A genetic survey of 1935 Turkish men with severe male factor infertility

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Abstract

Male factor infertility is the sole reason in approximately 25% of couples who suffer from infertility. Genetic factors such as numerical and structural chromosomal abnormalities and microdeletions of the Y chromosome might be the cause of poor semen parameters. The results of karyotype analyses and Y-chromosome microdeletions of 1935 patients with severe male factor infertility, which is the largest series from Turkey, were assessed retrospectively. The frequency of cytogenetic abnormalities among 1214 patients with non-obstructive azoospermia (NOA) and 721 patients with severe oligoasthenoteratozoospermia (OAT) were 16.40 and 5.83% respectively. The overall incidence of Y-chromosome microdeletion was 7.70%. The incidence of Y chromosome microdeletion in patients with NOA and OAT was 9.51 and 1.86% respectively. The abnormality rate increased with the severity of infertility. Some patients (n = 22) were carriers of both chromosomal abnormalities and Y-chromosome microdeletions. Results suggest the need for genetic screening and proper genetic counselling before initiation of assisted reproduction treatment.

Keywords: assisted reproduction techniques, karyotype analysis, male factor infertility, Y chromosome microdeletions

Introduction

Infertility affects approximately 15% of couples of reproductive age (Iammarrone et al., 2003) and 20–25% of reproductive infertility can be due to male factor alone (World Health Organization (WHO), 1997). It is a dilemma that even the most comprehensive work-up covering physical examinations, serological and hormonal tests, detailed semen analysis and imaging techniques may fail to detect the aetiology of reproductive disorders (Crosignani et al., 1993). A number of genetic and non-genetic conditions have been associated with male infertility; these include numerical and structural chromosomal abnormalities, Y chromosome microdeletions, mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene, congenital bilateral absence of the vas deferens (CBAVD), gonadotrophin deficiency, varicocele, testicular tumours, drugs, sperm autoimmunity, smoking, and exposure to toxins (Crosignani et al., 1993; WHO, 1997).

Male infertility appears to have a familial occurrence, especially among brothers and maternal uncles (Van Golde et al., 2004). It is estimated that genetic factors are involved in approximately 60% of male factor infertility cases (Lilford et al., 1994). Cytogenetic abnormalities such as numerical and structural chromosomal abnormalities (Chandley, 1998) and microdeletions of the Y chromosome (Lilford et al., 1994; Reijo et al., 1995; Vogt et al., 1996) are known to be amongst the genetic causes of low sperm concentrations. Structural abnormalities such as reciprocal and Robertsonian translocations can be inherited through generations by vertical transmission (Kobayashi et al., 1994; Vogt et al., 1996; Chang et al., 1999).

In the general male population the prevalence of chromosomal abnormalities ranges between 0.7 and 1.0% (Lange and Engel,
The frequency of karyotypic abnormalities increases with the severity of the semen parameters; the incidences among mild (oligospermic) and severe (azoospermic) groups reported as 4.6 and 13.7% respectively (Van Assche et al., 1996).

The entire length of the Y chromosome has been subdivided into seven deletion intervals (Figure 1). Each of these intervals is further subdivided into subintervals a, b and c. Some of the genes that are critical for spermatogenesis are located on the long arm of the Y chromosome in deletion interval 6 band 11.23 and deletion interval 5. More than 14 protein encoding Y genes in azoospermia factor (AZF) regions affect spermatogenesis and fertility, for instance, the *USP9Y* gene in AZFa, the RNA binding motif (*RBM*) gene in the AZFb region, and the deleted in azoospermia (*DAZ*) gene in the AZFc region (Kobayashi et al., 1994; Vogt, 1995; Vogt et al., 1996). Deletions removing the entire AZFa or AZFb regions are associated with Sertoli cell only syndrome (SCOS) and spermatogenic arrest respectively (Krausz et al., 2000; Kamp et al., 2001). AZFc deletions are, however, associated with normozoospermia, oligozoospermia, severe oligozoospermia and azoospermia (Oliva et al., 1998).

