CYTOGENETIC INVESTIGATIONS IN COUPLES WITH MALE STERILITY

ANCA MITROI[1]

[1]*Clinical Emergency County Hospital of Constanța*

Keywords: *chromosomal abnormalities, deletion, translocation*

Abstract: *The paper proposes the identification of the type and frequency of chromosomal abnormalities in couples with male factor infertility, as well as the identification of particularities in such couples. The study was realized on 26 couples in which the male partners had azoospermia or oligozoospermia. Both members of the couples were investigated through medical and familial anamnesis and conventional cytogenetic analyses. For the identification of couple's characteristics with chromosomal abnormalities, bivariate analyses were realized. Regarding this batch, chromosomal abnormalities with a frequency of 7.69% for males and 3.84% for females have been identified. The chromosomal abnormalities identified were: terminal deletion of Yq (n=1) and reciprocal translocation of autosomes (n=2). Bivariate analyzes revealed statistical significant differences in the male partner regarding the infertility duration, concentration of spermatozoa and familial anamnesis, positive for reproduction failure in the couples with chromosomal abnormalities.*

Cuvinte cheie: *anomalii cromosomiale, deleție, translocație*

Rezumat: *Lucrarea îşi propune identificarea tipului şi frecvenței anomaliilor cromosomiale la cuplurile cu sterilitate de cauză masculină, precum şi identificarea unor particularități ale acestor cupluri. Studiul a fost realizat pe 26 de cupluri cu sterilitate la care consultanzii au prezentat azoospermie sau oligospermie. Cuplurile au fost investigate prin efectuarea anamnezei medicale personale şi familiale şi efectuarea cariotipului constituțional pentru ambii membrii. Pentru a identifica caracteristicile cuplurilor cu anomalii cromosomiale a fost realizată analiza bivariată. Pe acest lot au fost identificate anomalii cromosomiale cu o frecvență de 7,69% la sexul masculin şi 3,84% la sexul feminin. Anomaliile cromosomiale de structură identificate au fost reprezentate de deleția terminală a cromosomului Y (n=1) şi translocații reciproce ale autosomilor (n=2). Analiza bivariată a relevat diferențe semnificative statistic la cuplurilor cu anomalii cromosomiale prezente la sexul masculin în ceea ce priveşte durata sterilității, concentrația spermatozoizilor şi anamneza familială pozitivă pentru tulburări ale funcției de reproducere.*

INTRODUCTION

According to the definition of the World Health Organization (WHO), couples are considered infertile if, no pregnancy occurs after one year of unprotected regular intercourse.(1) Sterility can be divided in female factor sterility (40%), male factor sterility (40%) and combined factors sterility (20%).(2)

There are many factors involved in the etiology of male factor sterility; among these, as it has been proved in the last decade, the genetic factors lie at the basis of an increased number of cases. Thus, constitutional chromosomal abnormalities affect 15% of males with azoospermia and 6% of males with severe oligozoospermia.(3)

Although the frequency of chromosomal abnormalities differs in studies according to the inclusion criteria, the following conclusions may be drawn: the frequency of constitutional chromosomal abnormalities increases at the same with the decrease of spermatozoa number in ejaculation and the frequency of sexual chromosomes anomalies is higher in the patients with non-obstructive azoospermia, while the structural autosomal anomalies are predominant in the patients with oligozoospermia.

PURPOSE OF THE STUDY

Given the important role of chromosomal abnormalities in male sterility etiology, the paper aims at identifying the type and frequency of chromosomal abnormalities involved in this type of reproduction failure, as well as at identifying the particularities of these couples.

MATERIAL AND METHODS

The observational retrospective study was accomplished on 26 couples with male factor sterility. The inclusion criteria were: presence of sterility of at least one year, plus azoospermia or oligozoospermia signalled in at least two sperm tests within an interval of 6-12 weeks. The exclusion criteria were the sperm analysis modifications due to an acquired obstruction of the genital ducts, genital infections, and congenital bilateral absence of vas deferens or abnormal results upon vasography, previous radiotherapy or chemotherapy, as well as the presence of gynaecologic cause in female sterility.

