Frequency of Chromosomal Abnormalities in Subpopulations of Infertile Males among Chinese Hakka Population

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Abstract

The frequency of chromosomal abnormalities is supposed to be elevated in infertile males as well as shown negative correlation of sperm concentration. Cytogenetic analysis, recommended by guidelines in infertile men, is expensive. Hence, it may be good for recognize riskiest men with chromosomal abnormalities, potentially by analyzing sperm concentration outside of the parameters. Aim to evaluate the frequency of chromosomal abnormalities in different subpopulations among Hakka population, we assessed several clinical characteristics of infertile men. A total of 1291 azoospermic men and men apply for in-vitro fertilization (IVF) treatment, were analyzed for semen parameters, hormone levels, Y chromosome microdeletions and medical history, related to chromosomal abnormalities. Chromosomal abnormalities were detected 46 of 1291 men (3.6%) in our study. Correlation was no shown between chromosomal abnormalities and sperm parameters except for sperm volume (OR 0.76, P=0.029). Significantly, azoospermia was related to an increased frequency of chromosomal abnormalities (OR 32.24, P<0.001). Elevated gonadotropic hormone levels, meaningfully, was related to the risk of carrying a chromosome abnormality (OR 4.38, P<0.001). Lower rate of chromosomal abnormalities was observed in infertile males with positive andrologic history (OR 0.21, P=0.003). Previous miscarriages, related to an increased frequency of chromosomal abnormalities, were discovered in non-azoospermic men (OR 4.78, P=0.003). The results indicated that azoospermic men with hypergonadotropic history as well as an eventless andrologic history have frequently risk of chromosomal abnormalities.

Keywords: Chromosomal abnormalities; Infertile male; Azoospermia; In-vitro fertilization

Introduction

Infertility, as a public health problem, affecting approximately 15% of families are incapable of pregnancy [1]. As early as 1979, Chandley et al. reported that in men with barren semen quality, the frequency chromosomal abnormalities is supposed to be higher than in the common population [2]. Klinefelter’s syndrome and Robertsonian translocations, are regarded as important causes of cytogenetic in male infertility [3]. Chromosomal abnormalities may increase the potential risks of infertility being transmitted from infertile men to their offspring that lead to a miscarriage or a child with congenital abnormalities [4]. According to the guidelines, cytogenetic analysis are considered as an essential examination before IVF treatment in patients with severe infertile problem [5,6].

Previous researches have reported frequency of chromosomal abnormalities in men with infertility varies from 3 to 19% in different population [3]. In some researches, cytogenetic analysis was done in all infertile men, but some researches did karyotype for men with exceedingly poor semen quality merely because karyotyping is expensive and take time. And therefore, it may be good for determine riskiest men with chromosomal abnormalities. Up to now, few researches have in consideration of semen parameters and clinical characteristics of infertile men as potential risk factors of chromosomal abnormalities.

In some researches on parameters of semen, it confirmed that sperm concentration demonstrates close association with the chance of carrying chromosomal aberrations [7-9]. These studies found that the frequency of chromosomal abnormalities increasing with sperm concentration reduced, and abnormalities observed in azoospermia at most [10-12]. However, similar association could not be proved in subgroups of infertile men in other studies [10,13]. Differences in the study populations (e.g. whether infertile men or couples) and in classes of sperm concentrations seem to cause such inconsistent results.

Both of sperm motility and morphology were studied the correlation of chromosomal abnormalities. No significant results in these parameters were implied between males with or without chromosomal abnormalities [9]. But some researches did observe a meaningful difference in oligozoospermia that men with a low total motile sperm count (TMSC) present higher prevalence of chromosomal aberrations [14,15].

These results of researches on sperm motility and morphology are obviously conflicting and greatly influenced by sperm concentration. Few researches have concentrated on effect of infertile men characteristics on chromosomal abnormalities. Few studies have been...
focused on whether previous miscarriages and positive andrological history is a prophet for chromosomal abnormalities in men with infertility. When increasing frequency of chromosomal abnormalities in men with subgroups of Y chromosome microdeletions, levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were significantly higher [16]. Men with these features were eliminated by some researchers, or no association were found in gonadotrophin levels between men with normal or abnormal karyotypes [17].

