Karyotype analysis in large-sample infertile couples living in Central China: a study of 14965 couples

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Abstract

Objective To explore that it is necessary to routinely detect chromosomes in fertile couples, we detected peripheral blood lymphocyte karyotype in 14965 infertile couples living in Central China and analyzed the incidence and type of chromosomal anomaly.

Methods G-banding karyotype analysis of peripheral blood lymphocytes was performed in 14965 couples who went to the outpatient department of our reproductive medical center for counseling on infertility between January 2004 and December 2011. Semen analysis was performed three times in all the men from the 14965 couples.

Results The rate of chromosomal anomaly in the 14965 infertile couples was 3.84 % (1150/29930). The rate of chromosomal anomaly in the men from 14965 couples was 6.84 % (1024/14965) and in the women 0.84 % (126/14965). The rates of chromosomal anomaly were 1.69 % in normal semen group, 11.82 % in light oligo-astheno-spermis group, 6.58 % in moderate to severe oligo-astheno-spermia group and 17.26 % in azoospermia group.

Conclusion Since the rates of chromosomal anomaly are 1.69 % and 11.82 % even in normal semen group and light oligo-astheno-spermia group, respectively, it is necessary to detect peripheral blood lymphocyte karyotype in all infertile couples.

Yan Liu and Xiang-dong Kong contribute equally to this study.

Capsule Since the rates of chromosomal anomaly are 1.69 % and 11.82 % even in normal semen group and light oligo-astheno-spermia group, respectively, it is necessary to detect peripheral blood lymphocyte karyotype in all infertile couples.

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Introduction

About 10 % of married couples have dysgenesis, and the causes of infertility from men or women all account for 50 %. The rate of chromosomal anomaly in the general population is 0.37 %–1.86 % [1–3]; while in the infertile population, it is as high as 3.95 %–14.3 % [4–8]. Chromosomal anomaly may decrease male potentia generandi and increase the history of female adverse pregnancy or delivery outcomes [9]. In order to provide an effective guidance for infertile couples, we carried out lymphocyte karyotype analysis in large-sample infertile couples.

In this study, lymphocyte karyotype analysis was performed in 14965 couples who went to the outpatient department of our reproductive medical center for counseling on infertility between January 2004 and December 2011. This is the largest sample-size known in the world. We can more accurately know the type and incidence of chromosomal anomaly in infertile couples living in Central China through the analysis of large sample data, providing a theoretical basis for that it is necessary to detect peripheral blood lymphocyte karyotype in all infertile couples.

Materials and methods

All study methods were approved by Institutional Review Board and Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All the subjects enrolled into the study gave written formal consent to participate.

Clinical data

Between January 2004 and December 2011, 14965 couples who went to the outpatient department of our reproductive medical center for counseling on infertility were enrolled in this study. The 14965 couples had primary infertility (couples lived together for more than 1 year, had a normal sexual life and did not adopt contraception without pregnancy), secondary infertility (couples did not adopt contraception without pregnancy for more than 1 year after they had been pregnant once) or a history of adverse pregnancy or delivery outcomes (women had three-time spontaneous abortions occurring in the same period of embryo development, or once gave birth to congenital malformation fetus and dead fetus).

Methods

Peripheral blood lymphocyte karyotype was detected in the 14965 couples and semen analysis was performed in all the men from the 14965 couples. Semen analysis was performed every 3–7 days and was repeated three times in each infertile male.

From each patient, 3 ml of peripheral venous blood was taken, and then incubated in 5 ml of medium containing heparin at 37 °C for 72 h. Lymphocytes were collected for G-banding karyotype analysis. Twenty cells in mitosis metaphase were selected for G-banding karyotype analysis in each patient. If there was a small proportion of chimeric karyotype, 50–100 cells in mitosis metaphase were selected. If the sex chromosome number was abnormal, more than 100 cells in mitosis metaphase were selected.

