Patients with abnormal sperm parameters have an increased sex chromosome aneuploidy rate in peripheral leukocytes

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BACKGROUND: Patients with oligoasthenoteratozoospermia (OAT) and normal karyotypes have an increased sperm aneuploidy rate. This may be due to an altered intratesticular environment that affects the chromosomal segregation mechanism(s). Alternatively, it may be due to a generalized meiotic and mitotic abnormality. In this case, patients with abnormal spermatogenesis should also have an increased somatic cell aneuploidy rate. To test this hypothesis, we evaluated peripheral leukocyte aneuploidy rate in patients with spermatogenic impairment.

METHODS: In all, 38 patients were enrolled, of whom 20 had OAT, 15 non-obstructive azoospermia and three Y chromosome (Yq) microdeletions (AZF). Eight healthy normozoospermic men with proven fertility were recruited as controls. Conventional karyotype analysis, AZF microdeletion evaluation and triple-colour FISH for chromosomes X, Y and 12 were conducted in all patients and controls. A total of 1000 lymphocytes were scored for each patient and control. RESULTS: All patients and controls had a normal karyotype. Sex chromosome aneuploidy rates in peripheral lymphocytes was significantly higher in patients with OAT (0.74 ± 0.09%), azoospermia (1.15 ± 0.15%) or Yq microdeleted (1.54 ± 0.40%), compared with controls (0.15 ± 0.03%) (P<0.05).

CONCLUSIONS: Patients with OAT, azoospermia or Yq microdeletions had a slight, but significant, increase of sex chromosome aneuploidy rate in lymphocytes, suggesting the presence of a generalized defective cell division mechanism. In contrast with recent observations, Yq microdeletions do not seem to predispose to a higher number of malsegregation events in somatic cells compared with patients with azoospermia.

Introduction

Karyotype abnormalities are frequently present among infertile men. It has been estimated that ~7% of the patients with oligozoospermia have chromosomal alterations, a figure 10 times higher than that found in normal men (Retief et al., 1984; Bourrouillou et al., 1985). The proportion of men with an abnormal karyotype (mainly Klinefelter syndrome and reciprocal or Robertsonian translocations) is greater (~12%) in patients with a more severe spermatogenetic failure, such as those with azoospermia or severe oligozoospermia (De Braekeleer and Dau, 1991).

Patients with an altered karyotype, if not azoospermic, produce an increased number of chromosomally abnormal spermatozoa (aneuploid, diploid and recombinant aneusomies). However, a normal somatic karyotype, evaluated by standard cytogenetic analysis, does not exclude the presence of aneuploid gametes. In this regard, several studies have shown that patients with oligoasthenoteratozoospermia (OAT) and normal karyotype produce a higher number of spermatozoa with an abnormal chromosome asset than normozoospermic men (for a review see Calogero et al., 2003). The increased sperm aneuploidy rate found in these patients may be ascribed to an altered intratesticular environment that disrupts the fine-tuned mechanisms controlling chromosome segregation. Alternatively, the increased sperm aneuploidy rate in patients with oligozoospermia or azoospermia may be due to an abnormality in the molecular mechanism(s) controlling cell division.

The mitotic checkpoint is indeed a failsafe mechanism for the cell to ensure accurate chromosome segregation during mitosis. An altered function of essential checkpoint proteins or mutations of their encoding genes lead to chromosome instability. Budding inhibited by benomyl (BUB) and mitotic arrest deficient (MAD) genes are essential components of the mitotic checkpoint pathway. BUB and MAD, by inhibiting...
ubiquitin ligase activity of the anaphase promoting complex/cyclosome (APC/C) during mitosis, ensure that cells with unaligned chromosomes do not prematurely enter anaphase (Chan and Yen, 2003). The spindle checkpoint monitors microtubule attachment and tension at kinetochores to ensure proper chromosome segregation (Shannon et al., 2002). Kinetochores, considered until a few years ago as rather inert structures that served only to attach mitotic chromosomes to microtubules, play an important active role not only in moving chromosomes along the microtubules of the mitotic spindle, but act also as dynamic and adaptable centres for regulating cell cycle progression through mitosis (Gorbsky, 1995).