Most studies excluding men with a history of genital infection, testicular biopsy, varicocele, children with congenital anomalies, enorchia and urethral fissure. Although these characteristics from a medical history of a man may not directly associate with infertility, it is likely that the characteristics and infertility have a coincident genetic beginning. A correlation between cryptorchidism, barren semen quality and genetic abnormalities has been advised in the testicular dysplasia [18]. Whether relation between these patient characteristics and chromosomal abnormalities is independence was not verified.

Until now, relation between Y chromosome microdeletions and chromosomal abnormalities in infertile men had been studied in different population [1,19,20]. The most regularly studied parameter related to the frequency of chromosomal abnormalities was sperm concentration in infertile men, but few systematic studies on relation with other sperm parameters, hormone levels, Y chromosome microdeletions and patients characteristic. The aim of our cohort study is to assess association sperm parameters and patient characteristics in males with azoospermic and non-azoospermic, to evaluate the frequency of chromosomal abnormalities in different subpopulations of infertile men among Hakka population.

Materials and Methods

Subjects

A total of 1,291 infertile men with azoospermia who were applying for IVF treatment were recruited from Hakka population in Meizhou of Guangdong Province in China. These patients visited Center for Reproductive Medicine of Meizhou People’s Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University, from January 2015 to December 2017. Couples applied for IVF due to male factor infertility is defined by the total number of spermatozoa < 20 million, or sperm concentration < 10 million/ml, or TMSC < 4 million, or when more than twice failure in artificial insemination procedure. All men took in a general questionnaire before treatment, including period of infertility, previous miscarriages and andrological history.

Patients whose cytogenetic and sperm analysis results were valid were included in the study. Our study was approved by the Ethics Committee of the Meizhou People’s Hospital (Huangtang Hospital), Meizhou Hospital Affiliated to Sun Yat-sen University. All participants for the study signed a written informed consent forms.

Cytogenetic analysis

Cytogenetic analysis was examined the cultured peripheral blood lymphocytes. Five GTG-banded metaphases with 440 banding resolution at least were analyzed for each patient and pictures were collected by karyotyping system (ZEISS, Hensoldt Wetzlar, Germany). If chromosome numerical mosaics were present, 100 metaphases were screened by microscope. In the case of complex chromosomal structure aberration, array comparative genome hybridization would be carried out on sample. Chromosomal heteromorphisms, as definition was stated in the International System for Human Cytogenetic Nomenclature 2009, were excluded from chromosome abnormality [21].

Sperm analysis

Sperm analysis was measured based on the criteria of 2010 World Health Organization [22]. Specimens were sent at indoor temperature to andrology laboratory within 1 hour of ejaculation and analyzed by semen analyzer (SQA-V GOLD, Los Angeles, USA). When the sperm density was lower than 5 million per ml, microscopic examination would be performed strictly. Sperm volume, sperm concentration and positive motility, and TMSC were collected. In statistical analyses, data was used only from first examination due to semen analysis of patients up to a maximum of seven included in our study.

Hormone analysis

Hormone analysis, including serum FSH, LH and testosterone were measured by electrochemiluminescence immunoassays (ECLIA) on the Automatic immune analyzer (Roche, Basel, Switzerland). According to our laboratory reference interval and cut-off levels related to researches on hormone levels and Y chromosome microdeletions before, cut-off levels were selected [19].

Y-chromosome microdeletions

Genomic DNA was separated from peripheral blood by the DNA extraction kit (Qiagen, Germany). The sequencing of Y chromosome microdeletions was performed by multiplex PCR, using Y chromosome microdeletions kit (Microread, Beijing, China) which including several sequence-tagged sites (STs): sY84, sY86, sY127, sY134, CDY2, SCMY, sY157, sY254, sY255, sY191, DAZ, CDY1, as well as internal controls consist of SRY and ZFXY genes. Finally, PCR products were detected by capillary electrophoresis (Applied Biosystems, USA).

Statistical analysis

Statistical analysis was carried out using SPSS version 20.0 software (Chicago, IL, USA). Data were performed in percentages and absolute counts. Associations were evaluated by a multivariate binary logistic regression analysis with method of entering and univariate binary logistic regression. Odds ratios (ORs), 95% confidence interval (CI) and P-values are shown in the results. Results were recognized statistically significant as P < 0.05.

