

# Chromosomal abnormalities in patients with oligozoospermia and non-obstructive azoospermia

Larysa Y. Pylyp · Lyudmyla O. Spinenko ·  
Natalia V. Verhoglyad · Valery D. Zukin

Received: 30 January 2013 / Accepted: 28 March 2013 / Published online: 11 April 2013  
© Springer Science+Business Media New York 2013

## Abstract

**Purpose** To assess the frequency and types of chromosomal abnormalities in 204 Ukrainian patients with non-obstructive azoospermia and oligozoospermia and 87 men with normozoospermia.

**Methods** Cytogenetic studies were performed on peripheral blood lymphocyte samples of 164 men with oligozoospermia, 40 men with non-obstructive azoospermia and 87 men with normozoospermia attending infertility clinic.

**Results** Chromosomal abnormalities were detected in 17 % of patients with sperm disorders: in 35 % of men with azoospermia and in 12.7 % of men with oligozoospermia. The frequency of chromosomal abnormalities in patients with sperm disorders was significantly higher, than in patients with normozoospermia ( $P=0.0001$ ). An increase in the incidence of chromosomal abnormalities with the decrease of sperm count was observed. Chromosomal abnormalities were detected in 1.1 % of patients with normozoospermia, 6.5 % of patients with mild oligozoospermia (sperm count  $5\text{--}15 \times 10^6/\text{ml}$ ), 18.4 % of patients with severe oligozoospermia (sperm count  $<5 \times 10^6/\text{ml}$ ) and 35 % of patients with azoospermia. A significant increase in the frequency of chromosomal abnormalities in patients with severe oligozoospermia was observed when compared to mild oligozoospermia ( $P=0.01$ ). A statistically significant association ( $P=0.02$ ) of chromosomal abnormalities and sex

chromosome abnormalities ( $P=0.0001$ ) with azoospermia when compared to oligozoospermia was observed.

**Conclusions** Our results highlight the importance of cytogenetic studies in patients with oligozoospermia (both mild and severe) and non-obstructive azoospermia. The presence of chromosomal abnormalities influences significantly the fertility treatment protocols, as well as provides a definite diagnosis to couples suffering from infertility.

**Keywords** Male infertility · Azoospermia · Oligozoospermia · Chromosomal abnormalities

## Introduction

Infertility is defined as inability to conceive after 1 year of regular unprotected intercourse [1]. It affects approximately 15 % of couples of reproductive age, 20–25 % of reproductive problems being contributed to male factor [2]. Male infertility is typically defined as abnormal semen analysis. However, abnormal semen parameters do not necessarily result in infertility but they correlate with lower probability of achieving pregnancy [3, 4]. Patients with reduced sperm counts and non-obstructive azoospermia are at increased risk of chromosomal abnormalities detection. Prevalence of chromosomal abnormalities in general population of infertile men ranges from 2 % to 7 % [5–7], which represents an 8–10-fold increase, when compared to the group of unselected newborns [8]. In groups of patients with azoo- and oligozoospermia numerical and structural chromosomal abnormalities are detected with frequencies ranging from 4 % to 23 % [6, 9, 10]. Variations in reported frequencies are due to different recruitment procedures (i.e. the severity of oligospermia), or interpretation of cytogenetic results (i.e. consideration of chromosomal variants as normal or pathological). Irrespective of the frequency of chromosomal abnormalities, the general consensus is that

**Capsule** Results of cytogenetic studies of Ukrainian men with infertility are presented. Increased incidence of chromosomal abnormalities was detected in patients with oligospermia and azoospermia warranting cytogenetic testing for these groups of patients.

