CEOG Clinical and Experimental Obstetrics & Gynecology

Cytogenetic analysis of 10,286 cases with male infertility

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Summary

Purpose: Chromosome analysis of 10,286 cases with male infertility and to discuss the genetic causes of male infertility. *Materials and Methods:* 10,286 patients with azoospermia and oligoasthenozoospermia were collected in the present center from January 2009 to January 2013.Peripheral blood lymphocyte culture and chromosome analysis were performed. *Results:* In all the 10,286 cases with azoospermia and oligoasthenozoospermia, 8,401 cases showed normal karyotype, 538 cases had chromosome polymorphism, accounting for 5.2% and 1,378 cases had chromosomal abnormalities with a frequency of 13.4%; *Conclusions:* Genetic factors are closely related to the occurrence of azoospermia and oligoasthenozoospermia, and chromosome analysis in patients with male infertility is necessary.

Key words: Male infertility; Azoospermia; Oligoasthenozoospermia; Chromosomal abnormality.

Introduction

According to the World Health Organization (WHO) standards, infertility caused by male factors in couples who lived together for more than one year but did not take any contraceptive measures, is defined as male infertility.

Male infertility can be divided into absolute sterility and partially (relative) infertility in terms of clinical manifestations.Absolute sterility has no fertility at all, such as azoospermia and relative infertility has fertility below the cut-off value to be pregnant, such as oligospermia and low sperm motility.

About 15% couples in childbearing age are infertile, among which male factors account for approximately 40%~ 50%.

In recent years, numerous research data show that genetic abnormality is also an important cause of male infertility. Chromosomal abnormalities or gene deletion can affect sperm production, resulting in azoospermia and oligoasthenozoospermia [1].

The present study aimed at chromosome analysis of 10,286 patients with azoospermia oroligoasthenozoospermia, in order to demonstrate the relationship between chromosome abnormality and male infertility.

Materials and Methods

The study included 10,286 patients with azoospermia and oligoasthenozoospermia referred to the present hospital for AID or ICSI from January 2009 to January 2013.

Chromosomal analysis was performed on metaphases obtained from phytohaemagglutinin (PHA)-stimulated peripheral blood-lymphocytes using standard techniques. GTG-banded metaphases were karyotyped and chromosomal anomalies were designated following standard nomenclature guideline (ISCN, 2009). 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) or other abnormality, additional cells were counted or C-banding were combined.

Results

Karyotype analysis of 10,286 cases with azoospermia and oligoasthenozoospermia showed that 8,385 had normal karyotype, 523 chromosome polymorphism (5.2%), and 1,378 abnormal karyotype with a chromosome abnormality rate (CAR) to be 13.4%. The chromosome polymorphism variants included Yqh+ in 184 cases, Yqh- in 91 cases, inv (9) (p12q13) in 65 cases, and other variants, such as variation of 1, 9, 16 constitutive heterochromatin area, satellite or satellite handle of D, G group, etc., in 183 cases).

Chromosomal abnormalities were present in 1,378 cases, which included Robertsonian translocation in 46 cases, autosomal translocation in 77 cases (26 cases involving chromosome 14, 12 cases chromosome 1, ten cases chromosome 22, seven cases chromosome 20, and seven cases chromosome 6), Y-autosomal translocation in 11 cases, X -autosomal translocation in three cases, 32 cases had pericentric inversion (eight cases with chromosome 1 and two cases with chromosome 5), two cases had ring chromosome, two cases had 46, i(X)(q10) karyotype Klinefelters Syndrome in 1,065 cases, ten cases had 47, XY, + mar karyotype, 47, XY+21 in one case, 47, XYY in 34 cases, 48, XXYY in two cases, 46, XX(male) in 69 cases, and one case had 48, XY, +2 mar karyotype.

Twenty-three cases were chimeric, abnormal karyotype results and their incidence in 10,286 cases with azoospermia and oligoasthenozoospermia are shown in Table 1.

Revised manuscript accepted for publication August 5, 2015

Table 1. — *Abnormal karyotype results and incidence from 10,286 cases with azoospermia and oligoasthenozoospermia.*

Karyotype	Case number	CAR (%)
47, XXY	1,065	10.35
Autosomal translocation	77	0.75
Y and autosomal translocation	11	0.11
X and autosomal translocation	3	0.03
Robertsonian translocation	46	0.45
46, XX(male)	69	0.67
Chimera	23	0.23
47, XY, +mar	10	0.10
47, XYY	34	0.33
48, XYYY	2	0.02
46, i(X)(q10), Y	2	0.02
Pericentric inversion	32	0.31
Ring chromosome	2	0.02
48, XY, +2mar	1	0.01

CAR: chromosome abnormality rate; RT: Robertsonian translocation.

Discussion

Spermatogenesis dysfunction can be caused by many factors, such as systemic disease, malnutrition, endocrine disorder, genetic defects, environmental factors, etc. With the wide application of cytogenetic technology in reproductive medicine, the correlation between chromosome abnormality and azoospermia/oligoasthenozoospermia is of more importance.