Results

The study contained 1291 men with available results of chromosomal analysis and semen analysis. The median age of the patients was 31.0 years (range 22-59). Chromosomal abnormalities were detected in 46 of the 1291 men (3.6%). Among these abnormalities, there were 31 men had gonosomal abnormalities, twenty seven 47,XY, one 46,XX, one 46,XY/47,XXX mosaics, one 46,X,del (Y) (q11.23) and one 46,XX,psudic (X;Y) (p11.1,p11.1). These aberrations also consisted of 15 men autosomal abnormalities: nine reciprocal translocations, four inversions, one Robertsonian translocations and one individual had four translocations as well as an inversion.

In our cohort of 1291 patients, at least one semen analysis was valid. 886 men (68.5%) had at least two, 423 men (32.7%) had three or more sperm analyses were recorded. The consequence of first semen parameters using multivariate binary logistic regression analysis is performed in Table 1. Of the semen parameters, median volume was 2.7 ml (range 0.1-11.2), median density was 34.1 million/ml (0-196.7), median positive motility was 26.0% (0-63) and median TMSC was 26.6 million (0-399.8). Research showed that only sperm volume statistically significant lower the risk of carrying a chromosomal
aberration (OR 0.76) while concentration, positive motility and TMSC were not associated with chromosomal abnormality. Compared to non-azoospermic men, azoospermia had an increased risk of a chromosomal abnormality (OR 32.24). All 31 men with gonosomal abnormal karyotype were found in azoospermia and 15 men with autosomal abnormalities were detected in non-azoospermia either.

Results of univariate regression analysis of patient characteristics were also provided in Table 1. FSH, LH and testosterone were examined in all the azoospermic men and only 12.7% of non-azoospermic men. Data showed that men with FSH > 10 IU/l or LH > 12 IU/l had a statistically significant correlation of the risk of a chromosomal abnormality, respectively (OR 4.38 and OR 7.96). Inversely, testosterone had a decrease risk of a chromosomal abnormality (OR 0.64).

Y chromosome microdeletions were carried out in 410/1291 men (31.8%) and 188/1291 men (14.6%) had previous miscarriages, neither of these two factors demonstrated an association with chromosomal abnormalities. Patient with a positive andrological history including genital infection, testicular biopsy, varicocele, children with congenital abnormalities. Patient with a positive andrological history including these two factors demonstrated an association with chromosomal abnormalities (OR 4.78).

Significantly, the prevalence of chromosomal abnormalities between azoospermia and non-azoospermia were different, and a univariate regression analysis for several variables was carried out in both groups. Results of 106 azoospermia were performed in Table 2, positive associations with chromosomal abnormalities were found in azoospermia with FSH > 10 IU/l or LH > 12 IU/l in our cohort (OR 26.1 and OR 20.8, respectively). On the contrary, testosterone and positive andrological history had a reduce risk of chromosomal abnormalities, respectively (OR 0.463 and OR 0.074). Unfortunately, Y chromosome microdeletions did not show meaningfully relation with chromosomal abnormalities.

Results of 1185 non-azoospermia were given in Table 3, negative association was found between chromosomal abnormalities and hormone levels, Y chromosomal microdeletions and positive andrological history. However, in this subgroup, positive association was discovered between previous miscarriage and chromosomal abnormalities (OR 4.78).