The deletion types of AZF a, b and c loci are the potential prognostic factors in patients recommended to undergo testicular surgery such as testicular sperm extraction (TESE) and microdissection−TESE procedures (micro-TESE). In more than half of men with AZFc deletions, mature spermatozoa can be obtained in the ejaculate or in the testis via surgery, whereas in patients with complete deletions in AZFa or AZFb regions, it is nearly impossible to obtain mature spermatozoa (Hopps et al., 2003).

So far, several retrospective studies have tried to establish the frequency of cytogenetic abnormalities and Y chromosome microdeletions in the Turkish population (Okutman-Emonts et al., 2004; Sargin et al., 2004; Vidian et al., 2004). However, sample sizes in these studies were inadequate to make inferences.

The purpose of this study was to evaluate the genetic risks in a large number of infertile Turkish men who were referred for assisted reproduction treatment.

### Materials and methods

#### Population

Cytogenetic and molecular screening test results of 1935 infertile men with oligoasthenoteratozoospermia (OAT) or non-obstructive azoospermia (NOA) who had been referred to Istanbul Memorial Hospital In-Vitro Fertilization (IVF) and Reproductive Genetics Centre between July 2000 and September 2007 were analysed retrospectively. Sperm samples were harvested 2–6 days after sexual abstinence and were subjected to evaluation for total count, percent motility and forward progression (WHO, 1999). Sperm morphology was evaluated according to strict criteria as outlined by Kruger et al. (1988) following Spermac staining.

Blood samples were taken for both cytogenetic analysis (*n* = 1935) and Y-chromosome microdeletion test (*n* = 1364). This study protocol was reviewed and approved by the Internal Review Board of Istanbul Memorial Hospital and written informed consent was obtained from each participant.

#### Cytogenetic analysis

Because of absence of spermatozoa and low sperm concentration (< 5 × 10⁹/ml) in a considerable numbers of patients, NOA and OAT were evaluated in 1214 and 721 patients respectively. Peripheral blood samples were collected from both partners and kept in lithium–heparin tubes for karyotyping. Blood samples

Figure 1. Schematic representation of the Y chromosome showing seven intervals and pseudoautosomal region (PAR) 1 and 2. Tiepolo and Zuffardi (1976) revealed the existence of deletions in the long arm of the Y chromosome and considered these regions an essential genetic factor for spermatogenesis. Later, this factor was named as azoospermia factor (AZF) (Ma et al., 1992; Vogt et al., 1992; Reijo et al., 1996). Interstitial microdeletions occur in three discrete regions on Yq: AZFa (proximal), AZFb (central) and AZFc (distal) (Vogt et al., 1996). The distal part of AZF is deleted in azoospermia (DAZ) (Reijo et al., 1995).
were put into Roswell Park Memorial Institute (RPMI) 1640 medium (Biological Industries, Ltd, Israel) and added with HEPES containing fetal bovine serum albumin, t-glutamine, penicillin–streptomycin and phytohemagglutinin. Forty-eight hours after incubation at 37°C, 150 µl of thymidine was added. During 17 h, culture was washed with RPMI medium without phytohaemagglutinin and left at 37°C for further incubation. Three hours later, 100 µl colcemide (10 µg/ml) was added for 50 min incubation before harvest. Following hypotonic treatment, cells were fixed by fresh cold fixative (methanol:glacial acetic acid, vol:vol, 3:1). Giemsa-Giemsa (GTG) banding was performed using trypsin (0.025% trypsin EDTA) and Giemsa’s solution (5%) (Merck and Co., Inc., Germany). A total of 20 metaphase plaques were analysed for each subject. The metaphase count was increased to 100 and fluorescent in-situ hybridization (FISH) was applied in cases of mosaicism. Numerical and structural karyotype analyses were performed at 550 band levels using Ikaros software (MetaSystems Inc., Germany).

FISH analysis

FISH analysis was conducted on metaphase plaques obtained from patients with structural abnormalities or numerical mosaicsisms. Centromeric (CEP), locus specific (LSI), whole chromosome painting (WCP), and telomeric (Tel) probes were used according to general FISH protocols. Probes for regions specific for sex chromosomes such as sex determining region Y (LSI SRY, Yp11.3), CEP X (DXZ1 alpha satellite) and WCP Y (Vysis Inc., Germany) were generally used to confirm the location of the SRY region. Co-denaturation of probe and DNA was performed at 72°C and hybridization was performed at 37°C in a hybridization chamber (Hybrite; Vysis Inc., Germany) for at least 6 h. Slides were washed in 0.4% saline sodium citrate for 2 min at 71°C. Following application of DAPI (4,6-diamidino-2-phenylindole II; Vysis) counterstain, images were analysed and captured in green, red, gold, aqua and DAPI fluorescence filters with an Olympus BX50 fluorescence microscope using commercial software (Iisis; Metasystems Inc., Germany).

