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Cytogenetic study of women with premature ovarian failure

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ABSTRACT

Etiology of premature ovarian failure (POF) is unclear in most patients. Since some cases are related to X-chromosome abnormalities, cytogenetical studies were conducted in patients with POF. Lymphocyte cultures from eleven patients were compared to cultures from age-matched controls. All individuals presented normal karyotypes. Frequencies of aneuploid and structural chromosome aberrations did not differ between the groups. The X chromosome was more frequently involved in aneuploidy and in premature centromere division in both groups.

INTRODUCTION

Premature ovarian failure (POF) has been defined by a triad of secondary amenorrhea, estrogen deficiency and elevated gonadotropin levels before the age of 40 (Aiman and Smentek, 1985; Coulam et al., 1986). In patients with this condition, ovarian histological features are very similar to those found in post-menopause ovaries resulting from the natural process of follicular atresia (Griffin, 1979).

Etiology and pathophysiology of this syndrome in most patients remain to be understood. No single cause accounts for all the cases on record. Some POF cases appear to have an autoimmune or environmental etiology (Alper et al., 1986). In cases with familial POF occurrence, genetic association is strongly suggested (Mattison et al., 1984). A possible genetic basis is also suggested by the association of family history and early menopause (Cramer et al., 1995). POF also occurs more frequently in fragile X carriers (Partington et al., 1996; Vianna-Morgante et al., 1996).

Some POF cases have been related to numerical or structural chromosome abnormalities, such as karyotypes 45,X, 45,X in mosaic forms, 47,XXX and structural X chromosome abnormalities (Alper et al., 1986; Gandar, 1988; Powell et al., 1994).

It has been shown that primordial germ cells seed the primitive gonad in a 45,X fetus, but degenerate shortly after formation of the primary follicles (Singh and Carr, 1966). In XXX females, the gonads also undergo early involution (Coulam, 1982). Thus, since these patients show variable degrees of gonadal failure, Devi et al. (1994) investigated aneuploid cell frequency in patients with POF by fluorescent in situ hybridization. Using an X chromosome alpha satellite probe a greater percentage of monosomy X cells was found, suggesting that, in
some patients, POF could be associated with a low level 45,X/46,XX mosaicism.

In the present study, 11 women with POF were investigated. Karyotype analysis and frequency of structural chromosome aberrations were considered as well as aneuploidy frequency. For the latter, a considerable amount of cells were analyzed in an attempt to detect possible low mosaicism. The X chromosome and its inactivation pattern were especially noted. In addition, we investigated the premature centromere division (PCD) frequency that has been associated with increased X chromosome aneuploidy (Fitzgerald, 1983; Méhes and Bühler, 1995).

SUBJECTS AND METHODS

POF women and controls

The study was performed on 11 women with POF ranging from 28 to 39 years old who met the following criteria for POF diagnosis: spontaneous secondary amenorrhea occurring before the age of 40, elapse of at least 12 months, menarche at normal age, increased levels of gonadotropins (FSH and LH) and low estrogen levels. Of the 11 women, six had ovarian biopsies that showed that the gonads were histologically composed of fibrous stroma with no follicles.

Eleven healthy women with normal menses, age-matched with each patient, were used as controls.

Lymphocyte cultures

Peripheral blood lymphocytes were cultured for 72 h in RPMI 1640 medium, supplemented with 20% calf serum and phytohemagglutinin P. Cultures for R-banding were grown in the presence of bromodeoxyuridine for 7 h prior to harvesting. Cytological preparation and slide mounting were performed by routine methods.

For each individual, a cytogenetic study was done through the analysis of 180 metaphase cells: 40 cells with solid staining, 40 with G-banding (Sanchez et al., 1973) and 100 with R-banding (Ribeiro and Melaragno, 1987). All counts were done by one observer in a blind test.

We scored the aneuploidy frequency in all cells analyzed. The X inactivation pattern in aneuploid cells was scored by R-banding. Structural chromosome aberrations and premature centromere division frequencies were scored on slides with solid staining and G-banding. When cells presented some alteration in solid staining, they were reanalyzed after destaining and G-banding to better reveal the chromosome aberration. C-group chromosomes that could not be identified were referred to as C chromosomes. Classification of the structural aberrations was carried out considering gap, break and rearrangement frequencies.

Data of the two groups were statistically compared by chi-square or Fisher tests.

RESULTS AND DISCUSSION

All POF women and controls had a normal female 46,XX karyotype, which is the most common POF karyotype.

Frequency comparisons between the groups for different types of acquired structural aberrations showed no significant differences (data not shown). The various aberration types identified had no preferential chromosome distribution among autosomal or sex chromosomes.