In keeping with this hypothesis, it has been reported that karyotypically normal patients with OAT have an increased mitotic instability (Gazvani et al., 2000). The present study was therefore undertaken to evaluate whether patients with a severe spermatogenesis impairment have an increased rate of somatic cell aneuploidy. To accomplish this, we evaluated the presence of sex chromosome aneuploidy by fluorescence in-situ hybridization (FISH) in leukocytes of selected patients with non-obstructive azoospermia or OAT and normal conventional karyotype. A group of fertile normozoospermic men served as controls. We also evaluated the presence of low level somatic mosaicsisms in patients with Yq microdeletions, since these patients have been reported to have an increased number of blood cells with a 45,XO karyotype (Siffroi et al., 2000).

Materials and methods

In all, 38 patients were enrolled in this study, of whom 20 had OAT, 15 non-obstructive azoospermia and three Yq microdeletions. Eight healthy men with proven fertility were recruited as controls. The protocol was approved by the Institutional Review Board and an informed written consent was obtained from each subject.

Conventional karyotype analysis of peripheral leukocytes was performed in all patients and controls. Venous blood samples were obtained from each of them and phytohaemagglutinin (PHA)-stimulated cultures were established using standard cytogenetic methods (Chromosome-Kit Euroclone Milano, Italy). Cytogenetic analysis was conducted in 20–30 metaphase plates.

Genomic DNA was extracted from peripheral leukocytes and Yq microdeletions were evaluated using 22 sequence-tagged site primers, spanning the intervals 5 and 6, which comprise the AZFa (5C), AZFb (5O-6B) and AZFc (6C-6F) regions, as reported previously (Calogero et al., 2001).

All patients and controls underwent triple-colour FISH for chromosomes X, Y and 12. Alpha-centromeric probes were purchased from Cytocell (Adderbury, UK). FISH was performed using a standard protocol. Briefly, leukocytes were spotted onto cleaned microscope slides and washed in 2× standard saline solution at 37°C, dehydrated in an ethanol series and allowed to dry. Probes were placed on the spotted area of the slide and a coverslip was applied. Slides were denatured a 75°C for 2 min and incubated at 37°C for 1 h. Afterwards, they were immersed in post-hybridization medium, counterstained with DAPI antifade and, after 10 min, viewed under a fluorescence microscope (Zeiss C, Oberkochen, Germany) with the appropriate set of filters: single band DAPI, FITC and Cy3. Leukocytes were considered as abnormal if they presented one (monosomy) or more than two (trisomy, etc.) distinct hybridization signals for the same chromosome (X, Y or 12), each equal in intensity and size and separated by at least one signal domain. Leukocytes were scored as nullisomic if they showed no signal for the heterochromosomes or the chromosome 12, in the presence of a regular signal of the other chromosome (chromosome 12 or X and Y, respectively). No FISH signal in a leukocyte showing DAPI stain was considered as no hybridization. Only slides with a hybridization rate >99% were scored. A total of 1000 leukocytes were scored for each patient and control.

Results were presented as mean ± SEM throughout the study. The data were analysed by one-way ANOVA followed by the Duncan’s multiple range test. The SPSS 9.0 for Windows software was used for statistical evaluation. A statistically significant difference was accepted when the P-value was <0.05.

Results

Patients with azoospermia or with Yq microdeletions had a mean age significantly lower than that of normozoospermic fertile men (P < 0.05, ANOVA followed by the Duncan test).

The main sperm parameters are reported in Table I. Leukocyte aneuploidies are reported in Figure 1. The rate of sex chromosome aneuploidies in peripheral leukocytes was significantly higher in patients with OAT (0.74 ± 0.09%), azoospermia (1.15 ± 0.15%) or Yq microdeletions (1.54 ± 0.40%) compared with controls (0.15 ± 0.03%) (P < 0.05, ANOVA followed by the Duncan test). Patients with Yq microdeletions had a significantly higher leukocyte aneuploidy rate compared with OAT, but not azoospermic patients (P < 0.05, ANOVA followed by the Duncan test).