| Characteristics | n | Abnormal karyotype (47) | Normal karyotype (1245) | Univariate analysis |
|-----------------|---|-------------------------|------------------------|---------------------|
|                 |   |                        |                        | OR                  |
| Azoospermia     | 106 | 29.00%                  | 70.10%                 | 32.24               |
|                 |     |                         |                        | 16.677-62.327       | <0.001 |
| Non-azoospermia | 1185| 1.30%                   | 94.70%                 | 1                   |
| Sperm volume per ml | 1291 | 2.4 (0.6-6.9)          | 2.8 (0-11.2)          | 0.76                |
|                 |     |                         |                        | 0.594-0.972         | 0.029  |
| Sperm concentration per million/ml | 1185 | 0 (0-102.8)            | 35.10 (0-196.7)       | 0.97                |
|                 |     |                         |                        | 0.957-0.991         | 0.003  |
| Sperm motility per % | 1185 | 0 (0-58.0)             | 27.00 (0-63.5)        | 0.95                |
|                 |     |                         |                        | 0.932-0.986         | <0.001 |
| TMSC per million | 1291 | 0 (0-343.4)            | 0 (0-399.76)          | 1.01                |
|                 |     |                         |                        | 1.009-1.028         | 0.291  |
| FSH per IU/l    | 252 | 31.45 (5.29-107.9)      | 6.27 (0.58-80.24)     | 1.06                |
|                 |     |                         |                        | 1.040-1.081         | <0.001 |
| LH per IU/l     | 252 | 67.40%                  | 5.30%                  | 4.38                |
|                 |     |                         |                        | 2.216-8.674         | <0.001 |
| LH > 10 IU/l    | 252 | 20.28 (3.71-55.88)      | 5.50 (0.26-37.84)     | 1.14                |
|                 |     |                         |                        | 1.092-1.166         | <0.001 |
| LH > 12 IU/l    | 252 | 60.90%                  | 2.80%                  | 7.96                |
|                 |     |                         |                        | 3.974-15.927        | <0.001 |
| Increased gonadotrophins* | 252 | 67.40%                  | 5.30%                  | 4.38                |
|                 |     |                         |                        | 2.216-8.674         | <0.001 |
| Testosterone per nmol/l | 252 | 4.23 (0.38-6.58)       | 4.24 (0.21-13.73)    | 0.64                |
|                 |     |                         |                        | 0.516-0.781         | <0.001 |
| Y chromosome microdeletions | 410 | 8.70%                  | 4.0%                   | 0.78                |
|                 |     |                         |                        | 0.264-2.296         | 0.65   |
| Previous miscarriage (s) | 1291 | 15.20%                  | 14.50%                 | 1.06                |
|                 |     |                         |                        | 0.465-2.395         | 0.898  |
| Positive andrological history* | 1236 | 8.70%                  | 30.40%                 | 0.21                |
|                 |     |                         |                        | 0.073-0.575         | 0.003  |

Note: Data are frequency or median (range).

Table 1: Sperm parameters and clinical characteristics in 1291 men with azoospermia or men applying for IVF in association with chromosomal abnormalities.

| Characteristics | n | Abnormal karyotype (31) | Normal karyotype (75) | Univariate analysis |
|-----------------|---|-------------------------|------------------------|---------------------|
|                 |   |                        |                        | OR                  |
| Sperm volume per ml | 106 | 2.3 (0.1-6.4)          | 2.1 (0.1-5.2)          | 0.963               |
|                 |     |                         |                        | 0.688-1.348         | 0.827  |
| FSH per IU/l    | 101 | 43.18 (9.21-107.9)     | 6.85 (1.63-80.24)     | 1.08               |
|                 |     |                         |                        | 1.049-11.116        | <0.001 |
| FSH > 10 IU/l   | 101 | 93.5%                   | 33.3%                  | 32.1               |
|                 |     |                         |                        | 5.743-118.62        | <0.001 |
| LH per IU/l     | 101 | 23.38 (3.85-55.88)     | 5.87 (2.19-29.92)     | 1.238              |
|                 |     |                         |                        | 1.140-1.345         | <0.001 |
| LH > 12 IU/l    | 101 | 83.9%                   | 18.7%                  | 20.8               |
|                 |     |                         |                        | 6.773-63.881        | <0.001 |
| Increased gonadotrophins* | 101 | 93.5%                   | 33.3%                  | 26.1               |
|                 |     |                         |                        | 5.743-118.62        | <0.001 |
| Testosterone per nmol/l | 101 | 2.35 (0.38-5.45)       | 4.40 (0.34-11.53)    | 0.483              |
|                 |     |                         |                        | 0.325-0.659         | <0.001 |
| Y chromosome microdeletions | 106 | 9.7%                   | 6.7%                   | 1.48               |
|                 |     |                         |                        | 0.331-6.608         | 0.609  |
| Positive andrological history* | 106 | 3.2%                   | 44.0%                  | 0.074              |
|                 |     |                         |                        | 0.016-0.330         | 0.001  |

Note: Data are frequency or median (range).