Molecular analysis

Out of 1935 cases that had been screened for cytogenetic abnormalities, 1364 were also analysed for Y chromosome microdeletions. The majority of patients who screened for microdeletions were azoospermic (n = 1041) and the rest had severe OAT (n = 323). Genomic DNA was obtained from peripheral blood leukocytes and analysed using an isolation kit (QIamp DNA mini kit; Qiagen) according to the manufacturer’s protocol. Microdeletions of the Y chromosome were screened using multiplex polymerase chain reaction (PCR). Promega kits containing 18 loci (from 2000 to 2003) and a homemade kit containing primers specific for 25 loci (thereafter) were employed. The following loci were screened: in the SRY region SY14; in the AZFa region SY81, SY82, SY83, SY84, SY86, SY87, SY88, SY182; in the AZFb region SY121, SY124, SY127, SY128, SY130, SY133, SY134, SY135; in the AZFc region SY143, SY145, SY152, SY157, SY158, SY254, SY255; and in the heterochromatic region SY160. Deletions in the fourth region (between AZFb and AZFc), which used to be termed AZFd and AZFf regions as now adopted.

For each sample, four different multiplex fluorescent PCR mixtures were prepared, which contained primer, PCR mix with buffer, dNTP and MgCl2, Taq polymerase, 30 ng DNA sample, and H2O with 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 s, 60°C for 60 s, and 72°C for 60 s, which was followed by final extension at 65°C for 45 min. 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 analyser (ABI Prism 3100; Applied Biosystems, USA) and the outputs were analysed with sequence analysis software (GeneScan, Applied Biosystems).

Statistical analysis

Data were subjected to the proc freq procedure of Statistical Analysis System (SAS, 1999). Differences in group frequencies were attained using the chi-squared test and significance was declared at P < 0.05.

Results

Table 1 presents detailed peripheral karyotype analysis with respect to semen parameters. The overall incidence of cytogenetic abnormalities was 12.45%. The frequencies of abnormalities increased with the severity of semen parameters, being 5.83 and 16.40% in OAT and NOA groups respectively. Klinefelter’s syndrome (47,XXY) was the most frequent abnormality (n = 138, 7.13%), especially in the NOA group (n = 133, 10.95%). Both reciprocal and Robertsonian translocations together (n = 25, 3.47%) were the most frequent abnormality in the OAT group.

The incidence of 46,XX males (n = 10), was nearly 1% among NOA patients (Figure 2). Six out of 10 males with this abnormality were also screened for Y-microdeletions; four patients were positive and two were negative for the SRY region. Further analysis using FISH technique revealed that the SRY region was translocated to one of the X chromosomes in all SRY+ cases (Figure 3). Another abnormality evaluated in this group was a 45,XO male (Figure 4), where the SRY region was associated with autosomal chromosome 2: 45,X,tas(Y;2) (p11.3;qter). So far as is known, this case is the first 45,XO male with SRY associated with the telomeric region of chromosome 2 (Figures 5 and 6).

The overall incidence of Y chromosome microdeletion was 7.70%. It was significantly different between OAT (1.86%) and NOA (9.51%) groups (P < 0.0001; Table 2). The most frequent region with deletions was the AZFc region.

A minority of patients (n = 22) had both karyotypic abnormalities and Y chromosome microdeletions (Table 3). One case of Klinefelter’s syndrome (47,XXY) was associated with partial deletions in the AZFa, AZFb and AZFc regions of the Y chromosome. Six 46,XX males and one 45,X male had no Y chromosome. Eight patients had mosaic forms of sex chromosomal abnormalities accompanied mostly by AZFb + c deletions.
Table 1. Distribution of normal and abnormal karyotypes in the infertile men studied.

