infertile men in order to assess the incidence and the type of chromosomal aberrations in male with infertility.

M. Ghorbel (✉) · S. Gargouri Baklouti · F. Ben Abdallah ·
N. Zribi · M. Cherif · R. Keskes · N. Belguith · H. Kamoun ·
F. Fakhfakh · L. Ammar-Keskes
Laboratory of Human Molecular Genetics, Faculty of Medicine,
Sfax, Tunisia
e-mail: myriamgh29@gmail.com

N. Chakroun · A. Sellami
Laboratory of Histology & Embryology, Faculty of Medicine,
Sfax, Tunisia

genetic cause of male infertility [40]; these microdeletions involve the loss of three regions associated with spermatogenic failure, namely AZFa, AZFb and AZFc. The incidence of Y-chromosome microdeletion varies from 1 to 50% among idiopathic and non-idiopathic azoospermic or severe oligozoospermic patients [13, 39, 40]. These data highlight the need for efficient genetic testing of infertile men to make couples aware of the risk of offspring inheritance of paternal genetic disorders. As recently reviewed by Fullerton et al. [15] several publications reported successful fertility treatment of men with KFS using testicular sperm extraction (TESE) combined with intra-cytoplasmic sperm injection (ICSI) [15], but concerns have been raised regarding the safety of ICSI to patients with genetic abnormalities [32, 43]. Therefore, genetic counselling may help to determine the prognosis and to choose the appropriate assisted reproduction technique that could minimize the risk of transmitting genetic abnormalities to future generations.

The present study was designed to evaluate the prevalence and the type of both chromosomal abnormalities and Y chromosome microdeletions among Tunisian infertile males with severe semen alterations and to discuss the importance of genetic investigations for the understanding of severe male infertility etiopathogenetic pathways.

Materials and methods

Both karyotyping and Y-microdeletion studies were carried out and a group of Tunisian infertile males ($n=76$) were involved in this retrospective analysis. These patients attended the University Hospital and School of Medicine at Sfax for clinical and biological investigations. They were subject to physical examination, hormonal estimation and sonographic evaluation. Semen analysis and genetic investigation (Karyotype and Y chromosomal microdeletions assesment) were performed on all patients.

Cases were classified into two groups according to semen analyses: azoospermia (54 cases) and severe oligospermia (22 cases) defined as the sperm cell count less than 5×10^6 cells/ml in seminal liquid. The diagnosis of oligozoospermia and azoospermia was confirmed by at least two consecutive spermograms performed on ejaculated semen collected at least 3 days apart. All cases of azoospermia or oligozoospermia resulting from obstructive causes were excluded from our study.

A spermogram was performed by a laboratory technician specially trained according to the standardized methods recommended by the World Health Organization (WHO) [42]. The following semen parameters were analyzed: volume, sperm concentration, sperm count, percentage of motile spermatozoa, and percentage of normal spermatozoa.

Cytogenetic studies were performed on peripheral blood cells using standard procedures: after 72-h culture with phytohemagglutinin stimulation, cultured cells were treated

with colchicine to obtain prometaphase chromosomes. The GTG banding technique was applied, and 20 metaphases were counted in each patient and controls.

Genomic DNA of each patient was extracted using a standard protocol. DNAs isolation was performed using the TSNT lyses buffer (1% Triton, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl pH8.0, and 1 mM EDTA) followed by phenol-chloroform extraction. Then DNAs were diluted and stored at 4°C prior to analysis.

Screening for classical Yq microdeletions was carried out on patients by Polymerase Chain Reaction (PCR) using a set of 6 pairs of oligonucleotide primers to amplify Y-specific STSs corresponding to different azoospermia factors (AZF) loci: 2 STSs located in AZFa (Sy84, Sy86), 2 STSs located in AZFb (Sy127, Sy134,) and 2 STSs located in AZFc (Sy254, Sy255). As positive control, we used one pair of primers amplifying one specific STS in SRY gene.

PCR was carried out on a total volume of 25 μ l. The reaction mixture included 100 ng of each DNA sample, 5 μ l of Amonum Sulfate PCR buffer (15 mM), 200 mM deoxynucleotidetriphosphates (dNTPs), 1 μ M of each primers and 1 unit of Taq DNA polymerase. Thermocycling consisted of an initial denaturation of 5 min at 94°C followed by 35 cycles of 40 s at 94°C, 50 s at 55°C, 50 s at 72°C and finally, 10 min at 72°C. PCR products were analysed by electrophoresis on 2% of agarose gels containing ethidium bromide and visualized under ultraviolet light. Negative PCR amplifications were repeated at least 3 times to confirm the deletion of a given marker.

Each individual was screened for two AZFc specific STSs (sY1291 and sY1191). Gr/gr deletion was identified by the absence of amplification of marker sY1291 and presence of marker sY1191. The b2/b3 deletions were characterized by the absence of sY1191 and the presence of sY1291. The b1/b3 deletion was characterized by the absence of amplification of both sY1291 and sY1191 according to Giachini et al. [17].

