Male infertility in Northeast China: a cytogenetic study of 135 patients with non-obstructive azoospermia and severe oligozoospermia

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Abstract

Purposes To detect the frequency and types of chromosomal anomalies with non-obstructive azoospermia and severe oligozoospermia in Northeast China, and to compare the frequencies with other regions of China and the world. To investigate the general characteristics of this population.

Methods Eighty-one men with non-obstructive azoospermia and 54 men with severe oligozoospermia were recruited. Karyotype analyses were performed on peripheral blood lymphocytes using standard G-banding. Measurements of follicle-stimulating hormone, testosterone, prolactin, and inhibin B were obtained.

Results The frequency of chromosomal anomalies for patients with non-obstructive azoospermia (17.28%) was comparable with studies from Europe, Africa, Asia, and other regions of China. However, the frequency for patients with severe oligozoospermia (9.26%) was slightly higher than reported from other Asian countries. The infertile men were more likely than the fertile to smoke and consume alcohol, and to have significantly lower levels of inhibin B.

Conclusions For infertile men in Northeast China, chromosome analysis is a necessary part of routine genetic testing, and the contributing effects of high smoking and alcohol consumption rates of this population should be discussed during genetic counseling.

Keywords Chromosomal abnormality · Non-obstructive azoospermia · Severe oligozoospermia · Alcohol · Inhibin B

Introduction

Infertility is defined as the inability of a couple to conceive after 1 year of unprotected sexual intercourse. It affects approximately 10%–15% of couples, and male factors are responsible for about 40%–50% of these cases [1–3]. Chromosomal anomalies are considered as important causes of male infertility [4–6]. The reported frequencies of chromosomal anomalies are 10.00–23.62% and 1.10–13.33% in non-obstructive azoospermia and severe oligozoospermia, respectively [1–3, 7–14]. Moreover, the practise of using intracytoplasmic sperm injection (ICSI) by these severely infertile men to father their own children carries the risk of passing on genetic disorders to their offspring, including chromosomal anomalies [4, 5].

Azoospermia, the absence of sperm in semen, may due to a physical obstruction in the posttesticular genital tract, or may have non-obstructive causes that could be genetic. To the best of our knowledge, few if any researches have focused on the types and frequency of chromosomal anomalies, especially including the results of reproductive hormones and other characteristics in men with non-obstructive azoospermia and severe oligozoospermia in Northeast China.

This study aimed to find the frequency and types of chromosomal anomalies and the general characteristics of non-obstructive azoospermic and severe oligozoospermic men in Northeast China, using standard cytogenetic methods. Furthermore, we compared our results with reports from other regions of China and the world.

Capsule For infertile men in Northeast China, it is essential to do a chromosome analysis and pay attention to the contributing effects of high smoking and alcohol consumption rates during routine genetic work.

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Materials and methods

Patients

One hundred thirty-five infertile men from Northeast China who sought help for infertility from our center were included in this study. Each of these patients was interviewed about their medical histories, reproductive problems, and underwent a physical examination. Patients were questioned regarding their cigarette smoking and alcohol drinking habits. Semen samples were obtained after a 7-day period of ejaculatory abstinence, and semen analysis was performed according to the World Health Organization guidelines [15]. Each patient underwent semen analysis at least twice before a diagnosis of non-obstructive azoospermia or severe oligozoospermia was made. Blood samples were stored for cytogenetic analysis and hormonal evaluations. Patients with obstructive azoospermia were excluded from this study. Severe oligozoospermia was diagnosed with a sperm count $<5 \times 10^6/\text{mL}$. The control group included 60 fertile men, aged 21 to 38 years. Every man in the control group had fathered at least one child. All underwent the same examinations and analyses as the men in the infertile group.

Chromosome analysis

Chromosome analysis was performed using G-banding in peripheral blood lymphocyte culture. After a 72-h incubation period, lymphocytes were cultured in RPMI-1640 (GIBCO, Invitrogen, USA), phytohemagglutinin (Shanghai Yihua Medical Technology, China), and fetal bovine serum (Beijing Dingguo Biotechnology, China), followed by treatment with colcemid. Then G-banding of metaphase chromosomes was performed. At least 20 metaphases were analyzed for each patient. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature [16].