In the literature, karyotype-phenotype correlation has proven to be useful in genetic mapping. The study of women with constitutional chromosome aberrations suggested that the existence of a region on the long arm of the X chromosome (Xq13-q26), named the “critical region”, must be intact for normal ovarian functioning (Therman et al., 1990; Mumm et al., 1996).

Based on the study of females with structural X chromosome abnormalities, Tharapel et al. (1993) deduced the location of a possible Xq POF gene at Xq26.1-q27. Powell et al. (1994) proposed POF locus heterogeneity and argued that there may be a second gene for ovarian function located at Xq13.3-q21.1.

Concerning the aneuploidy frequency (hypodiploid and hyperdiploid cell frequencies), we also found no significant difference between the POF and control groups (Table I). Analysis of chromosome distribution involved in aneuploidy in both groups revealed that X chromosomes were significantly more involved than any other chromosomes (Figure 1). The involvement of X chromosomes with other chromosomes in hypodiploid cells (36 of 105) and hyperdiploid cells (19 of 31) was significantly greater than expected ($\chi^2 = 226.61$ and 241.25, respectively) if the loss or gain of chromosomes occurred randomly among the 23 chromosome pairs.

**Table I** - Number and frequency of hypodiploid and hyperdiploid cells and cells with premature centromere division (PCD) in POF patient and control groups.
<table>
<thead>
<tr>
<th></th>
<th>Hypodiploid cells</th>
<th>Hyperdiploid cells</th>
<th>Cells with PCD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Total of cells</td>
</tr>
<tr>
<td>POF</td>
<td>57</td>
<td>2.88</td>
<td>1.980</td>
</tr>
<tr>
<td>Controls</td>
<td>48</td>
<td>2.42</td>
<td>1.980</td>
</tr>
<tr>
<td>Chi-square test</td>
<td>$\chi^2 = 0.79$</td>
<td>$\chi^2 = 0.81$</td>
<td>$\chi^2 = 1.84$</td>
</tr>
</tbody>
</table>

**Figure 1** - Distribution of chromosome loss (A) and gain (B) observed in aneuploid cells from POF and control groups.

Similarly, we found no significant difference in premature centromere division frequencies between the two groups of individuals (Table I), but there was a preferential involvement of the X chromosome compared to other chromosomes ($\chi^2 = 37.08$).

These data agree with most of the studies about X aneuploidy increasing with age in female peripheral blood preparations (Ford and Russel, 1985; Nowinski et al., 1990; Kormann-Bortolotto et al., 1993; Guttenbach et al., 1995). An increase in X chromosome premature centromere division, also found in aging, appears to represent irregular centromeric behavior, a mechanism of nondisjunction causing X aneuploidy in somatic cells (Fang et al., 1975). Fang *et al.* (1975) found age effects in X chromosome loss in the mitotic complement of the human ovary, but they did not find a correlation between ovarian X loss and clinical menstrual state of the patients. In fact, increased X aneuploidy occurred in both POF and control groups in the present study, suggesting no relation to the cause of POF in these patients.

Our results do not support those of Devi *et al.* (1994), who found an increase of monosomic cells in women with...
Concerning the X inactivation pattern in aneuploid cells with loss and gain of X chromosomes, Abruzzo et al. (1985) found that in over 85% of those cells the inactive X chromosome was involved. They argue that either the inactive X chromosome has a special propensity for undergoing mitotic errors or that mitotic errors occur at random followed by subsequent selection. When we analyzed the X inactivation pattern in aneuploid cells in POF patients and controls, we found an absence of the inactive X chromosome in all 18 hypodiploid cells (10 POF and 8 controls). This preferential involvement of the inactive X chromosome did not occur in hyperdiploid cells. We found four 47,XXX cells in two POF patients and one 48,XXXX cell in the control group. All the cells had two active X chromosomes. As a result, we believe that alteration in the replication pattern is not related to the cause of POF in these patients.

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RESUMO

A etiologia da insuficiência ovariana prematura (POF) ainda é desconhecida na maioria dos pacientes. Uma vez que alguns casos estão associados a anormalidades do cromossomo X, estudamos citogeneticamente pacientes portadores de POF. Comparamos as culturas de linfócitos de onze pacientes com as de indivíduos controles de mesmas idades. Todos os indivíduos estudados apresentaram cariótipos normais. As frequências de células aneuplóides e com aberrações cromossômicas estruturais não diferiram entre os grupos. Verificamos uma maior frequência de aneuploidia e de divisão prematura do centrômero do cromossomo X nos dois grupos.

REFERENCES


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