L. Y. Pylyp (✉) · L. O. Spinenko · N. V. Verhoglyad · V. D. Zukin  
Clinic of Reproductive Medicine “Nadiya”, M. Kryvonosa St. 19a,  
Kyiv 03037, Ukraine  
e-mail: l.pylyp@ivf.com.ua

the probability of detecting an abnormality in a patient's karyotype is negatively correlated to the sperm count [11]. Assisted reproduction technologies in general and intracytoplasmic sperm injection in particular, currently are a treatment option for patients with reduced sperm counts. However, intracytoplasmic sperm injection increases the chances of bypassing chromosomal abnormalities to the offspring, thus increasing the probability of developing malformations of genetic origin [12]. Therefore, cytogenetic analysis of patients with azoospermia and oligozoospermia is mandatory before infertility treatment with assisted reproduction technologies.

The purpose of the current study was to evaluate the frequencies and types of chromosomal abnormalities in groups of patients with azoospermia and oligozoospermia and to compare our results with other reports.

## Material and methods

From January 2009 to August 2012 164 men with oligozoospermia, 40 men with azoospermia and 87 men with normozoospermia were recruited into the study. The mean age in groups was  $34.9 \pm 6.6$ ;  $36 \pm 6.3$ ;  $33.9 \pm 5.8$  respectively and did not differ significantly. Semen analysis was performed according to World Health Organisation [13]. Azoospermia and severe oligozoospermia were defined as a total absence of sperm cells in seminal liquid and sperm cell count  $\leq 5 \times 10^6/\text{ml}$  respectively; patients with sperm count more than  $5 \times 10^6/\text{ml}$  but less than  $15 \times 10^6/\text{ml}$  were considered as mild oligozoospermic.

Cytogenetic studies were performed as a part of routine evaluation of males with severe infertility. Chromosomal analysis was performed on phytohaemagglutinin (PHA)-stimulated peripheral lymphocyte cultures using standard cytogenetic methods. 20 GTG-banded metaphases with minimum resolution of 550 bands per haploid set were analyzed in each case. In cases of suspected mosaicism, the number of metaphases was increased to 50. Only structural and numerical rearrangements were considered abnormal; polymorphic variants were classified as normal. Genetic counseling was performed to inform patients about the risk of transmitting chromosomal abnormalities (if present) to the offspring and preimplantation genetic diagnosis was offered to couples with chromosomal abnormalities.

Differences in group frequencies were assessed by  $\chi^2$ -test and significance was declared at  $P \leq 0.05$ .

## Results

Chromosomal abnormalities were identified in 21 patients with oligozoospermia and 14 patients with azoospermia,

corresponding to the frequencies of 12.8 % and 35 % respectively. As a whole, we observed chromosomal abnormalities in 17.1 % of patients, from which 6.3 % showed numerical and 10.8 % showed structural abnormalities.

Klinefelter syndrome was detected in 64 % (9/14) of patients with azoospermia. Other gonosomal abnormalities in this group included terminal deletion of chromosome Y (delYq11.2-qter) in one patient (7 %) and karyotype 47, XYY in one patient (7 %). Autosomal abnormalities in patients with azoospermia were presented by 3 cases (22 %) of balanced structural rearrangements (2 Robertsonian translocations and 1 reciprocal translocation).

Structural autosomal rearrangements constituted 91 % (19/21) of chromosomal abnormalities detected in patients with oligozoospermia, including 2 inversions, 7 Robertsonian translocations and 10 reciprocal translocations. In 9 % of patients (2/21) numerical abnormalities were detected: two mosaic cases with karyotype 47,XXY/46,XY and 47,XYY/46,XY.

A significant increase in the frequency of chromosomal abnormalities was detected in patients with severe oligozoospermia ( $< 5 \times 10^6/\text{ml}$ ) when compared to patients with mild oligozoospermia ( $< 5 \times 10^6/\text{ml}$ ): 18.4 % vs 6.5 % ( $P=0.01$ ) (Table 1).

The frequency of chromosomal abnormalities in patients with normozoospermia was 1.1 % (1/87). One patient in this group showed a pericentric inversion of chromosome 10 – inv(10)(p12q21).