Hormonal analysis

Reproductive hormone levels, including serum follicle-stimulating hormone (FSH), testosterone (T), prolactin (PRL) and inhibin B, were measured for all infertile and fertile men. FSH, T, and PRL were measured by electrochemiluminescence immunoassay, using a Roche Elecsys 2010 serum chemistry analyzer (Germany), according to the manufacturer's instructions. Inhibin B was measured using an inhibin B ELISA kit purchased from ADL, USA. Normal reference ranges for FSH, T, and PRL were 0.8–15 mIU/mL, 3.2–15.6 ng/mL and 70–480 $\mu\text{IU/mL}$, respectively. The normal range levels of inhibin B were 125–

225 pg/mL and 80–220 pg/mL for men between the ages of 20–29 years and 30–44 years, respectively.

Statistical analyses

Statistical analyses were performed with SPSS 11.5 software (Chicago, IL, USA). Chi-square tests were used to compare chromosomal anomalies, smoking, and alcohol consumption. Sample values of inhibin B for men with chromosomal anomalies and fertile controls were all normally distributed. Thus, *t*-test was used to compare the level of inhibin B between these two groups. Besides this, all other comparisons of hormone levels used the Mann–Whitney test, because at least values of one sample was abnormally distributed. A probability (*P*)-value <0.05 was considered statistically significant and all *P*-values were two-sided.

Results

Of 135 infertile men, 60% (81/135) were found to have non-obstructive azoospermia, and 40% (54/135) had severe oligozoospermia. There were no differences in the mean ages of patients with azoospermia, oligozoospermia and the control men (29.01 ± 4.30 , 28.81 ± 5.07 , and 29.80 ± 4.33 years, respectively).

In the non-fertile groups, 17.28% (14/81) of the non-obstructive azoospermic, and 9.26% (5/54) of the severe oligozoospermic men had chromosomal anomalies. 16 men were found to have Klinefelter's syndrome with a 47, XXY karyotype, and two mosaic forms: 46, XY/47, XXY and 46, XX/47, XXY. Thirteen of these patients were azoospermic men (81.25%). One man with severe oligozoospermia was found to be 46, XY/46, XX. None of the control group had any chromosomal anomalies.

Table 1 lists the hormone levels of the entire population involved in this study. The levels of inhibin B were significantly lower in the infertile men compared to the fertile men. The levels of FSH were significantly higher in infertile men than fertile, and these levels in the non-obstructive azoospermic group were more than twice that of the severe oligozoospermic group. However, between the two types of infertility there was no significant difference in T levels. There was also no significant difference in PRL levels among the three groups.

The prevalence of smoking and alcohol consumption were significantly higher in the group of infertile men as a whole than in the fertile men (smoking: $\chi^2 = 11.704$, $P = 0.001$; alcohol: $\chi^2 = 13.774$, $P = 0.000$). In particular, alcohol consumption was significantly higher in those with azoospermia compared to the fertile controls ($\chi^2 = 13.773$, $P = 0.000$), and in those with oligozoospermia compared to the controls ($\chi^2 = 7.860$, $P = 0.005$).

Table 1 Hormone levels of 81 non-obstructive azoospermic, 54 severe oligozoospermic, and 60 control men

Group	FSH, mIU/mL			T, ng/mL			PRL, μ U/mL			INH B, 20–29 years, pg/mL			INH B, 30–44 years, pg/mL		
	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max	Med	Min	Max
Infertile men	9.20 ^b	0.40	55.00	2.60 ^c	0.25	27.00	320.00	11.08	2000.00	83.86 ^c	33.94	269.41	78.40 ^c	28.92	292.85
Non-obstructive azoospermia	13.50 ^{a,b}	0.40	55.00	2.40 ^c	0.32	27.00	330.00	11.08	2000.00	87.05	44.13	269.41	79.31	28.92	180.00
Severe oligozoospermia	5.20	0.70	42.00	2.90 ^c	0.25	8.80	310.00	29.00	840.00	76.59	33.94	258.30	74.61	34.79	292.85
Chromosomal anomalies	33.00 ^b	0.70	55.00	1.70 ^c	0.25	27.00	310.00	18.69	1200.00	69.38 ^c	44.13	104.00	72.11 ^c	65.30	109.28
Fertile control	3.05	0.80	14.50	3.80	3.20	14.00	322.50	230.00	476.00	171.50	121.57	218.64	168.70	113.60	211.60

FSH follicle-stimulating hormone; T testosterone; PRL prolactin; INH B inhibin B

^a Significantly higher compared with severe oligozoospermia, $P=0.000$ ^b Significantly higher compared with fertile control, $P=0.000$ ^c Significantly lower compared with fertile control, $P=0.000$

Discussion

Although it has long been recognized that chromosomal anomalies are closely associated with male infertility, few studies in the literature have paid specific attention to the prevalence of these anomalies. This is especially so for comparisons between the frequencies of specific anomalies, reproductive hormones and living habits in non-obstructive azoospermic and severe oligozoospermic men in Northeast China.