Semen analysis was performed three times according to the criteria made by World Health Organization in 2010 [10]. According to the results of semen analysis, the men from the 14965 couples were divided into 4 groups including normal semen group (semen density $\geq 15 \times 10^6/\text{ml}$ and forward motile sperm $\geq 32\%$) slight oligo-astheno-spermia group ($10 \leq$ semen density $< 15 \times 10^6/\text{ml}$ and/or $20\% \leq$ forward motile sperm $< 32\%$), moderate to severe oligo-astheno-spermia group ($0 <$ semen density $< 10 \times 10^6/\text{ml}$ and/or $0\% <$ forward motile sperm $< 20\%$) and azoospermia group (no spermatozoa).

Statistical analysis

Statistical treatment was performed with SPSS 16.0 software (Chicago, USA). χ^2 test was used in the comparison of chromosomal anomaly between this study and other published studies. Statistical significance was established at $P < 0.05$.

Results

Rate of chromosomal anomaly

The rate of chromosomal anomaly was 3.84 % (1150/29930) in the 14965 couples, 6.84 % (1024/14965) in the men from 14965 couples and 0.84 % (126/14965) in the women from the 14965 couples. The rate of chromosomal anomaly was 9.29 % (800/8606) in primary infertility, 5.47 % (285/5213) in secondary infertility and 5.25 % (60/1146) in adverse pregnancy or delivery history (Table 1). The rate of chromosomal anomaly was significantly higher in primary infertility group (9.29 %) than in secondary infertility group (5.47 %) and adverse pregnancy or delivery history group (5.25 %).

Type of chromosomal anomaly

Of the 1150 cases with chromosomal anomaly, autosomal number and structure were abnormal in 263 cases accounting for 22.87 % (263/1150), sex chromosome number and structure were abnormal in 469 cases (40.78 %), chromosome polymorphism occurred in 376 cases (32.70 %), unknown source chromosome occurred in 6 cases (0.52 %), chimera occurred in 17 cases (1.48 %), true hermaphroditism occurred in 18 cases (1.57 %) and 46,XY occurred in one women receiving bone marrow transplantation.

In the women from the 14965 couples, autosomal structural abnormality (0.39 %, 59/14965) was the most followed by chromosomal polymorphism (0.37 %, 55/14965).

In the men from the 14965 couples, numerical abnormality of sex chromosomes (3.09 %, 463/14965) was common including Klinefelter's syndrome in 446 cases (98.45 %, 446/453), 47,XXY combined with polymorphism in 10 cases, 47,XYY in 5 cases and 48,XXYY in 2 cases. Chromosomal polymorphism (2.15 %, 321/14965) took the second place and autosomal structural abnormality (1.36 %, 203/14965) took the third place.

The rate of chromosomal anomaly was the highest in azoospermia group (17.26 %) in which numerical abnormality of sex chromosome was dominant mainly with Klinefelter's syndrome. Although the rate of chromosomal anomaly was higher in slight oligo-astheno-spermia group than in moderate to severe oligo-astheno-spermia group, chromosomal polymorphism (6.72 %) was dominant in slight oligo-astheno-spermia group (Table 2).

Correlation of semen parameters with the incidence of chromosomal anomalies in men with primary infertility, secondary infertility and adverse pregnancy or delivery outcomes

Table 3 shows the rates of chromosomal anomaly in the men with different semen analysis and infertile causes. In the

Table 1 Distribution of chromosomal anomaly in primary infertility, secondary infertility and adverse pregnancy or delivery history [n (%)]

Type	Chromosomal anomaly in men	Chromosomal anomaly in women	Chromosomal anomaly in couples	Case
Primary infertility	744(8.64 %)*	55(0.64 %)	1(0.01 %)	801
Secondary infertility	259(4.97 %)	22(0.42 %)	4(0.08 %)	289
Adverse pregnancy and delivery history	16(1.41 %)	44(3.84 %)**	0(0 %)	60
Case	1019	121	5	1150

*indicates $P<0.05$, compared with secondary infertility and adverse pregnancy or delivery history

**indicates $P<0.05$, compared with primary infertility and secondary infertility

men with primary infertility, there were significant differences in the rate of chromosomal anomaly among normal semen group, light oligo-astheno-spermia group, moderate to severe oligo-astheno-spermia group and azoospermia group. In the men with secondary infertility, the rate of chromosomal anomaly was significantly lower in normal semen group than other groups.