Table 2: Sperm parameters and patient characteristics in 79 azoospermic men in association with chromosomal abnormalities.
High levels of gonadotrophins results from testicular dysplasia and > 10 IU/l or LH > 12 IU/l were related to chromosomal abnormalities. In our study, although a huge research group of patients were included, we have only a limited number of hormone results valid due to the design of the retrospective study and the situation of China. We observed that FSH was positively correlated to chromosomal abnormalities, but it was not crucial because of the small size of patients in this group, FSH was positively correlated to chromosomal abnormalities. Moreover, we found that testosterone decreased the frequency of chromosomal abnormalities in azoospermia. Whether the correlation between gonadotrophin levels or testosterone and chromosomal aberrations in azoospermia as a common test to distinguish the risk of carrying a chromosomal abnormality should be confirmed in the future studies. No correlation was shown between FSH, LH or testosterone and chromosomal aberrations in non-azoospermia group.

In our study, 59 of 1291 men (13.4%) were detected as having Y chromosome microdeletions, which is consistent with the observation reported by Zhang et al. [30], but no correlation was found between males with Y chromosome microdeletions and chromosomal abnormalities. In non-azoospermic group, males with previous miscarriages history have the highest frequency of chromosomal abnormalities, and this is in agreement with the consequence found in previous study [18]. Males with autosomal abnormalities are more likely to miscarry of his partner.

In azoospermic men, postive andrological history including genital infection, testicular biopsy, varicocele, children with congenital anomalies, cryptorchism and hypospadias lowered the risk of a chromosomal abnormality (OR 0.21). It indicated that azoospermia unrelated to Y chromosome microdeletions, which is consistent with the observation reported by Zhang et al. [30], but no correlation was found between males with Y chromosome microdeletions and chromosomal abnormalities. In non-azoospermic group, males with previous miscarriages history have the highest frequency of chromosomal abnormalities, and this is in agreement with the consequence found in previous study [18]. Males with autosomal abnormalities are more likely to miscarry of his partner.

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based on univariate regression analysis and performed it in Figure 1. It performed that azoospermia with high gonadotrophin levels increased the risk of a chromosomal abnormality, while positive andrological history that can explain the barren sperm quality lowered the risk. Also, non-azoospermia with previous miscarriages history had the highest risk of chromosomal abnormalities. Whether these consequences are applicable to all infertile men remains to be further study.

**Conclusion**

We observed that congenital frequency of chromosomal abnormalities is 3.6% in infertile men in our cohort, sperm volume can imply lower risk of chromosomal abnormalities, sperm concentration is not a good prophet for chromosomal abnormalities while the absence of sperm is. Sperm motility does not associate with chromosomal abnormalities. Frequency of chromosomal abnormalities in azoospermia is 41.3%. If gonadotrophin levels are high (FSH>10 IU/l and/or LH >12 IU/l) and testosterone are low, risk of chromosomal abnormality adds significantly. Azoospermia had a positive andrological history, low gonadotrophin levels and high testosterone levels, the risk reduced. Frequency of chromosomal abnormalities in non-azoospermia is 1.3%, and men had previous miscarriages history, the risk increased. Our finding allow a more specific risk evaluate on chromosomal abnormalities in subpopulations of infertile men among Hakka population. Whether cytogenetic analysis should be offered in men should be based on cost-benefit analysis, which the cost of unhealth events (such as miscarriages) because of chromosomal abnormalities in males should be considered. Data acquired in our study can be used in these future economic models.

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**Contributors**

Pingsen Zhao conceived and designed the experiments; Xiaodong Gu and Man Yang recruited subjects and collected clinical data. Pingsen Zhao, Xiaodong Gu and Huaxian Wang conducted the laboratory testing. Pingsen Zhao and Xiaodong Gu prepared the manuscript.

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**Competing Interests**

None declared.

**Patient Consent**

Patient consent is obtained.

**Ethics Approval**

The study was approved by the Ethics Committee of the Meizhou People’s...
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