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Category of male infertility</th>
<th>Total (n = 1935)</th>
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<tbody>
<tr>
<td></td>
<td>NOA (n = 1214)</td>
<td>OAT (n = 721)</td>
</tr>
<tr>
<td>Normal (46,XY)</td>
<td>973 (80.15)</td>
<td>645 (89.46)</td>
</tr>
<tr>
<td>Normal variable features/polymorphisms</td>
<td>42 (3.46)</td>
<td>34 (4.72)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>199 (16.40)</td>
<td>42 (5.83)</td>
</tr>
<tr>
<td>Klinefelter syndrome (47,XXY)</td>
<td>133 (10.96)</td>
<td>5 (0.69)</td>
</tr>
<tr>
<td>Mosaic Klinefelter</td>
<td>10 (0.82)</td>
<td>6 (0.83)</td>
</tr>
<tr>
<td>Other sex chromosome mosaicisms</td>
<td>13 (1.07)</td>
<td>2 (0.28)</td>
</tr>
<tr>
<td>45,X0 (n = 1) and 46,XX (n = 10)</td>
<td>11 (0.91)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Other X chromosome abnormalities</td>
<td>10 (0.82)</td>
<td>1 (0.14)</td>
</tr>
<tr>
<td>Reciprocal translocation</td>
<td>12 (0.99)</td>
<td>13 (1.80)</td>
</tr>
<tr>
<td>Robertsonian translocation</td>
<td>1 (0.08)</td>
<td>12 (1.66)</td>
</tr>
<tr>
<td>Inversions</td>
<td>4 (0.33)</td>
<td>1 (0.14)</td>
</tr>
<tr>
<td>Markers</td>
<td>1 (0.08)</td>
<td>1 (0.14)</td>
</tr>
<tr>
<td>Other abnormalities</td>
<td>4 (0.33)</td>
<td>1 (0.14)</td>
</tr>
</tbody>
</table>

Values are number (%).
NOA = non-obstructive azoospermia; OAT = oligoasthenoteratozoospermia.

Figure 2. Karyotype analysis results of a 46,XX, SRY positive male.
Figure 3. Fluorescence in-situ hybridization technique enabled identification and localization of the SRY region on one of the X chromosomes. CEP X is in spectrum green, whereas LSI SRY is in spectrum red.

Figure 4. Karyotyping revealed that in addition to the absence of the Y chromosome, only one X chromosome was present in all of the metaphase plaques analysed; 45, X0.

Figure 5. Fluorescence in-situ hybridization (FISH) analysis results of 45,X,tas(Y;2)(p11.3;qter). FISH study revealed that the SRY (spectrum red) region was not translocated on the X chromosome (CEP X, spectrum green) but was on an autosomal chromosome which was presumed to be chromosome 2.

Figure 6. Further fluorescence in-situ hybridization study using probes specific to centromeric (CEP 2, spectrum gold) and telomeric loci (Tel 2q, spectrum gold) of chromosome 2 revealed that the SRY region was associated with the telomeric region of chromosome 2, which was still present in its location together with SRY (evident from WCP probe, spectrum green). The WCP probe binds both SRY region and a homologous region on the X chromosome.
Discussion

The chromosomal abnormality rate in this study (12.4%), was similar to the study of Nakamura and colleagues (2001) in Japan (12.6%), but lower than reported in the study of Kleiman and colleagues (1999) in Israel (16.6%) and higher than reported in the studies of Yoshida and colleagues (1997) in Japan (6.2%) and Quilter and colleagues (2003) in the UK (9.7%). Among all cytogenetic abnormalities, Klinefelter’s syndrome was the most frequent abnormality detected, which is in agreement with other studies conducted on infertile men (Nakamura et al., 2001; Elghezal et al., 2006). The second most frequent abnormality was related to chromosomal rearrangements (2.2%) such as reciprocal and Robertsonian translocations and inversions. The high rate of translocations in this population could be associated with the high proportion of patients with male infertility as well as repeated implantation failures or recurrent pregnancy losses. Patients were referred to the clinic, which is a reference clinic as well as a genetic diagnostic centre in Turkey performing more than 500 cycles per year for preimplantation genetic diagnosis (PGD), mostly for aneuploidy screening and translocations.