## Discussion

In the present study, chromosomal abnormality rate in patients with oligo- and azoospermia constituted 17 %, which was close to the rate reported by Kleiman and colleagues [14] in Israel (16.6 %) and higher than previously reported by Kumpete and colleagues [15] in Turkey (12 %), Wang and colleagues [16] in China (8.5 %), Rao and colleagues [17] in India (7.9 %), Gekas and colleagues [7] in France (6.9 %). In the studied group, 35 % of patients with azoospermia and 12.8 % of patients with oligozoospermia displayed chromosomal abnormalities. It should be mentioned, that most studies report approximately 15–17 % (from 11 % to 24 %) of patients with azoospermia and 2–16 % of patients with oligozoospermia to have chromosomal abnormalities [6, 7, 18–24]. Such variability among different series is likely to be related to a dissimilar composition of the studied populations, mostly to the severity of male factor. Moreover, the relative increase of prevalence of chromosomal abnormalities observed in our group, compared to other reports, could be explained by the specificity of the studied cohort: a high proportion of infertile patients was referred to our clinic with miscarriage and unsuccessful previous in vitro fertilization

**Table 1** Types and frequencies of chromosomal abnormalities in groups of patients with azoospermia, oligozoospermia and normozoospermia

Type of infertility	Number of patients	Chromosomal abnormalities, N(%)	Autosomal abnormalities, N(%)	Sex chromosome abnormalities, N(%)
Azoospermia	40	14 (35) <sup>a</sup>	3 (7.5)	11 (27.5) <sup>b</sup>
Severe oligozoospermia ( $\leq 5 \times 10^6$ /ml)	87	16 (18.4) <sup>c</sup>	11 (12.6)	5 (5.8)
Mild oligozoospermia ( $5\text{--}15 \times 10^6$ /ml)	77	5 (6.5) <sup>d</sup>	5 (6.5)	0 (0)
Total	204	35 (17.1)	19 (9.3)	16 (7.8)
Normozoospermia	87	1 (1.1)	1 (1.1)	0 (0)

<sup>a, c</sup>  $p=0.0001$  compared to control group

<sup>b</sup>  $p=0.0001$  compared to oligozoospermia group

<sup>d</sup>  $p=0.05$  compared to control group

attempts for preimplantation genetic diagnosis. The incidence of chromosomal abnormalities in these subgroups of patients is known to be increased, which explains the increase in chromosomal abnormalities observed.

Chromosomal abnormalities detected in the present study are comparable with those reported in other studies of infertile men: gonosomal abnormalities were mainly detected in men with azoospermia, while autosomal aberrations were mainly reported in non-azoospermic men [10, 25–27]. We observed a strong and statistically significant association of numerical chromosomal abnormalities with azoospermia ( $P=0.0001$ ). The association of gonosomal abnormalities, in particular Klinefelter syndrome and primary testicular failure is well known [28, 29]. In studied group, 64 % of patients with azoospermia were presented with Klinefelter syndrome. Balanced structural chromosome abnormalities do not normally result in azoospermia, but usually present the phenotype varying from severe oligozoospermia to normozoospermia. In the present study, out of 22 structural chromosomal abnormalities detected only 4 (18 %) resulted in azoospermia, 1 (4.5 %) had no effect on spermatogenesis (a patient from the control group) and 77.5 % led to decreased sperm count. Carriers of balanced chromosomal rearrangement, while normal phenotypically, may experience fertility problems due to the production of unbalanced gametes, which increase the risk of miscarriage and live birth of children with malformations of genetic origin [30]. The relative frequency of formation of normal/balanced or unbalanced gametes depends on the type of rearrangement, chromosomes involved, size of regions involved in the rearrangement, presence of heterochromatin, location of breakpoints and likelihood of recombination to occur within the translocated segments. The proportion of unbalanced gametes varies in each case from 19 to 90 % and is dependent on sperm count [31, 32]. The levels of unbalanced gametes are

significantly higher than empiric risks of having an unbalanced offspring due to the natural selection of balanced gametes and early abortion of unbalanced products of conception. Still, in carriers of chromosomal abnormalities the risk of miscarriage and live birth of children with unbalanced karyotype is high and preimplantation genetic diagnosis should be offered to such patients.

