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Cytogenetic abnormalities in Tunisian women with premature ovarian failure

Anomalies chromosomiques et insuffisance ovarienne prématurée en Tunisie

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ABSTRACT

To identify the distribution of chromosome abnormalities among Tunisian women with premature ovarian failure (POF) referred to the department of Cytogenetic at the Pasteur Institute of Tunis (Tunisia), standard cytogenetic analysis was carried out in a total of 100 women younger than 40 affected with premature ovarian failure. We identified 18 chromosomal abnormalities, including seven X-numerical anomalies in mosaic and non-mosaic state (45,X; 47,XXX), four sex reversal, three X-structural abnormalities (terminal deletion and isochromosomes), one autosomal translocation and one supernumerary marker. The overall prevalence of chromosomal abnormalities was 18% in our cohort. X chromosome aneuploidy was the most frequent aberration. This finding confirms the essential role of X chromosome in ovarian function and underlies the importance of cytogenetic investigations in the routine management of POF.

Mots clés :
Insuffisance ovarienne prématurée
Caryotype
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RÉSUMÉ

L'insuffisance ovarienne prématurée (IOP) est une affection peu fréquente touchant 1 % des femmes âgées de moins de 40 ans. Elle est définie comme la présence d'une aménorrhée primaire ou l'apparition d'une aménorrhée secondaire avant l'âge de 40 ans et associe une hypooestrogénie et une élévation des gonadotrophines. Nous nous sommes proposé d'identifier les causes génétiques chez 100 femmes atteintes d'IOP adressées au laboratoire de cytogénétique de l'institut Pasteur de Tunis. Pour chaque patiente, un caryotype sanguin en bandes RHG a été réalisé. Nous avons décelé 18 % d'anomalies

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chromosomiques touchant essentiellement le chromosome X (anomalies de nombre et/ou de structure), quatre cas de réversion de sexe et une translocation autosomique. Ces chiffres incitent à la considération de l'étude cytogénétique au cours du bilan de l'insuffisance ovarienne prématurée, encore non systématique dans notre environnement.

1. Introduction

Premature ovarian failure (POF, OMIM 311360) is defined as the cessation of ovarian function before the age of 40, associated with elevated gonadotrophin serum levels [1]. The diagnostic is based on elevated FSH in a menopausal range (usually $\geq$ 40 IU/l) detected on at least twice within a few weeks (Conway 2000). POF occurs in one per 1000 women aged 30 and in one per 100 women by aged 40. This disorder may be characterized by primary amenorrhoea or secondary amenorrhoea for at least 4–6 months and is considered as a common pathology leading to infertility [2]. In fact, POF leads to 10% of ovulatory female sterility [1]. POF can be observed as a syndromic form associated with other features or as an isolated condition. The etiology of POF is highly heterogeneous, including genetic, metabolic, infectious, autoimmune, and iatrogenic (anticancer treatment) causes [3]. The most common genetic cause of POF is X chromosome abnormalities ranging from numerical defects, deletions, X-autosome translocations, and isochromosomes [3–5]. Chromosomal abnormalities have been recognized as a frequent cause of POF, with varying percentages in reported series [6–8]. However, in a large proportion of cases, no etiology is found and POF is classified as idiopathic [3].

The aim of this study was to investigate the frequency and type of chromosome abnormalities in Tunisian POF patients in order to assess the efficacy of cytogenetic screening.

2. Patients and methods

2.1. Patients

Between January 2002 and December 2012, 100 POF patients were referred by clinicians for cytogenetic analysis to the Cytogenetic Department at the Pasteur Institute of Tunis.

Inclusion criteria were primary amenorrhoea or secondary amenorrhoea for more than 4 months prior to the age of 40, with FSH serum levels higher than 40 IU/L. All of the patients referred to our department underwent a complete clinical assessment, including complete medical and gynaecological history, in order to exclude any other related pathology. Women with clinical signs of Turner's syndrome were excluded as well as women with personal history of autoimmune disease or clinical antecedents of pelvic surgery. Written informed consent was obtained from all participants.

2.2. Methods

2.2.1. Conventional cytogenetic analysis

Metaphase chromosome spreads were obtained from phytohaemagglutinin-stimulated peripheral blood lymphocytes. Karyotype analysis was performed on RHG-banded metaphase chromosomes using a standard protocol that generated 500–550 band resolutions. A minimum of 20 metaphases per patient were analyzed. If any cell among the 20 showed a non-model cell (45,X or 47,XXX), an additional 30 cells were counted. Chromosome polymorphisms, for example pericentric inversion of chromosome 9 and centromeric heterochromatin variants, were recorded, but classified as normal. Chromosomal abnormalities have been reported in accordance with the current international standard nomenclature (ISCN 2009).

2.3. Fluorescent in situ hybridization (FISH) analysis

FISH study was performed using alpha satellite probes of chromosome X and Y (alpha satellite DXZ 1 probe/Green Q Biogen, chromosome Y alpha satellite DYZ 3 probe/Red Q Biogen) and using LSI SRY, p11.3 Spectrum Orange, Abbot-Vysis. The application of the probes was done according to the manufacturer's instructions. A range of 100 nuclei and metaphases were taken into account.

3. Results

One hundred POF patients were included in this study. The average age was 26.95 $\pm$ 6.4 years (16–40) at the time of cytogenetic exploration. Most of our patients (n = 60; 60%) presented with secondary amenorrhoea (SA), while the others presented with primary amenorrhoea (PA).

Table 1 shows the characteristics of each group. These patients do no present any specific somatic anomalies, except for one woman who displayed a blepharo-phimosis epicantus syndrome.

We detected 18 chromosomal abnormalities (18%) using karyotype analysis. Among these patients, one case showed an autosomal abnormality, another woman had a supernumerary marker at a mosaic state and 16 cases had gonosomal abnormalities (88.8%). The rest of patients (82) had normal karyotype.

The frequency of karyotypic abnormalities in patients with PA (13/40, 32.5%) was higher than the frequency of patients with SA (5/60, 8.3%).

The distribution and details of chromosomal abnormalities are summarized in Table 2.

The most common abnormality was numerical X chromosome abnormalities, which were found in seven cases (38.8%). Moreover, we detected different kinds of structural X chromosome abnormalities: one X(q) deletion, one isochromosome [i(Xq)] and one mosaic isodicentric that was confirmed by FISH. Furthermore, in this latter case, FISH allowed the detection of a monosomic X cell line in a proportion of 12% of observed cells.