Rare karyotype in the world

In the 14965 couples, 1150 cases had chromosomal anomaly. Of the 1150 cases, 8 cases had rare karyotype in the world which was simultaneously involved in autosomes and sex chromosomes, or more than 3 chromosomes (Table 4).

Discussion

Chromosomal anomaly is an important cause of infertility. Chromosomal anomaly can produce abnormal gametes during meiosis, leading to a history of adverse pregnancy (recurrent pregnancy loss) or delivery outcomes (birth of

congenital malformation fetus or dead fetus) [9, 11]. The rate of chromosomal anomaly in general population is 0.37 %–1.86 % [1–3]. The rate of chromosomal anomaly in infertile population is 2–3 times the rate of chromosomal anomaly in general population [4–6, 8, 9]. This study indicated that in infertile couples living in Central China, the rate of chromosomal anomaly was 3.84 %.

Comparison of chromosomal anomaly in infertile population between this study and other published papers

Chromosomal anomaly in infertile couples in Europe, Africa and other regions of China was listed in Table 5. Although the rate of chromosomal anomaly is different between various regions, the rate of chromosomal anomaly was all significantly higher in infertile couples than in general population. The rate of chromosomal anomaly in this study was 3.84 % (1150/29930).

Most of previous studies on chromosomal anomaly were carried out in specific infertile couples. For example, Riccaoni et al. [8] investigated chromosome anomaly in 2710 couples receiving assisted reproductive treatment in Italy; Kayed et al.

Table 2 Distribution of chromosomal anomaly in normal group, slight oligo-astheno-spermia group, moderate to severe oligo-astheno-spermia group and azoospermia group [n (%)]

Type of chromosomal anomaly	Normal semen group	Light oligo-astheno-spermia group	Moderate to severe oligo-astheno-spermia group	Azoospermia group	Total cases
Autosomal structural/numerical abnormality	17(0.23 %)	104(3.15 %)	59(2.37 %)	23(4.98 %)	203(1.36 %)
Sex chromosomal structural/numerical abnormality	–	13(0.57 %)	71(2.85 %)	379(14.16 %)*	463(3.09 %)
Mosaic	–	–	2(0.08 %)	13(0.49 %)	15(0.10 %)
Unknown source chromosomes	1(0.01 %)	–	2(0.08 %)	1(0.04 %)	4(0.02 %)
Chromosomal polymorphism	109(1.45 %)	154(6.72 %)*	30(1.20 %)	28(1.05 %)	321(2.15 %)
Sex reversal	–	–	–	18(0.67 %)	18(0.12 %)
Total chromosomal anomaly	127(1.69 %)*	271(11.82 %)**	164(6.58 %)***	462(17.26 %)	1024(6.84 %)
Total cases	7504	2293	2491	2677	14965

*indicates $P<0.05$, compared with other three groups

**indicates $P<0.05$, compared with moderate to severe oligo-astheno-spermia group and azoospermia group

***indicates $P<0.05$, compared with azoospermia group

Table 3 The rates of chromosomal anomaly in the men with different semen analysis and infertile causes

Infertile causes	Normal group	Light oligo-astheno-spermia group	Moderate to severe oligo-astheno-spermia group	Azoospermia group	Total chromosomal anomaly	Total cases
Primary infertility	0.23 %(10/4397)*	25.03 %(184/735)**	11.17 %(89/797)***	17.26 %(462/2677)	745	8606
Secondary infertility	3.89 %(117/3009)*	6.65 %(85/1279)	6.60 %(61/925)	–	263	5213
Adverse pregnancy and delivery	–	0.72 %(2/279)	1.82 %(14/769)	–	16	1146
Total chromosomal anomaly	127	271	164	462	1024	
Total cases	7504	2293	2491	2677		14965