In this study, the proportion of chromosomal anomalies in non-obstructive azoospermic men (17.28%) was higher than in severe oligozoospermic men (9.26%). This was consistent with the results of other studies, suggesting that the prevalence of chromosomal anomalies decreases as the sperm count increases [8–12]. A previous study from our department showed that the prevalence of abnormal karyotypes was 8.5% for patients diagnosed with azoospermia, oligozoospermia or oligoasthenoteratozoospermia (OAT) syndrome [17].

Table 2 lists the results of studies of the frequency of chromosomal anomalies in infertile men in other parts of China and some regions of Europe, Africa, and Asia. In order to ensure comparability, all of the articles included in the table were selected to contain the same study objects as the current study (i.e., non-obstructive azoospermia and severe oligozoospermia with a spermatozoa count $<5 \times 10^6/\text{mL}$). From Table 2 it could be seen that our data in non-obstructive azoospermic men were similar to those obtained from a review of the literature ($P>0.05$). However, the frequency of anomalies in severely oligozoospermic men that we obtained (9.26%) was relatively higher than the results obtained in other countries of Asia (1.10%, 1.85%, and 6.52% for Kuwait, Thailand, and India, respectively) [9–11].

Foresta et al. [7] showed that the increased risk of chromosomal anomalies in children conceived by ICSI, compared to those conceived naturally, may be due to the increased frequency of chromosomal anomalies in their infertile fathers. Because patients with non-obstructive azoospermia and severe oligozoospermia can be candidates for ICSI, the prevalence of chromosomal anomalies in this population becomes important. Thus the results of comparison in the current study are valuable. The high frequency of chromosomal anomalies found in this study for this region alerted clinical workers to the importance of careful genetic counseling and chromosomal analysis, especially before ICSI.

In our study, the most common type of chromosomal abnormality found was Klinefelter's syndrome, occurring in 82.41% of all patients with chromosomal anomalies. Klinefelter's syndrome is recognized as the most common reason for chromosome-related male infertility. Yet sperm

Table 2 Comparison of chromosomal anomalies' frequencies between this study and studies in other regions of China and the world

Region	Authors	Non-azoospermic men	Severe oligozoospermic men
Southeast Turkey, Europe	Balkan M et al. [1]	13.46% (7/52)	^b
Eastern Turkey, Europe	Ceylan G et al. [3]	^b	13.33% (4/30)
Italy, Europe	Foresta C et al. [7]	^b	5.60% (42/750)
Tunisia, Africa	Elghezal H et al. [8]	23.62% (81/343)	8.85% (44/497)
Kuwait, Asia	Mohammed F et al. [9]	19.44% (21/108)	1.10% (2/181) ^a
Thailand, Asia	Vutyavanich T et al. [10]	10.00% (5/50)	1.85% (1/54)
India, Asia	Nagvenkar P et al. [11]	14.29% (6/42)	6.52% (3/46)
Israel, Asia	Kleiman SE et al. [2]	16.13% (10/62)	^b
Hong Kong, China, Asia	Ng PP et al. [12]	21.13% (15/71)	4.46% (10/224)
Taiwan, China, Asia	Chiang HS et al. [13]	23.13% (31/134)	^b
Mainland, China, Asia	Zhou-Cun A et al. [14]	14.06% (36/256)	^b
Northeast China, Asia	The current study	17.28% (14/81)	9.26% (5/54)

^a Significantly lower frequency compared with the current study, $\chi^2=6.956$, $P=0.008$

^b Excluded results, because of the different study objects found between the corresponding study and this study

retrieval made it possible for some 47, XXY patients to father children via ICSI [1]. What is more, the genetic risk to the offspring of these men was presumed to be low [12].