Chromosomal rearrangements can affect fertility due to impaired gametogenesis and/or production of chromosomally unbalanced gametes. In the present study, the translocation rate was lower in the NOA group than the OAT group (1.1 versus 3.5%). Van Assche and colleagues (1996) also reported a lower incidence of translocations in patients with NOA than in those with OAT (1.06 versus 3.46%). These results show that this type of chromosomal rearrangement may not always cause spermatogenic arrest. For translocation carrier couples, poor prognosis is related to a reduced chance of producing normal or balanced gametes. Attempts have been made to reveal any possible correlation between chromosomal abnormalities in gametes and embryos. Using FISH on sperm cells, it is possible to determine the rate of chromosomally unbalanced sperm cells in the ejaculate. The rate of unbalanced spermatozoa and gametes in ejaculates has been reported as 72.1 (Estop et al., 1995) and up to 25.0% (Escudero et al., 2000) respectively. The PGD technique can be offered to couples who are translocation carriers. By analysing one cell on day 3 of embryonic development, it is possible to detect the balanced or normal embryos for transfer. With experienced staff, PGD decreases...
Table 4. Y chromosome microdeletion rate determined in idiopathic azoospermic and severe oligoazoospermic patients from different countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>n</th>
<th>Deletion (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>683</td>
<td>12.9</td>
<td>–</td>
</tr>
<tr>
<td>China</td>
<td>101</td>
<td>11.0</td>
<td>Fu et al., 2002</td>
</tr>
<tr>
<td>China</td>
<td>62</td>
<td>19.4</td>
<td>Song et al., 2005</td>
</tr>
<tr>
<td>Taiwan</td>
<td>94</td>
<td>11.7</td>
<td>Lin et al., 2000</td>
</tr>
<tr>
<td>Taiwan</td>
<td>180</td>
<td>10.6</td>
<td>Lin et al., 2002</td>
</tr>
<tr>
<td>Japan</td>
<td>63</td>
<td>15.8</td>
<td>Kobayashi et al., 1994</td>
</tr>
<tr>
<td>India</td>
<td>83</td>
<td>9.6</td>
<td>Dada et al., 2003</td>
</tr>
<tr>
<td>India</td>
<td>100</td>
<td>12.0</td>
<td>Athalye et al., 2004</td>
</tr>
<tr>
<td>Oceania</td>
<td>65</td>
<td>7.7</td>
<td>–</td>
</tr>
<tr>
<td>New Zealand</td>
<td>65</td>
<td>7.7</td>
<td>Kerr et al., 2000</td>
</tr>
<tr>
<td>Europe</td>
<td>530</td>
<td>9.7</td>
<td>–</td>
</tr>
<tr>
<td>France</td>
<td>53</td>
<td>9.4</td>
<td>Seifer et al., 1999</td>
</tr>
<tr>
<td>Spain</td>
<td>50</td>
<td>16.0</td>
<td>Oliva et al., 1998</td>
</tr>
<tr>
<td>Slovenia</td>
<td>226</td>
<td>4.4</td>
<td>Peterlin et al., 2002</td>
</tr>
<tr>
<td>Finland</td>
<td>201</td>
<td>9.0</td>
<td>Aho et al., 2001</td>
</tr>
<tr>
<td>N. America</td>
<td>168</td>
<td>12.5</td>
<td>–</td>
</tr>
<tr>
<td>USA</td>
<td>108</td>
<td>7.0</td>
<td>Girardi et al., 1997</td>
</tr>
<tr>
<td>USA</td>
<td>60</td>
<td>18.0</td>
<td>Najmabadi et al., 1996</td>
</tr>
<tr>
<td>Middle East</td>
<td>444</td>
<td>6.2</td>
<td>–</td>
</tr>
<tr>
<td>Israel</td>
<td>105</td>
<td>6.7</td>
<td>Nakamura et al., 2001</td>
</tr>
<tr>
<td>Turkey</td>
<td>208</td>
<td>9.1</td>
<td>Vician et al., 2004</td>
</tr>
<tr>
<td>Turkey</td>
<td>71</td>
<td>5.6</td>
<td>Okutman-Emonts et al., 2004</td>
</tr>
<tr>
<td>Turkey</td>
<td>60</td>
<td>3.3</td>
<td>Sargin et al., 2004</td>
</tr>
<tr>
<td>Total</td>
<td>1890</td>
<td>10.4</td>
<td>–</td>
</tr>
<tr>
<td>Turkey</td>
<td>1364</td>
<td>7.7</td>
<td>Present study</td>
</tr>
</tbody>
</table>

abortion rates and increases pregnancy and implantation rates among couples with chromosomal rearrangements (Otani et al., 2006; Beyazyurek et al., 2007).