The couples that fulfilled the above criteria fully completed a multiple choice questionnaire which contained data

[1]Corresponding author: Anca Mitroi, B-dul. Tomis, nr. 145, Constanța, 550330, România, e-mail: anatomie_patologica@yahoo.com, tel +40723398798
Article received on 28.10.2011 and accepted for publication on 31.01.2012
ACTA MEDICA TRANSILVANICA March 2012;2(1):211-213

about medical and family anamnesis, physical examination and laboratory investigations for both members of the couple.

Both members were cytogenetically investigated through karyotyping conducted by G banding using the peripheral blood lymphocyte cultures according to the standard protocols.(4) In order to obtain a high level of G banding resolution, cell cycle synchronization with 5-fluorodeoxiuridine and thymidine was realized. The spread banding metaphases without chromosome overlapping were analyzed with an Axioskope 4 Zeiss microscope and karyotyping was realized with Ikaros Meta Systems software. In each case, 30-50 metaphases were analyzed and at least five cells were karyotyped.

In order to establish the statistical significance between the variables of the couples with chromosomal abnormalities and the variables of the couples without chromosomal abnormalities, we used bivariate analyses, the t-student test or the χ^2test. The level of significance was $p<0.005$.

RESULTS

The 26 couples included in the study presented the following characteristics: the mean age of the male partner was 34.5±4.12 years and the mean age of the female partner was 31.35±3.58 years. The majority of couples (88%) presented primary sterility and only two cases were diagnosed with secondary sterility. The sterility duration varied between 1.5 and 11 years, and the mean duration was of 4.29±2.74 years. The semen analysis revealed: azoospermia in 8% of cases, severe oligozoospermia for 27% of cases, moderate oligozoopsermia for 34% of the cases and mild oligozoospermia for 31% of cases. Family anamnesis was positive for reproduction failure in 19% of couples. Physical examination revealed no particularities; only in one case, bilateral testicular volume was reduced. Cytogenetic analyses revealed the presence of structural chromosome abnormalities in one member for 3 couples (11.53%). In two couples, the chromosomal abnormality was detected in the male partner and for one couple, in the female partner. General frequency of chromosomal abnormalities was of 5.78%. The frequency for males was of 7.69% (n=2) and of 3.84% for females (n=1). The types of structural chromosome abnormalities were: unbalanced abnormalities (terminal deletion of Yq) and balanced rearrangements (reciprocal translocations of autosomes).

The karyotypes identified in our patients group are presented in table I.

Table no. 1. Chromosomal abnormalities detected in the couples with male factor sterility (n=26)

Chromosomal abnormality	Females with chromosomal abnormality(n=1)	Males with chromosomal abnormality (n=2)
Deletions	-	46,XdelY(q11.23→qter)
Autosomes reciprocal translocation	46,XX,t(3;18)(q24;q11)	46,XY,t(11,22)(q23;q11.2)

Terminal deletion of Yq was identified in a 40-year old male with azoospermia and couple infertility for ten years. The family anamnesis revealed in the patient's family, the presence of a brother with sterility due to mumps orchitis.

Reciprocal translocation t (11;22) was identified in a 27-year old proband with severe oligozoospermia and couple infertility for 9 years. The family anamnesis revealed the presence in the patient's family of another brother who had a malformed stillbirth in the reproduction history. The brother karyotype was 46,XY.

Reciprocal translocation t (3;8) was identified in a 34-year old female partner who had hyperprolactinemia. The couple presented primary infertility for 5 years and was included in the study because the male partner presented oligozoospermia.