In the current study, one mosaic form of Klinefelter's syndrome found in azoospermic patients was 46, XX/47, XXY, which is a rare Klinefelter's variant. One azoospermic man was found with a Y chromosomal polymorphism: 46, XY, (Y<21), which may account for his sperm production disorders, as genes associated with germ cell development have been mapped to the long arm of the Y chromosome. However, whether Y chromosomal variants contribute to male infertility is still controversial [11]. 46, XY, t(Y, 4)(p11;p14) was found in one severe oligospermic men in this study. The reciprocal translocation between a sex chromosome and an autosomal chromosome has been said to be the usual reason for infertility [2].

The levels of FSH in the non-obstructive azoospermic group were significantly higher than in the severe oligozoospermic group (Table 1). This result was consistent with the large study by Kosar et al. [4]. As shown in Table 1, patients with chromosomal abnormalities were prone to have significantly higher levels of FSH, and lower levels of T and inhibin B, than either fertile men or infertile men of either type, averaged as a whole. Until the present study, no report in the literature has shown that the levels of these reproductive hormones could be used to predict the presence of chromosomal aberrations.

We found no significant difference in inhibin B levels between the azoospermic and oligozoospermic groups. However, levels of inhibin B were significantly lower in the infertile group as a whole (i.e., both azoospermic and oligozoospermic men) than in fertile men. This was consistent with the result found in another study of men

in the northeastern United States [18]. FSH, with or without luteinizing hormone (LH), and inhibin B have been compared to determine which one is a better marker of spermatogenesis. Both Pierik et al. [19] and Kumanov et al. [20] concluded that inhibin B was a better measurable marker than FSH (and LH in Kumanov's essay). However, Myers et al. [18] found inhibin B to be similar to FSH as a useful marker of spermatogenesis in the northeastern United States [18]. Our study found that inhibin B held no priority over FSH, as FSH levels could discriminate between azoospermic and oligozoospermic patients, while inhibin B could not. The patients enrolled in this study all suffered from non-obstructive azoospermia or severe oligozoospermia, severe forms of infertility. Further study is required to determine whether FSH, which was found to be better than inhibin B, is a marker good enough for evaluating different degrees of spermatogenesis dysfunction in men from Northeast China.

This study found that our patients with non-obstructive azoospermia and severe oligozoospermia were much more likely to smoke than the fertile men. Vine et al. [21] investigated more than 1,000 men in the United States, and the data revealed that the sperm concentration of smokers was reduced 13–17% compared to non-smokers. Thus smoking may be one reason for the decrease of sperm count in our patients. This could be due either to exposure to high levels of the numerous mutagenic, clastogenic, carcinogenic, and toxic compounds in tobacco smoke, or other damages to sperm DNA [22]. The results of studies by Vutyavanich et al. [10] and Kosar et al. [4] were in agreement with ours, that there was a higher rate of smoking consumption in azoospermic and oligozoospermic men.

It was also found that a greater percentage of the infertile men as a group, as well as those with either non-obstructive azoospermia or severe oligozoospermia, consume alcohol than the men of the fertile control group. This result was contrary to the result of Martini et al. [23], who found no difference in seminal parameters between fertile controls and the infertile patients who stated they drank alcohol. This difference may be due to the regional and cultural habits of drinking alcohol among most men living in this region. Or this may be due to the different methods used in these two studies: in our study, the groups were divided according to sperm count, while in Martini et al.'s, the groups were divided according to the amount of alcohol consumed each day.

In conclusion, the frequency of chromosomal anomalies in non-obstructive azoospermic men in Northeast China was comparable to their counterparts in Europe, Africa, and Asia. But the percentage of chromosomal abnormalities in men with severe oligozoospermia in Northeast China was slightly higher than infertile men with this condition from other Asian countries. The high frequency of chromosomal anomalies strongly suggests that chromosome analysis should be a required part of routine genetic testing for infertile men in Northeast China, especially before using assisted reproduction techniques. The contributing effects of high smoking and alcohol consumption rates in men from Northeast China should be discussed with patients during genetic counseling.