Heterochromatin polymorphisms were observed in 3.9% of all men screened for karyotype abnormalities (Table 1). The question arises as to whether these polymorphisms are less innocent than might be expected. In support of this, the incidence of heterochromatin polymorphism is higher in infertile than in fertile men (Yakin et al., 2005). A well-known example of this type of variation is a regular pericentric inversion on chromosome 9; inv(9)(p12;q13). Although this variation is not considered to have an effect on fertility, there have been some studies demonstrating its association with an infertility factor (Sasagawa et al., 1998; Tomaru et al., 1999; Davalos et al., 2000), the mechanism has not been identified. In addition to inv(9), heterochromatic region polymorphisms on chromosomes 1,9,16 and Y are also associated with impaired spermatogenesis (Nazarenko and Sukhanova, 1991; Bobrow, 1995).

The impairment of adjacency between X and Y chromosomes during meiotic divisions has been proposed to have an effect in spermatogenic arrest in the case of microdeletions (Yoge et al., 2004). Significant impairment in X/Y alignment was observed in patients with microdeletions in AZF b + c regions compared with patients with isolated AZFc deletions, suggesting that either the deletion or the absence of the genes involved were probably responsible for meiosis impairment (Yoge et al., 2004).

The deletion rate is highly variable in the literature (Table 4). In the present study, DNA analysis of AZFa, AZFb and AZFc regions showed that 7.70% of infertile patients had Y-microdeletion; with a higher incidence in the NOA group than the OAT group (9.51 versus 1.86%; Table 2). Out of 105 patients with microdeletions, 97 patients (92.38%) were carrying partial or complete deletions in AZFc region, as reported elsewhere (Schlegel, 2002; Simoni et al., 2008). Y chromosome microdeletion rates may vary from 0 to 25.90% (Simoni et al., 2008) and the rate in this study (7.70%) is at the mid-point of other reports worldwide (Girardi et al., 1997; Simoni et al., 1998; Athalye et al., 2004). This rate is higher than two previous reports (Okutman-Emonts et al., 2004; Sargin et al., 2004) and lower than one previous report (Vician et al., 2004) conducted in Turkey (Table 4). The variability is probably related to differences in clinical selection criteria, molecular strategies, and the number of loci used for screening as well as sample size.

The deletion types of AZF a, b and c loci on Yq11 are the potential prognostic factors in patients planned to undergo TESE/micro-TESE procedures. For men carrying complete deletions in AZFa, AZFb regions, it is virtually impossible to obtain mature spermatozoa (Simoni et al., 2004). Similar to the present results, in 56% of men with deletions in the AZFc region,
mature spermatozoa could be retrieved via testicular biopsy (Hopps et al., 2003). Therefore, Y chromosome microdeletion screening provides clinicians with the information needed to avoid unnecessary surgical procedures and alter treatment choices. Micro-TESE was performed on 30 patients with microdeletions after informing them about the rates of sperm retrieval. Two patients with complete AZFb + c and one patient with partial AZFa + b + c deletions chose to undergo TESE. No spermatozoa were obtained in two patients with partial AZFa, two patients with complete AZFb + c and one patient with partial AZFa + b + c deletions. In the remaining 25 patients with AZFc deletions, mature spermatozoa were obtained in 14 patients (56%). Eight patients had already become fathers of eight children (five girls and three boys) via intracytoplasmic sperm injection (ICSI). Boys were not monitored for AZF deletions, but the parents were informed about the likelihood of occurrence of the same problem in their children. Out of 30, 15 patients had microdeletions involving exactly the same regions (SY152, SY157, SY158, SY254, SY255), mature spermatozoa were, however, found in six patients and no spermatozoa were obtained in nine patients.