All negative PCR reactions were repeated for at least three times. As positive control, we used one pair of primers amplifying one specific STS in SRY gene.

Results

Conventional cytogenetic analysis identified various numerical and structural chromosome abnormalities in 15 (19.7%) azoospermic and oligospermic patients (Table 1). The occurrence of chromosomal abnormality was respectively 22.2% and 13.6% among males with azoospermia ($n=12$) and severe oligospermia ($n=3$) (Table 3). The most common was KFS, accounting for 10 of the 15 cytogenetic defects (66.6%). Seven of (KF) patients (6 azoospermic and 1 severe oligospermic) had non mosaic form (47, XXY) and 3 azoospermic patients had mosaic form (47, XXY/ 46,

Table 1 Numerical and structural chromosome abnormalities identified in azoospermic and severe oligospermic infertile males

Karyotype	Number of patients	Semen Analysis	Testis biopsy	FSH	Y microdeletion
46,XX	1	Azoospermia	SCO	18.6	–
	1	Azoospermia	–	–	No
47,XXY	3	Azoospermia	SCO	25.9 ; 67 ; 24	No
	1	Azoospermia	SCO	34	b1/b3
	1	Azoospermia	–	–	No
	1	Azoospermia	–	20.9	No
	1	Severe Oligospermia	–	2.93	No
46,XY/47,XXY	1	Azoospermia	–	–	No
	1	Azoospermia	–	33.8	No
	1	Azoospermia	SCO	–	–
Chromosome 12 inversion	1	Azoospermia	SCO	33	No
Chromosomal marker	1	Severe Oligospermia	–	–	No
Del Y chromosome long arm	1	Severe Oligospermia	–	–	Yes (- STS157)
Total	15				

XY). Among these 10 patients, 5 azoospermic men harbored in their testis biopsy the Sertoli Cell Only (SCO) syndrome and 6 of them had High levels of FSH. No classical Y chromosome microdeletions were found in all these 10 patients. But one KF azoospermic patient had partial AZFc deletion (b1/b3). Four other chromosomal abnormalities were identified in 3 azoospermic patients (2 men with 46, XX karyotype and 1 man with chromosome 12 inversion), and in 2 severe oligospermic patients (1 case of chromosome marker and 1 case of a total deletion of Y chromosome long arm). All chromosomal abnormalities detected were summarized in Table 2.

We found that the total frequency of Y chromosomal microdeletions (classical and partial) was 17.1%, with respective frequencies in azoospermic and severe oligospermic groups, 11.1% and 31.8%. The most frequent of Y chromosomal deletions were the partial ones 27.2% in oligospermic and 11.1% in azoospermic (Table 3).

Discussion

The occurrence of karyotypic abnormalities among infertile men depends on a number of factors. The most important is

the criterion for selection of patients based on the sperm count. It is well-known that the sperm count is inversely related to the existence of chromosomal anomaly [32]. Our retrospective analysis of cytogenetic results of 76 infertile patients diagnosed with various non obstructive spermatogenic defects revealed constitutional chromosomal abnormalities in 15 patients (19.73%). As mentioned in the literature, chromosomal abnormality incidence was higher in the azoospermic group (22.2%) than in the oligozoospermic group (13.6%); this confirms that the incidence of chromosomal abnormality increases as sperm count decreases [4]. Indeed, in Ceylan et al. report, chromosomal abnormality incidence was 33.3% in the azoospermic group and 13.3% in severe oligozoospermic group [4]. Akgül et al. found that chromosomal abnormalities were detected in 17.4% of 86 azoospermic cases and in 6.8% of 73 oligozoospermic cases in a regional study in Turkey [2]. Samli et al. reported chromosomal abnormality in 47 (12%) of 383 non-obstructive azoospermia cases and in 20 (4%) of 436 oligospermic patients [34]. More recently, chromosomal abnormalities were detected in 5.4% of 92 azoospermic cases and no patients of 23 oligozoospermic cases [32] (Table 4). According to the literature, the most common type of karyotype abnormality found in our infertile cases is

Table 2 Results of chromosomal analysis in the azoospermic and severe oligospermic groups

Patients groups	Karyotype					
	46,XY	46,XX	47,XXY	46,XY/47,XXY	chromosome 12 Inversion	Chromosome marker
Azoospermia (N=54)	77.7% (N=42)	3.7% (N=2)	11.1% (N=6)	5.5% (N=3)	1.8% (N=1)	0%
Severe oligospermia (N=22)	86.3% (N=19)	0%	4.5% (N=1)	0%	0%	4.5% (N=1)

Table 3 Results of Y chromosome microdeletion analysis in the azoospermic and severe oligospermic groups