Figure no. 1. GTG banding karyograme, 46,XdelY(q11.23→qter) karyotype

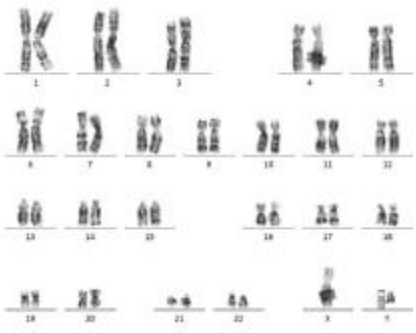

Table no. 2. Bivariate analyses of couples with male factor sterility and chromosomal abnormalities in males

Chromosomal abnormalities in males from couples with sterility			
	not present	present	P
	N=23	N=2	
Male partner mean age	34,04±4,14	38,5±2,12	0,151
Female partner mean age	31,09±3,72	33±1,41	0,316
The mean span of sterility	3,80±2,41	9,5±0,71	0,003
Male partner mean weight (kg)	84,57±11	81±1,41	0,657
Male partner mean height (m)	1,79±0,06	1,8±0,01	0,793
Male partner body mass index (kg/m^2)	26,41±2,95	25±0,04	0,513
Female partner mean weight (kg)	1,66±0,05	1,70±0,04	0,260
Female partner mean height (m)	59,17±5,77	65±7,07	0,189
Female partner body mass index (kg/m^2)	21,54±1,74	22,69±3,41	0,407
Mean sperm concentration (x 10^6/ml)	4,07±2,54	0,1±0,14	0,041
Smoker	39%(9)	50% (1)	0,654
Anterior fertility	13% (3)	0	0,553
Personal medical history	39%(9)	50%(1)	0,654
Positive familial anamnesis	13%(3)	100%(2)	0,042

Bivariate analyses between couples with chromosomal abnormalities in male partners and those without cytogenetic abnormalities (table II) revealed the presence of statistical significant differences in sterility duration which was greater in the couples with chromosomal abnormalities, spermatozoa concentration which was significant lower in the patients with chromosomal abnormalities and family anamnesis positive for reproduction failure in couples with chromosomal abnormalities.

DISCUSSIONS

For many couples, infertility is associated with reduced sperm concentration, which in certain cases is due to chromosomal abnormalities.(5) There are numerous studies in which the presence of azoospermia or oligozoopsermia is associated with the existence of numerical or structural gonosomes abnormalities, as well as with structural chromosome abnormalities (reciprocal or Robertsonian translocations); chromosomal abnormalities are identified in 3-13% of patients with sperm concentration abnormality.(6-12) The frequency of chromosomal abnormalities in the males

included in our group is similar with that one identified in other studies.(6-12) Identification of chromosomal abnormalities in the females from couples with male factor sterility is not accidental because sterility is a characteristic of couples, and the presence of the chromosomal abnormalities in the female partner is citied by other studies which investigated the couples with male factor infertility, cytogenetically.

Reciprocal translocations were identified in a male who presented severe oligozoopsermia and in a female with hyperprolactinemia.

Gametogenesis in the male carrier of reciprocal translocation appears more vulnerable than in the female. An important element in this male vulnerability may be the meiotic integrity of the X-Y bivalent, synapsing and recombining at the pseudoautosomal regions at the tips of Xp and Yp. Unpaired autosomal segments might disturb this integrity, leading to disruption of spermatogenesis.(14)

Regarding the terminal deletion of Yq chromosomes identified in our patient, the spermatogenesis failure is explained by the deletion of AZF regions a, b, and c. the most commonly seen deletion involves the AZFc region, in Yq11.23. AZFa or AZFb deletions are more severe in terms of effects than AZFc. The reduction of fertility may be relative, at least for AZFc deletions, and at a younger age, and perhaps with a partner of "excellent" fertility; a man with a deletion may become a parent.(15)

One of the possible explications for a longer duration of infertility in couples with chromosomal abnormalities as against the couples with no chromosomal abnormalities is due to genetic unbalanced of gametes, even after the application of assisted reproductive technologies.