It was noteworthy that eight cases carried sex chromosomal mosaicsisms including 45,X0 cell lines and Y chromosome microdeletions (Table 3). An association between Y-chromosome microdeletions and 45,X0/46,XY chromosomal mosaicism has been proposed before (Siffroi et al., 2000; Alvarez-Nava et al., 2008). Moreover, Alvarez-Nava and colleagues (2008) found a higher incidence of Y-chromosome microdeletions on gonadal DNA than on peripheral blood lymphocyte DNA in 11 patients with 45,X0/46,XY gonadal dysgenesis, suggesting that microdeletions in the Y chromosome might be associated with Y chromosomal instability leading to mitotic loss of the Y chromosome (Siffroi et al., 2000; Alvarez-Nava et al., 2008). The PGD technique can also be considered as a treatment option for cases with both a high rate of sex chromosomal mosaicism and Y chromosome microdeletions because there is a risk of transmitting this unstable Y chromosome that may lead to gonadal dysgenesis in the offspring.

Polymorphisms (Ferlin et al., 2007) and expression profiles of several candidate genes (Kleiman et al., 2007) in testicular tissue of azoospermic patients are associated with impaired spermatogenesis. Although Y chromosome microdeletions have direct effects on spermatogenesis, microdeletions of Yq involving the DAZ gene are associated with variable phenotypic expressions that can include evidently normal fertility (Chang et al., 1999) and thereby permitting vertical transmission. In the present study, despite having deletions in the same loci in AZFc region, spermatozoa were retrieved from six patients, whereas no spermatozoa could be obtained from nine patients after micro-TESE procedures. The presence of polymorphisms or mutations in other genes related to spermatogenesis may contribute to variable phenotypic expression accompanied by AZFc deletions.

There is growing evidence that draws attention to the paternal contribution to embryonic development, implantation and abortion. Morphological abnormalities of head and tail as well as poor motility are associated with increased chromosomal abnormalities in spermatozoa (Calogero et al., 2001; Kahrman et al., 2004, 2006; Tempest et al., 2004) and embryos (Kahrman et al., 2004, 2006). Furthermore, FISH studies reveal that aneuploidy rate is high in sperm samples obtained from TESE and its incidence increases with the severity of infertility (Gianaroli et al., 2005b). There is a significant proportional increase in gonosomal aneuploidy in embryos derived from testicular sperm, despite no overall increase in chromosomal aneuploidy (Gianaroli et al., 2005a). Men with high levels of DNA fragmentation are also at greater risk for low blastocyst rates and failure to initiate an ongoing pregnancy (Virro et al., 2004) and have significantly higher IVF abortion rates (Lin et al., 2007). Therefore, the fragmentation and aneuploidy tests provide invaluable prognostic information to physicians counselling couples before IVF and/or ICSI cycles.

In conclusion, among Turkish infertile men with idiopathic male factor infertility, the frequency of cytogenetic abnormality and Y chromosome microdeletion was 12.45 and 7.70% respectively, suggesting the need for genetic screening and counselling in these particular cases. To give the appropriate treatment to couples requiring ICSI, peripheral blood karyotyping and Y chromosome microdeletion analysis should be considered before initiating IVF cycles.

References


Escudero T, Lee M, Carrel D et al. 2000 Analysis of chromosome
abnormalities in sperm and embryos from two 45,X/47,XYt(13;14) (q10;q10) carriers. Prenatal Diagnosis 20, 599–602.

Estop AM, Van Kirk V, Cieply K 1995 Segregation analysis of four translocations, t(2;18), t(3;15), t(5;7), and t(10;12), by sperm chromosome studies and a review of the literature. Cytogenetics and Cell Genetics 70, 80–87.


Lin MH, Kuo-Kuang LR, Li SH et al. 2007 Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertility and Sterility 90, 352–359.


Tempest HG, Homa ST, Dalakiouridou M et al. 2004 The association between male infertility and sperm disomy: evidence for variation in disomy levels among individuals and a correlation between particular semen parameters and disomy of specific chromosome pairs. Reproductive Biology and Endocrinology 2, 82–91.


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