C. R. Biologies 337 (2014) 223–228

Contents lists available at ScienceDirect

Comptes Rendus Biologies

www.sciencedirect.com

Genetics/Génétique

Chromosomal evaluation in a group of Tunisian patients with non-obstructive azoospermia and severe oligozoospermia attending a Tunisian cytogenetic department

Anomalies chromosomiques et infertilité masculine : étude rétrospective de 476 hommes tunisiens azoospermiques ou oligozoospermiques sévères

Ahlem Amouri [a,b,*,1], Wajih Hammami [a,b,1], Olfa Kilani [a,b], Abderrezzak Bouzouita [c], Wiem Ayed [a,b], Mounir Ben Meftah [d], Mohamed Khrouf [d], Anis Fadhlaoui [d], Sonia Abdelhak [b], Fethi Zhioua [d], Mohamed Habib Jaafoura [e,f]

[a] *Department of Histology and Cytogenetics, Institut Pasteur de Tunis, 13, place Pasteur, BP 74, 1002 Tunis Belvédère, Tunisia*
[b] *Laboratory of Biomedical Genomics and Oncogenetics, Institut Pasteur de Tunis, Tunis, Tunisia*
[c] *Department of Urology, Charles-Nicolle University Hospital (Medical University of Tunis), boulevard du 9-Avril-1938, Bab Saadoun, Tunis, Tunisia*
[d] *Department of Obstetrics and Gynecology, Aziza Othmana University Hospital (Medical University of Tunis), place du Gouvernement, La Kasba, 1008 Tunis, Tunisia*
[e] *Laboratory of Electronic Microscopy, Faculty of Medicine, 1007 Tunis, Tunisia*
[f] *Department of Pathology, Institut Kassab d'Orthopédie, Ksar Said, Tunisia*

ARTICLE INFO

Article history:
Received 5 March 2013
Accepted after revision 15 February 2014
Available online 21 March 2014

Keywords:
Male infertility
Chromosome abnormalities
Oligozoospermia
Azoospermia

ABSTRACT

Male infertility is the cause in half of all childless partnerships. Numerous factors contribute to male infertility, including chromosomal aberrations and gene defects. Few data exist regarding the association of these chromosomal aberrations with male infertility in Arab and North African populations. We therefore aimed to evaluate the frequency of chromosomal aberrations in a sample of 476 infertile men with non-obstructive azoospermia ($n = 328$) or severe oligozoospermia ($n = 148$) referred for routine cytogenetic analysis to the department of cytogenetics of the Pasteur Institute of Tunis. The overall incidence of chromosomal abnormalities was about 10.9%. Out of the 52 patients with abnormal cytogenetic findings, sex chromosome abnormalities were observed in 42 (80.7%) including Klinefelter syndrome in 37 (71%). Structural chromosome abnormalities involving autosomes (19.2%) and sex chromosomes were detected in 11 infertile men. Abnormal findings were more prevalent in the azoospermia group (14.02%) than in the severe oligozoospermia group (4.05%). The high frequency of chromosomal alterations in our series highlights the need for efficient genetic testing in infertile men, as results may help to determine the prognosis, as well as the choice of an assisted reproduction technique. Moreover, a genetic investigation could minimize the risk of transmitting genetic abnormalities to future generations.

* Corresponding author. Department of Histology and Cytogenetics, Institut Pasteur de Tunis, 13, place Pasteur, BP 74, 1002 Tunis Belvédère, Tunisia.
E-mail addresses: amouri.ahlem@pasteur.rns.tn, amouriahlem@yahoo.fr (A. Amouri).
[1] These authors contributed equally.