In the present study, we detected that sperm concentration shows the best correlation with presence of chromosomal abnormalities. We observed an increase of chromosomal abnormalities rate with the sperm count decrease: chromosomal abnormalities were detected in 1.1 % of patients with normozoospermia, 12.8 % of patients with oligozoospermia and 35 % of patients with azoospermia. Interestingly, when patients of oligozoospermic group were divided into subgroups with severe oligozoospermia ( $\leq 5 \times 10^6$ /ml) and mild oligozoospermia ( $> 5 \times 10^6$ /ml) the increase in frequency of chromosomal abnormalities with the decrease of sperm count was observed: from 6.5 % in men with mild oligozoospermia to 17.1 % in men with severe oligozoospermia ( $P=0.02$ ). Still, a significant increase in the frequency of chromosomal abnormalities was detected in mild oligozoospermic group ( $P=0.05$ ) when compared to men with normozoospermia. That is why, we believe that patients with both mild and severe oligozoospermia should be referred for cytogenetic analysis. Moreover, since one case of structural chromosomal abnormality in normozoospermic group was observed, patients with normal semen parameters and unexplained infertility should also be referred for cytogenetic studies.

In summary, the presented data allows for a more specific risk estimate of chromosomal abnormalities in subgroups of men with infertility. Our results demonstrate the elevated incidence of chromosomal abnormalities in groups of patients with azoospermia and oligozoospermia (sperm counts less than  $15 \times 10^6$ /ml). Therefore, cytogenetic studies in

these groups of patients need to be performed before the assisted reproduction treatment.