Table I. Karyotype, age and sperm parameters of patients and controls

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>Normozoospermic (n = 8)</th>
<th>OAT (n = 20)</th>
<th>Azoospermia (n = 15)</th>
<th>Yq microdeletions (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.7 ± 1.7</td>
<td>33.9 ± 1.0</td>
<td>31 ± 0.9*</td>
<td>31.7 ± 2.2*</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>4.2 ± 0.3</td>
<td>3.2 ± 0.2</td>
<td>2.8 ± 0.3</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.9 ± 0.02</td>
<td>8.1 ± 0.1</td>
<td>8.0 ± 0.1</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Density (million/ml)</td>
<td>72.4 ± 10.2</td>
<td>3.2 ± 0.8</td>
<td>0</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Total motility (%)</td>
<td>60.9 ± 3.4</td>
<td>28.6 ± 3.6</td>
<td>NA</td>
<td>40*</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>51.6 ± 0.6</td>
<td>13.7 ± 2.2</td>
<td>NA</td>
<td>50*</td>
</tr>
<tr>
<td>Normal forms (%)</td>
<td>34.0 ± 2.2</td>
<td>12.6 ± 1.3</td>
<td>NA</td>
<td>30*</td>
</tr>
</tbody>
</table>

Results are mean ± SEM. Normo = normozoospermic fertile men; OAT = oligoasthenozoospermia; Yq = microdeletions of the Y chromosome long arm (Yq); NA = not assessable. *P < 0.05 versus Normo.

*Only one datum was available since 2 Yq microdeleted patients were azoospermic.
Leukocyte aneuploidy in infertile men

A wide range of numerical chromosomal abnormalities was found in the leukocytes of patients with abnormal sperm parameters or Yq microdeletions (Table II). The most frequent karyotype found in patients and controls was 45,X0, followed by 47,XXY, which ranked second in OAT, azoospermics and control, and 45,Y0, which ranked second in patients with Yq microdeletions. Overall, sex chromosomes abnormalities accounted for the total aneuploidy rate in normal men, whereas they were 0.59%, 0.96% and 1.36% in patients with OAT, azoospermia or Yq microdeletions, respectively. The rate of malsegregation events involving the X chromosome (1.49%) was similar to that of the chromosome Y (1.51%) in patients and controls.

Discussion

We found that patients with OAT or azoospermia had a significantly greater number of leukocytes with sex chromosome aneuploidy than fertile normozoospermic men. Our data underline a progressive increase of somatic cell aneuploidy rate in patients with a more severe spermatogenic impairment, such as those with azoospermia. Indeed, patients with OAT had a five times higher leukocyte sex chromosome aneuploidy rate than normal subjects, whereas it was about eight times higher in azoospermic patients. The increased sex chromosome aneuploidy rate in somatic cells of patients with impaired spermatogenesis provides evidence for the presence of a generalized abnormality in the molecular mechanisms controlling cell division. This results in a mitotic/meiotic instability that leads to chromosome malsegregation events. This hypothesis is further supported by a study showing an increased sex chromosome aneuploidy rate in leukocytes of patients with severe oligo-asthenozoospermia. Indeed, a 13-fold increase in heterochromosome aneuploidies was found in this group of patients with “unexplained” oligo-asthenozoospermia compared with normal donors (Gazvani et al., 2000).

Although the molecular nature and the site of action of the meiotic abnormality is not clearly known, it predisposes the chromosome to malsegregation events during cell division (both mitosis and meiosis). Malsegregation affects the various chromosomes differently. Indeed, sex chromosomes appear to be more at risk of partition errors than the autosomes. 12 evaluated in this study. Indeed, the vast majority of karyotype abnormalities found in leukocytes were sex chromosome monosomies and trisomies. The only abnormal karyotype involving chromosome 12 was its monosomy, which ranged from 0.6 to 1 leukocyte per patient, whereas no such an alteration was found in normal men (see Table II). The more frequent heterochromosome involvement in malsegregation events does not seem to be ascribable to a technical-related problem, since it has also been reported by Gazvani et al. (2000). These authors found chromosome 18, the autosome used in the triple-colour FISH in their study, to be affected caly.