1631-0691/$ – see front matter © 2014 Académie des sciences. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.crvi.2014.02.006

Mots clés :
Infertilité masculine
Anomalies chromosomiques
Oligozoospermie
Azoospermie

RÉSUMÉ

On estime à près de 10 % la fréquence de l'infertilité masculine dans la population humaine. Les causes de cette infertilité sont multiples, notamment génétiques, et plus particulièrement chromosomiques. Dans ce travail, nous avons exploré 476 patients tunisiens présentant des troubles non obstructifs de la spermatogenèse (328 azoospermiques et 148 ayant une oligospermie sévère). Une étude du caryotype constitutionnel est réalisée chez l'ensemble de ces patients, avec marquage chromosomique en bandes RHG. Nous avons pu révéler ainsi 52 anomalies chromosomiques (fréquence 10,9 %), réparties en 46 anomalies gonosomales et 6 anomalies autosomiques. La fréquence de ces anomalies est plus élevée dans le groupe des azoospermies, chez qui la constitution 47,XXY prédominait. Les autres anomalies étaient autosomales, correspondant à des translocations, une inversion et des chromosomes marqueurs surnuméraires. Nos résultats confirment la forte prévalence des anomalies chromosomiques chez les hommes atteints de troubles sévères de la spermatogenèse. Nos chiffres sont comparables à ceux décrits dans la littérature, incitant à la considération de l'étude cytogénétique au cours du bilan d'infertilité masculine et justifiant la pratique systématique du caryotype avant toute tentative de procréation médicalement assistée.

1. Introduction

Infertility is a major health problem affecting up to 15% of couples of reproductive age [1]. For many years, it was assumed that most reproductive problems could be attributed to the female partner, but research in recent years has demonstrated that 30–50% of infertility is caused by a male factor [2].

The term "male infertility" does not constitute a defined clinical syndrome, but rather a collection of different conditions exhibiting a variety of etiologies. Genetic factors accounts for 10–15% of severe male infertility [3,4]. Among the genetic abnormalities found in infertile men, those involving chromosome anomalies amount to about 8%, the most frequent one being the 47,XXY karyotype that characterizes the Klinefelter Syndrome [5]. Because modern artificial reproduction techniques like intracytoplasmic sperm injection (ICSI) can help couples to overcome infertility, it is imperative to analyze the underlying genetic causes of male infertility.

The aim of this study was to determine the frequency of chromosomal abnormalities in a group of Tunisian infertile men attending the Pasteur Institute of Tunis.

2. Materials and methods

2.1. Patients

A total of 476 Tunisian infertile patients with idiopathic oligozoospermia or azoospermia were enrolled in the study. These infertile men with sperm disorders were referred for karyotyping to the department of histology and cytogenetics at the Pasteur Institute of Tunis between 2006 and 2012. Patients were checked for the history of relevant medical disorders, e.g., diabetes, renal, liver disease, radiation, endocrine abnormality (e.g., hypogonadotropic hypogonadism), exposure to toxins and/or medication affecting spermatogenesis, acquired and congenital structural defects of urogenital system; history of surgical intervention of genital tract. All cases of azoospermia or severe oligozoospermia resulting from endocrine or obstructive cases were excluded from our study. Upon verifying that sperm density was lower than 5×10^6/mL, patients were asked to sign and informed consent form for genetic analysis.

2.2. Karyotyping

Cytogenetic analysis was performed from phytohemagglutinin-stimulated lymphocyte cultures by routine laboratory protocol. For microscopic analysis, R-banded metaphase spreads were analyzed and abnormalities recorded according to the current International System for Human Cytogenetic Nomenclature [6]. A resolution of 550 to 700 bands per haploid karyotype was used for the routine analysis.

For each patient, at least 20 well-spread metaphases were analyzed and two to five metaphases were karyotyped. When at least one of the 20 showed a loss or gain of a chromosome, especially X or Y chromosome, the number of analyzed metaphases was increased to 30. If a second abnormal cell was observed, the analysis was considered complete; otherwise, the number of metaphases was increased to 50. Sex chromosome mosaics occurring at a level of less 5% were not considered as well as pericentric inversions of chromosome 9 or other structural chromosome variants and polymorphisms that were considered as normal cytogenetic events.

The statistical analysis was performed using the Chi^2 test. A P-value < 0.05 was considered to indicate statistical significance.

3. Results

The present study only entailed 476 men with non-obstructive subfertility. They included men with azoospermia ($n = 328$; 68.91%) and severe oligozoospermia with sperm counts lower than 5 million/mL ($n = 148$; 31.09%).