*indicates $p < 0.05$, compared with other groups

** indicates $p < 0.05$, compared with moderate to severe oligo-astheno-spermia group and azoospermia group

*** indicates $p < 0.05$, compared with azoospermia group

[7] and Peschka et al. [12] respectively carried out chromosome analysis in 2650 and 781 couples who underwent intracytoplasmic sperm injection (ICSI) in Egypt and Germany. Butnariu et al. [4] analyzed chromosomal anomaly in 266 couples who had reproductive diseases in France. Zhang [13] and Hou [14] respectively explored chromosomal anomaly in 1624 and 1218 infertile patients in Northern China and Eastern China. To date, little research has been done on the type and incidence of chromosomal anomaly in non-selected infertile population. We carried out lymphocyte karyotype analysis in 14965 couples who went to the outpatient department of our reproductive medical center for counseling on infertility between January 2004 and December 2011. This could more accurately reflect the type and incidence of chromosomal anomaly in infertile population.

The rate of chromosomal anomaly is higher in this study than in other studies performed by Clementini et al. and Riccaboni et al. [6, 8] in Italy, which may be due to regional disparity. However, the rate of chromosomal anomaly is lower in this study than in other studies performed by Butnariu et al. and Peschka et al. [4, 12], which may be due to the different inclusion criteria for the study population. In the study performed by Peschka [12], the study population was the couples receiving ICSI mainly due to severe oligo-astheno-spermia. In the patients with oligo-astheno-spermia, the rate

of chromosomal anomaly is 8 %–15 % [15, 16] which is more than 6 times the rate of chromosomal anomaly in general population, resulting in that the rate of chromosomal anomaly is higher in the patients with ICSI than in infertile population.

Type and incidence of chromosomal anomaly in infertile men and women

In this study, the rate of chromosomal anomaly was significantly higher in men (6.84 %, 1024/14965) than in women (0.84 %, 126/14965). Klinefelter's syndrome is the most common type in male chromosomal anomaly [17]. In this study, the detection rate of Klinefelter's syndrome was 2.98 % (446/14965). Klinefelter's syndrome is caused by sex chromosome non-disjunction during the meiosis of germ cells. Testicular biopsy indicates glass-like change in the contorted seminiferous tubules, marked reduction of spermatogenous cells and pseudo-adenomatous stromal cells in Klinefelter's syndrome. The main clinical manifestation is azoospermia in the men with Klinefelter's syndrome. Autosomal abnormalities can interfere with spermatogenesis, affecting spermatogenesis with severe oligo-astheno-spermia [15, 18]. Autosomal abnormalities are the most common type in female chromosomal anomaly. Although a few scholars reported that chromosomal anomaly in women could affect ovarian function [19], especially the abnormality occurring in the key site of X chromosome; most scholars believed that autosomal balanced translocation or Robertsonian translocation in women did not affect ovarian function [20]. Therefore, the rate of chromosomal anomaly in infertile population is higher in men than in women.

Type and incidence of chromosomal anomaly in infertile men

In the men from the 14965 couples, the numerical abnormality of sex chromosomes (3.09 %, 463/14965) was dominant including Klinefelter's syndrome in 446 cases (98.45 %,

Table 4 Rare karyotype in the world

Rare karyotype in the world
45,X,-Y,der(Y,15)(q10;q10)
47,XXY,t(7;12)(p22;q14)
47,XXY,t(2;14)(q34;q23)
48,XXXY,t(3;16)(p24;q24)
46,XY,der(2),t(1;2)(q31;q12),t(2;7;8)(q32;p15;q24)
46,XY,inv(17)(p22,q11),t(11;15)(p11;q11.1)
46,XY,t(9;17)(q34;q21),inv(9)(p12,q13),add(15)(p13)
46,XX,inv(9)(p11;q12),t(14;15)(q22;q24)