Table II. Number of leukocytes with chromosome abnormalities in fertile normozoospermic men and in patients with OAT, azoospermia or microdeletions of the chromosome Y long arm

<table>
<thead>
<tr>
<th></th>
<th>Normozoospermic (%)</th>
<th>OAT (%)</th>
<th>Azoospermia (%)</th>
<th>Yq microdeletions (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>47,XXY</td>
<td>0.5 ± 0.2 (0.05)</td>
<td>2.1 ± 0.3 (0.20)</td>
<td>3.1 ± 0.5 (0.28)</td>
<td>3 ± 0.6 (0.29)</td>
</tr>
<tr>
<td>47,XYY</td>
<td>0 ± 0 (0)</td>
<td>0.5 ± 0.3 (0.05)</td>
<td>1.5 ± 0.3 (0.13)</td>
<td>1.7 ± 1.2 (0.16)</td>
</tr>
<tr>
<td>45,X0</td>
<td>0.6 ± 0.2 (0.06)</td>
<td>2.4 ± 0.4 (0.23)</td>
<td>3.8 ± 0.4 (0.36)</td>
<td>5.3 ± 1.9 (0.52)</td>
</tr>
<tr>
<td>45,Y0</td>
<td>0.3 ± 0.2 (0.02)</td>
<td>0.8 ± 0.2 (0.07)</td>
<td>1.7 ± 0.3 (0.14)</td>
<td>3.7 ± 2.0 (0.36)</td>
</tr>
<tr>
<td>46,XX</td>
<td>0 ± 0 (0)</td>
<td>0.2 ± 0.1 (0.02)</td>
<td>0.3 ± 0.2 (0.03)</td>
<td>0.3 ± 0.3 (0.03)</td>
</tr>
<tr>
<td>Sex chromosome nullisomy</td>
<td>0 ± 0 (0)</td>
<td>0.2 ± 0.2 (0.02)</td>
<td>0.2 ± 0.1 (0.02)</td>
<td>0 ± 0 (0)</td>
</tr>
<tr>
<td>Chromosome 12 monosomy</td>
<td>0 ± 0 (0)</td>
<td>0.6 ± 0.3 (0.05)</td>
<td>0.8 ± 0.3 (0.08)</td>
<td>1 ± 0.6 (0.10)</td>
</tr>
</tbody>
</table>

Figure 1. Leukocyte aneuploidy rate (mean ± SEM) for sex chromosomes in patients with oligoasthenoteratozoospermia (OAT; n = 20), azoospermia (Azoo; n = 15) and Y chromosome long arm microdeletions (Yq; n = 3) compared with fertile men with normal sperm parameters (Normo; n = 8).
of Turner’s syndrome (Siffroi et al., 2000). In addition, these findings have a relevant clinical implication for those Yq microdeleted patients who undergo assisted reproductive techniques, since they are at increased risk of generating an aneuploidy offspring. We also found that patients with Yq microdeletions had a high leukocyte aneuploidy rate; however, it was not statistically different from that observed in patients with azoosperma. It is noteworthy that two out of the three Yq microdeleted patients studied had azoospermia. In addition, the aneuploidy rates of chromosomes X and Y were similar. Hence, our data do not provide evidence for the contention that structural abnormalities predispose the Y chromosome to increased mitotic errors. We do not have a definitive explanation for this discrepancy, which may well relate to the low number of Yq microdeleted patients that were enrolled in this study.

Increased sex chromosome abnormalities in peripheral blood of patients with impaired spermatogenesis provides evidence for the existence of a subtle generalized mitotic/meiotic instability, which may disrupt cell division by interfering with either the arrangement of microtubules on the mitotic spindle or the sequence of centromere separation. Such instability predisposes the chromosomes to non-disjunction, which appears to affect sex chromosomes more frequently than autosomes. Sex chromosome aneuploidy may be eliminated through a selective degeneration of the abnormal cell line. This could be the mechanism by which some infertile men develop oligozoospermia or azoospermia.

In conclusion, patients with OAT or non-obstructive azoospermia have a slight, but significantly, increased sex chromosome aneuploidy rate in blood cells, suggesting the presence of a generalized meiotic instability, which leads to chromosome malsegregation. In contrast with recent observations, Yq microdeletions did not seem to predispose to a higher number of malsegregation events in somatic cells compared with patients with azoospermia.

References

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