## References

- World Health Organisation. WHO manual for the standardized investigation and diagnosis of the infertile couple. Cambridge: UK, Cambridge University Press; 2000.
- Ferlin A, Raicu F, Gatta V, Zuccarello D, Palka G, Foresta C. Male infertility: role of genetic background. *Reprod Biomed Online*. 2007;14:734–45.
- deKretser DM. Male infertility. *Lancet*. 1997;349:787–90.
- Huynh T, Mollard R, Trounson A. Selected genetic factors associated with male infertility. *Hum Reprod Update*. 2002;8:183–98.
- Baschat AA, Kupker W, al Hasani S, Diedrich K, Schwinger E. Results of cytogenetic analysis in men with severe subfertility prior to intracytoplasmic sperm injection. *Hum Reprod*. 1996;11:330–3.
- Elghezal H, Hidar S, Braham R, Denguezli W, Ajina M, Saad A. Chromosome abnormalities in one thousand infertile males with nonobstructive sperm disorders. *Fertil Steril*. 2006;86:1792–5.
- Gekas J, Thépot F, Turleau C, Siffroi JP, Dadoune JP, Briault S, 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.
- Nielsen J, Wohlert M. Chromosome abnormalities found among 34910 newborn children: results from a 13-year incidence study in Arhus, Denmark. *Hum Genet*. 1991;87:81–3.
- 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;90:152–6.
- O'Flynn, O'Brien KL, Varghese AC, Agarwal A. The genetic causes of male factor infertility: a review. *Fertil Steril*. 2010;93:1–12.
- Clementini E, Palka C, Iezzi I, Stuppia L, Guanciali-Franchi P, Tiboni GM. Prevalence of chromosomal abnormalities in 2078 infertile couples referred for assisted reproductive techniques. *Hum Reprod*. 2005;20:437–42.
- Georgiou I, Syrrou M, Pardalidis N, Karakitsios K, Mantzavinos T, Giotitsas N, et al. Genetic and epigenetic risks of intracytoplasmic sperm injection method. *Asian J Androl*. 2006;8:643–73.
- World Health Organisation (2010) WHO laboratory manual for the examination and processing of human semen. ed. 5. Geneva, Switzerland.
- Kleiman SE, Yogev L, Gamzu R, Hauser R, Botchan A, Lessing JB. Genetic evaluation of infertile men. *Hum Reprod*. 1999;14:33–8.
- Kumtepe E, Beyazyurek C, Cinar C, Ozbey I, Ozkan S, Cetinkaya K, et al. A genetic survey of 1935 Turkish men with severe male factor infertility. *Reprod Biomed Online*. 2009;18:465–74.
- Wang RX, Fu C, Yang YP, Han RR, Dong Y, Dai RL, et al. Male infertility in China: laboratory finding for AZF microdeletions and chromosomal abnormalities in infertile men from Northeastern China. *J Assist Reprod Genet*. 2010;27:391–6.
- Rao KL, Babu KA, Kanakawalli NK, Padmalatha VV, Deenadayal M, Singh L. Prevalence of chromosome defects in azoospermic and oligoastheno-teratozoospermic South Indian infertile men attending an infertility clinic. *Reprod Biomed Online*. 2006;10:467–72.
- Akgul M, Ozkinay F, Ercal D, Cogulu O, Dogan O, Altay B, et al. Cytogenetic abnormalities in 179 cases with male infertility in Western Region of Turkey: report and review. *J Assist Reprod Genet*. 2009;26:119–22.
- Chantot-Bastaraud S, Ravel C, Siffroi JP. Underlying karyotype abnormalities in IVF/ICSI patients. *Reprod Biomed Online*. 2008;16:514–22.
- Dul EC, van Ravenswaaij-Arts CMA, Groen H, van Echten-Arends J, Land JA. Who should be screened for chromosomal abnormalities before ICSI treatment? *Hum Reprod*. 2010;25:2673–7.
- Ferlin A, Arredi B, Foresta C. Genetic causes of male infertility. *Reprod Toxicol*. 2006;22:133–41.
- Lessing JB. Genetic evaluation of infertile men. *Hum Reprod*. 1999;14:33–8.
- Martin RH. Cytogenetic determinants of male fertility. *Hum Reprod Update*. 2008;14:379–90.
- Yatsenko AN, Yatsenko SA, Weedin JW, Lawrence AE, Patel A, Peacock S, et al. Comprehensive 5-year study of cytogenetic aberrations in 668 infertile men. *J Urol*. 2010;183:1636–42.
- Dohle GR, Halley DJ, Van Hemel JO, van den Ouwel AM, Pieters MH, Weber RF, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. *Hum Reprod*. 2002;17:13–6.
- Dul EC, Groen H, van Ravenswaaij-Arts CMA, Dijkhuizen T, van Echten-Arends LJA. The prevalence of chromosomal abnormalities in subgroups of infertile men. *Hum Reprod*. 2012;27:36–43.
- Van Assche E, Bonduelle M, Tournaye H. Cytogenetics of infertile men. *Hum Reprod*. 1996;11:1–24.
- Maiburg M, Repping S, Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. *Fertil Steril*. 2012;98:253–60.
- Oates RD. The natural history of endocrine function and spermatogenesis in Klinefelter syndrome: what the data show. *Fertil Steril*. 2012;98:266–73.
- Stephenson M, Sierra S. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. *Hum Reprod*. 2006;21:1076–82.
- Anton E, Vidal F, Blanco J. Role of sperm FISH studies in the genetic reproductive advice of structural reorganization carriers. *Hum Reprod*. 2007;22:2088–92.
- Ferfourri F, Selva J, Boitrelle F, Gomes DM, Torre A, Albert M, et al. The chromosomal risk in sperm from heterozygous Robertsonian translocation carriers is related to the sperm count and the translocation type. *Fertil Steril*. 2011;96:1337–43.