Table 5 Rate of chromosomal anomaly in other countries and other regions of China [% (n/n)]

Authors	Publication date	Countries	Rate of chromosomal anomaly	Rate of chromosomal anomaly in men	Rate of chromosomal anomaly in women
Peschka, et al.	1999 [12]	Germany	15.68 % (245/1562)	11.91 % (93/781)	19.46 % (152/781)
Clementini, et al.	2005 [6]	Italian	1.97 % (82/4156)	2.02 % (42/2078)	1.92 % (40/2078)
Kayed, et al.	2006 [7]	Egypt	1.18 % (64/1218)	5.21 % (138/2650)	0.91 % (24/2650)
Riccaboni, et al.	2008 [8]	Italian	1.37 % (74/5420)	1.5 % (41/2710)	1.3 % (23/2710)
Butnariu, et al.	2010 [4]	France	8.08 % (43/532)	7.52 % (20/266)	8.65 % (23/266)
Hou Xiaoni	2010 [14]	Eastern China	5.24 % (64/1218)	6.98 % (47/673)	3.12 % (17/545)
ZhangLin, et al.	2011 [13]	Northern China	11.33 % (184/1624)	16.20 % (132/815)	6.43 % (52/809)
The current study	–	Central China	3.84 % (1150/29930)	6.84 % (1024/14965)	0.84 % (126/14965)

446/453). The incidence of chromosomal polymorphism (2.15 %, 321/14965) took the second place and it was about two times the incidence of chromosomal polymorphism in general population. Y chromosome polymorphism and D/G genome polymorphism are common. Most scholars believe no clear association between chromosome polymorphism and infertility [21, 22]. However, some scholars believe that chromosome polymorphism can cause carriers to give birth to offspring with unbalanced translocation karyotype or to have a history of adverse pregnancy or delivery outcomes [23]. Autosomal structural abnormalities (1.36 %, 203/14965) took the third place and mainly were balanced translocation and Robertsonian translocation. Most scholars believe that autosomal structural abnormalities can not affect individual growth and development, but may form abnormal gametes which give rise to triploid embryos, leading to a history of adverse pregnancy or delivery outcomes, or Down's syndrome children [24].

In this study, the rate of chromosomal anomaly was lower in normal semen group than other three groups, but was higher in azoospermia group than other three groups. Cavkayta et al. [25] have reported that the rate of chromosomal anomaly is far higher in the men with moderate to severe oligo-asthenospermia than in general population, and chromosomal anomaly often leads to male spermatogenic dysfunction and reduces male reproductive ability. However, this study indicated that the rate of chromosomal anomaly was higher in slight oligo-asthenospermia group (11.82 %) than in moderate to severe oligo-asthenospermia group (6.58 %), which was caused by that chromosomal anomaly mainly was chromosomal polymorphism in slight oligo-asthenospermia group and chromosomal polymorphism did not markedly affect male fertility [22, 23].

Type and incidence of chromosomal anomaly in infertile women

In this study, autosomal structural abnormality (0.39 %, 59/14965) was dominant in infertile women. MauHolzmann

et al. [26] have indicated that chromosomal balanced translocation does not affect meiosis in women, but can still lead to a history of adverse pregnancy or delivery outcomes; and it is unclear whether chromosomal balanced translocation affects ovarian function. Chromosomal polymorphism (0.37 %, 55/14965) took the second place in women, but it is also unclear whether chromosomal polymorphism affects female reproductive ability. Chromosomal rearrangements can lead to a history of adverse pregnancy, delivery outcomes, or a birth of children with chromosomal disorder. Therefore, it is also important to detect female chromosomal karyotype in infertile couples.