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References

1. Balkan M, Tekes S, Gedik A. Cytogenetic and Y chromosome microdeletion screening studies in infertile males with oligozoospermia and azoospermia in Southeast Turkey. *J Assist Reprod Genet.* 2008;25:559–65.
2. Kleiman SE, Yorgev L, Gamzu R, Hauser R, Botchan A, Lessing JB, Paz G, Yavetz H. Genetic evaluation of infertile men. *Hum Reprod.* 1999;14(1):33–8.
3. Ceylan GG, Ceylan C, Elyas H. Genetic anomalies in patients with severe oligozoospermia and azoospermia in eastern Turkey: a prospective study. *Genet Mol Res.* 2009;8(3):915–22.
4. Kosar PA, Ozcelik N, Kosar A. Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia. *J Assist Reprod Genet.* 2010;27:17–21.
5. Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF, Govaerts LC. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. *Hum Reprod.* 2002;17(1):13–6.
6. Martin RH. Cytogenetic determinants of male fertility. *Hum Reprod Updat.* 2008;14(4):379–90.
7. Foresta C, Garolla A, Bartoloni L, Bettella A, Ferlin A. Genetic abnormalities among severely oligospermic men who are candidates for intracytoplasmic sperm injection. *J Clin Endocrinol Metab.* 2005;90(1):152–6.
8. Elghezal H, Hidar S, Braham R, Denguezli W, Ajina M, Saad A. Chromosome abnormalities in 1,000 infertile males with non-obstructive sperm disorders. *Fertil Steril.* 2006;86(6):1792–5.
9. Mohammed F, Al-Yatama F, Al-Bader M, Tayel SM, Gouda S, Naguib KK. Primary male infertility in Kuwait: a cytogenetic and molecular study of 289 infertile Kuwaiti patients. *Andrologia.* 2007;39(3):87–92.
10. Vutyavanich T, Piromlertamorn W, Sirirungsri W, Sirisukkasem S. Frequency of Y chromosome microdeletions and chromosomal abnormalities in infertile Thai men with oligozoospermia and azoospermia. *Asian J Androl.* 2007;9(1):68–75.
11. Nagvenkar P, Desai K, Hinduja I, Zaveri K. Chromosomal studies in infertile men with oligozoospermia & non-obstructive azoospermia. *Indian J Med Res.* 2005;122(1):34–42.
12. Ng PP, Tang MH, Lau ET, Ng LK, Ng EH, Tam PC, Yeung WS, Ho PC. Chromosomal anomalies and Y-microdeletions among Chinese subfertile men in Hong Kong. *Hong Kong Med J.* 2009;15(1):31–8.
13. Chiang HS, Wei HJ, Chen YT. Genetic screening for patients with azoospermia and severe oligo-asthenospermia. *Int J Androl.* 2000;23(2):20–5.
14. Zhou-Cun A, Yang Y, Zhang SZ, Zhang W, Lin L. Chromosomal abnormality and Y chromosome microdeletion in Chinese patients with azoospermia or severe oligozoospermia. *Yi Chuan Xue Bao.* 2006;33(2):111–6.
15. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge: Cambridge University Press; 1999.
16. Mitelman F, ISCN, editors. An international system for human cytogenetic nomenclature. Basel: Karger; 1995.
17. Wang RX, Fu C, Yang YP, Han RR, Dong Y, Dai RL, Liu RZ. Male infertility in China: laboratory finding for AZF microdeletions and chromosomal abnormalities in infertile men from Northeastern China. *J Assist Reprod Genet.* 2010;27(7):391–6.
18. Myers GM, Lambert-Messerlian GM, Sigman M. Inhibin B reference data for fertile and infertile men in Northeast America. *Fertil Steril.* 2009;92(6):1920–3.
19. Pierik FH, Vreeburg JT, Stijnen T, De Jong FH, Weber RF. Serum inhibin B as a marker of spermatogenesis. *J Clin Endocrinol Metab.* 1998;83(9):3110–4.
20. Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. *Fertil Steril.* 2006;86(2):328–32.
21. Vine MF, Margolin BH, Morrison HI, Hulka BS. Cigarette smoking and sperm density: a meta-analysis. *Fertil Steril.* 1994;61:35–43.
22. Giwercman A, Giwercman YL. Environmental factors and testicular function. *Best Pract Res Clin Endocrin Metab.* 2011;25:391–402.
23. Martini AC, Molina RI, Estofan D, Senestrari D, Fiol de Cuneo M, Ruiz RD. Effects of alcohol and cigarette consumption on human seminal quality. *Fertil Steril.* 2004;82(2):374–7.