The causal relation existing between chromosomal abnormalities and male infertility are very well known, so the incidence of chromosomal abnormalities is correlated inversely proportional with sperm concentration (16), fact observed in our study, where chromosomal abnormalities are detected in cases with azoospermia or severe oligozoopsermia.

The presence of reproduction failure in family anamnesis gives evidence of the necessity for cytogenetic investigation of proband's family.

CONCLUSIONS

The frequency and the type of chromosomal abnormalities detected in our study are in accordance with other study and the detection of chromosomal abnormalities in female is not randomly, because sterility is a characteristic of the couple. The probability of chromosomal abnormalities in males in the infertile couples is high if the duration of sterility is increased, sperm concentration is much reduced and there are other cases of reproduction failure among the family members.

REFERENCES

1. World Health Organization. WHO Manual for the standardized investigation and diagnosis of the infertile couple. Cambridge, UK: Cambridge University Press. 1993.
2. Speroff L, Glass R, Kase N. Female infertility. In Kase NG (ed): Clinical gynecologic endocrinology and infertility, 6th ed. Baltimore: Lippincott Williams & Wilkins. 1999:1013-1042.
3. Van Assche E, Bonduelle M, Tournaye H et al. Cytogenetics of infertile men. Hum Reprod. 1996;11(Suppl 4):1-26.
4. Dracopli D, Haines J, Korf B et al. Short protocols in human genetics – a compendium from current protocols in human genetics, Wiley. 2004:4.2 – 4.4,4.14 – 4.17.
5. Gekas J, Thepot F, Turleau C et al. Chromosomal factors of infertility in candidate couples for ICSI: an equal risk of constitutional aberrations in women and men. Hum Reprod. 2001;16:82-90.
6. Causio F, Fischetto R, Schonauer L, Leonetti T. Intracytoplasmic sperm injection in infertile patients with structural cytogenetic abnromalities. J Reprod Med. 1999;44: 59- 864.
7. De Braekeleer M, Dao T. Cytogenetic studies in male infertility: a review. Hum Reprod 1991;6:245-250.
8. Dohle G, Halley J, Van Hemel J et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Repord. 2002;17:13-16.
9. Meschede D, Lemcke B, Exeler J et al. Chromosome abnormalities in 447 couples undergoing intracytoplasmatic sperm injection – prevalence, types, sex distribution and reproductive relevance. Hum Reprod. 1998;13:576-582.
10. Peschka B, Schwanitz G, Van der Ven K. Types and frequency of consitutional chromosome aberrations in couples undergoing ICSI. Hum Reprod. 1999;14:2257-2263.
11. Stuppia L, Gatta V, Calabrese G et al. A quarter of men with idiopathic oligo-azoospermia display chromosomal abnormalities and microdeletions of different types in interval 6 of Yq11. Hum Genet. 1998;102:566-570.
12. Van der Ven K, Peschka B, Montag M et al. Increased frequency of congenital chromosomal aberrations in female partners of couples undergoing intracytoplasmatic sperm injection. Hum Reprod. 1998;13:48-54.
13. Hale D. Is X-Y recombination necessary for spermatocyte survival during mammalian spermatogenesis? Cytogenet Cell Genet 1994;65:278-282.
14. Guichaoua M, Quack B, Speed R et al. Infertility in human males with autosomal translocations: meiotic study of a 14;22 Robertsonian translocation. Hum Genet. 1990;86:162-166.
15. Krausz C, Mc Elreavey K. Y chromosome microdeletion in fertile males. Hum Reprod 2001;16:1306-1307.
16. Chandley A, Edmond P, Christie S. Cytogenetics and infertile in man. Karyotype and seminal analysis: results of a five-year survey of men attending a sun fertility clinic. Ann Hum Genet. 1975;39:231-254.