Rates of chromosomal anomaly in the couples with primary infertility, secondary infertility or a history of adverse pregnancy or delivery outcomes

Niroumanesh et al. [27] reported that chromosomal anomaly occurred in 8 % of female and in 5 % of male among 100 couples with spontaneous abortion. Lyer et al. [28] found chromosomal rearrangements in 3.5 % of 2150 couples with a history of adverse pregnancy or delivery outcomes. In this study, the rate of chromosomal anomaly in the couples with a history of adverse pregnancy or delivery outcomes was 1.14 % in men and 3.84 % in women, which were lower than that in above reports. In this study, the rate of chromosomal anomaly was higher in primary infertility group than in secondary infertility group and adverse pregnancy or delivery history group, because Klinefelter's syndrome is the most common in male chromosomal anomaly and it is characterized by spermatogenic dysfunction.

Correlation of semen parameters with the incidence of chromosomal anomalies in men with primary infertility, secondary infertility and adverse pregnancy or delivery outcomes

In the men with primary infertility, the rate of chromosomal anomaly in light oligo-asthenospermia group was as high

as 25.03 % (184/735) which was higher than that in other groups. In light oligo-astheno-spermia group, chromosomal anomaly mainly was chromosomal polymorphism and the effects of chromosomal polymorphism on male fertility are inconclusive. Our data indicated that chromosomal polymorphism was increased in infertile couples. In this study, chromosomal polymorphism usually exhibited light oligo-astheno-spermia. In the men with primary infertility, the rate of chromosomal anomaly (17.26 %, 462/2677) in azoospermia group was also high because most chromosomal anomaly in azoospermia group was Klinefelter's syndrome which is common in infertile men.

In the men with secondary infertility, the rates of chromosomal anomaly were significantly higher in light oligo-astheno-spermia and moderate to severe oligo-astheno-spermia groups than in normal group. In moderate to severe oligo-astheno-spermia group, the most of chromosomal anomaly was abnormal autosomal structure which led to the formation of abnormal gamete with severe oligo-astheno-spermia [15, 18].

Detection of peripheral blood lymphocyte karyotype

Clementini et al. [6] and Riccaboni et al. [8] believe that it is not necessary to carry out karyotype analysis in all infertile patients, but the men whose sperm number is less than 20×10^6 require peripheral blood lymphocyte karyotype analysis. However, in the men with normal semen, the rate of chromosomal anomaly still was 1.69 % (127/7504), which possibly leads to adverse pregnancy or delivery outcomes. Therefore, it is necessary to detect peripheral blood lymphocyte karyotype in all infertile couples.

Conventional G-banding karyotype analysis fails to identify micro-changes in chromosomes and uniparental disomy, because its resolution is only 50 Mb. Novelli et al. [29] described that single nucleotide polymorphism detection for peripheral blood lymphocytes could identify 9 %–12 % of genovariations in the couples who had normal G-banding karyotype analysis and a history of adverse pregnancy and delivery outcomes.

Limitation and strong points

Although we used the data from Nielsen, Forabosco, Ravel and sperm donors as healthy control, we did not establish the healthy control group in this study due to lack of the data of chromosomal anomalies in healthy couples in child-bearing period. The infertile couples in this study were all from outpatients and underwent different examinations according to their various clinical manifestations, but did not receive the same and comprehensive examinations except peripheral blood lymphocyte karyotype. Therefore, the causes of infertility were unclear in some females and the

rates of chromosomal anomaly in different infertile causes failed to be analyzed. These are the limitations of this study.

This study is the largest sample-size known in the world.

Conclusion

We analyze peripheral blood lymphocyte karyotype in infertile couples living in Central China. This is the largest sample-size known in the world. Since the rates of chromosomal anomaly are 1.69 % and 11.82 % even in normal semen group and light oligo-astheno-spermia group, respectively; it is necessary to detect peripheral blood lymphocyte karyotype in all infertile couples.

Declaration of interest There was no conflict of interest in this article.

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