Chromosome 14 break 26 times, chromosome 1 break 12 times, chromosome 22 break ten times, chromosome 20 break seven times, chromosome 6 break seven times in balanced translocation cases; can we guess that these autosomal fracture have relations with spermatogenesis? This may require further research.

Chromosome abnormality has higher detection rate and varied types in male infertility patients. Klinefelter syndrome (also known as congenital testicular dysgenesis, 47, XXY) was the most common sex chromosome abnormality in male infertility patients [2], accounting for 10.35% in male infertility, as high as 30% in patients with male agenesis or gonad dysplasia. In this study, the authors found 1,065 cases of 47, XXY with an incidence of 10.35%, representing the most common karyotypic abnormalities of male infertility, which was consistent with the previous studies. Klinefelter's syndrome resulted from non-dysjunction of one of the parental X chromosomes during germ cells meiosis or early embryonic cell mitosis. Generally, non-dysjunction of paternal autosomomes accounts for about 10%, while in patients with Klinefelter's syndrome,the incidence rises to 50% [3]. The intervention of an excess X chromosome resulted in male infertility and the more excess X chromosomes existed, the worse the symptoms represented.

46, XX male is a rare chromosome aberration, and the authors detected 69 cases in this study (0.67%). AZF gene tests were further performed, and the authors found that all of these patients had SRY gene. Therefore, whether sterility resulted from translocation of testis determining factor to other chromosome or not still needs further study.

The present authors detected 47 cases 47, XYY with the incidence of 0.33%. Most 47, XYY males had normal fertility, different from Klinefelter's syndrome, and they occasionally represented cryptorchidism, hypoplasia of testis, spermatogenesis dysfunction, subfertility, hypospadias, etc. In addition, the authors also found 11 patients with autosome-Y chromosome reciprocal translocation, whose infertility might have resulted from AZF region deletion of Y chromosome by reciprocal translocation. However, all of these 11 patients had no AZF micro-deletion. These patients had mumps history and cotton seed oil edible history, but whether the abovementioned histories associated with their spermatogenic failure or not still needs further research.

The present authors found 5.2% patients with chromosome polymorphism (538 cases). Chromosome polymorphisms mainly were heterochromatin variation, especially the constitutive heterochromatin containing highly repetitive DNA. The constitutive heterochromatin primarily located at the centromere, telomere, satellite, secondary constriction, and long arm of Y chromosome. DNA contained in constitutive heterochromatin at the molecular level mainly were "noncoding" highly repetitive sequences, which did not contain structural genes with transcription activity. Based on that the variation of heterochromatin was thought to be normal variation with no phenotypic effects. Nevertheless, in recent years, studies have shown that heterochromatin plays an important role in centromeric function, which is essential for the sister chromatids union and chromosome separation. The euchromatin translocated to the heterochromatin are a by chromosome rearrangement, would induce euchromatin heterochromatination, thereby inhibition of gene expression and abnormal infertility [4].

Chromosomal inversion, a common chromosome abnormality, was reconnected after 180-degree inversion of the central fragment when the chromosome broke two times at the same time. Pericentric inversion was defined when it occurs between the short arm and the long arm. Although every chromosome has the possibility to suffer from pericentric inversion, pericentric inversion in chromosome 9 was the most frequently seen in population (1%) [5]. The present authors found eight cases that had pericentric inversion of chromosome 1 in this study, and all of them presented as thenospermia, oligospermiaorazoospermia. Therefore, chromosome 1 might be relevant with sperm production, which still needs further research.There were 65 cases of chromosome 9 pericentric inversion inv (9) (p12q13), one of the chromosomomal polymorphisms in, which accounted for 0.63%, presenting asthenospermia, oligospermiaorazoospermia. Although there is no loss of genetic material, position effect due to the alteration of gene order could be induced by chromosome 9 pericentric in-

version can cause, thus leading to infertility, sterility, abortion, stillbirth, etc. So chromosome polymorphism may be the cause of male infertility, which should be paid attention to by clinicians. Sperm donor however, who have normal sperm quality and Yqh+ karyotype can have normal children [6].

Ring chromosome was formed by reunion of the ends of each chromosome arm after it break. It is also a structurally chromosomomal abnormality with deletion. Loss of azoospermia factor 1 (AZF) in the formation of ring chromosome would inevitably lead to male dyszoospermia [7]. In the present study, the authors found a ring chromosome formed by Y chromosome and chromosome 15 with the phenotype of oligospermia and asthenospermia.

Given that chromosomal abnormalities are closely associated with male infertility, chromosome analysis has important diagnostic value for patients with asthenospermia, oligospermia, and andazoospermia. Cytogenetic analysis not only can elucidate etiology for infertililty, avoid other invalid test for patients, but also help clinicians provide better genetic counseling and correct fertility guidance for such patients.

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