Infertile patients	Number of patient	Karyotype	Y chromosome microdeletion (classical and partial)	
			Type	Frequency (%)
Azoospermia (N=54)	1	47,XXY	b1/b3	11.1
	5	46,XY	gr/gr	
Severe oligospermia (N=22)	1	Del Y chromosome long arm	STS 157	31.8
	3	46,XY	gr/gr	
	2	46,XY	b2/b3	
	1	46,XY	b1/b3	
Total	13	–	–	17.1

represented by KFS (16.6% and 4.5% in azoospermic and severe oligospermic groups, respectively) (Table 4). Ferlin et al. reported that the prevalence of KFS among infertile men is up to 5% in severe oligozoospermia and 10% in azoospermia [9]. It has always been assumed that more than 90% of non-mosaic 47, XXY males are azoospermic [32]. Indeed, in Ferlin et al. report, 74.4% of mosaic 47,XXY/46, XY patients were azoospermic, whereas the remaining had severe oligospermia [9]. In the current study, the mosaic form was detected in only three azoospermic.

KFS is a form of primary testicular failure and hypogonadism with testicular hypotrophy and elevated gonadotropin plasma levels [24]. Our results showed effectively that most of KF patients had a high FSH level (6/10).

In this study we report a pericentric inversion of chromosome 12 associated with non obstructive azoospermia. This anomaly was reported earlier in patients with malignant hematologic disorders [23, 35] and in patients with familial pericentric inversion [18, 38, 41]. Also, a case of primary male infertility associated with centric inversion of chromosome 12 was described [36]. The patient was diagnosed as having KFS by conventional cytogenetic analysis, which also showed an abnormal chromosome 12. In our case report, the pericentric inversion of chromosome 12 was associated with a very severe testicular phenotype including the absence of germinal cells in all seminiferous tubules. This

phenotype may cause the inactivation, by deletion of genes located within or near of the breakpoints, and acting on the spermatogenesis process.

As mentioned in the literature, there are some conflicting reports on the occurrence of Y chromosome microdeletions in KF patients [3, 7, 37] and the current study, failed to find microdeletions in AZF region in KF patients. In Ceylan et al. report, AZF deletions in KF patients were detected in the AZFc region [5]. Other authors observed that KF patients had multiple types of AZF deletions: in AZFa, AZFb and AZFc [19, 25].

In the present study, the total frequency of Y chromosomal microdeletions (classical and partial) was 17.1%; the most frequent deletions were the partial ones and the frequency of classical deletions was only 1.3%. Similar low frequencies in oligospermic or azoospermic men were reported in others studies [28, 29, 33].

Partial AZFc deletions result in the absence of several AZFc genes and gr/gr deletion has been suggested to be an important genetic risk factor for spermatogenic failure [10, 33]. As reported earlier in Hadj Kacem et al study [19], gr/gr deletion was the most frequent in both non KF azoospermic (11.9%) and severe oligospermic patients (15.7%). But in contrast to their findings, none of the KF patients harbored gr/gr deletion. In Moroccan population, partial deletions of AZFc (gr/gr) were observed in 4.70% of infertile men ($n=149$) and in 3.98% of control group [21]. Gr/gr deletion was also found to be equally present both in the patients and in the control group in a study of infertile men from Sri Lanka [12].

Concerning the other partial AZFc deletions, and according to the literature data and to our findings, they seem to be less frequent than gr/gr deletion. Indeed, we found that b1/b3 deletion was detected in only one azoospermic KF patient and one severe oligospermic 46, XY patient. It was totally absent in HadjKacem-Loukil et al. study [19]. Hucklenbroich et al. found b1/b3 deletions both in controls and in patients with spermatogenic failure [20]. Also, in our report b2/b3 deletions were identified in only two severe oligospermic men (2/76, 2.6%). Imken et al found this deletion in only two azoospermic subjects (2/149,

Table 4 Chromosomal abnormalities frequencies in different studies of literature

	% Chromosome abnormality		% Klinefelter
	Azoospermia	Oligospermia	
Samli et al., 2006 [34]	12	4	–
Ceylan et al., 2009 [4]	33.3	13.3	52.9
Akgül et al., 2009 [2]	17.44	6.8	66.6
Pinar et al., 2010 [32]	5.4	0	80
Yatsenko et al., 2010 [43]	13.3	5.2	49
Our study	22.2	13.6	66.6

1.34%) [21]. The Hucklenbroich et al. findings showed that b2/b3 deletions were significantly more frequent in the normozoospermic (5/170, 2.9%) than in the oligo-/azoospermic men (2/348, 0.5%) [20].

In conclusion

Genetic factors may contribute in up to 75% male infertility, so the analysis of genetic factors that impact on male factor infertility (karyotype analysis, cystic fibrosis mutation detection and Y chromosome microdeletion analysis) will provide valuable insights into the creation of targeted treatments for patients and the determination of idiopathic infertility causes.

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