

Cytogenetic Abnormalities in Infertile Men in the Prešov Region (Slovakia)

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ABSTRACT Chromosome anomalies belong to genetic factors, which participate on etiology of reproductive failure. The aim of the study was to investigate the frequency of chromosome abnormalities in infertile men in the Prešov region in Slovakia (1998-2014). Karyotyping using G-banding and C-banding methods was performed in 1426 subjects including 948 infertile men and 478 controls. Karyotype analyses revealed chromosomal abnormalities in 2.6 percent of infertile men. Detected frequency of sex chromosome abnormalities in men with diagnosed azoospermia was 11.5 percent compared with 1.0 percent of chromosome abnormalities in men with severe oligospermia ($p < 0.01$). Heterochromatin variants were identified in 13 percent of infertile men. Detected frequency of heterochromatin variants of infertile men was significantly higher than in controls ($p < 0.0001$). The results of the study might suggest the role of chromosome anomalies in human fertility. All these findings support genetic screening of infertile men before starting assisted reproductive treatments.

INTRODUCTION

Infertility affects approximately 15 percent of couples in reproductive age, approximately 50 percent of which might be attributed to male factors (Ananthapur et al. 2014). The most common causes of male infertility are abnormal sperm delivery, chromosomal abnormalities, defective pituitary gland function, infections of the male accessory glands and overexposure to certain environmental factors (Mierla et al. 2014). Human infertility is closely linked to the chromosomal abnormalities (Minocherhomji et al. 2009; Šípek et al. 2014). Chromosomal heteromorphisms, known as chromosomal polymorphisms, include varying sizes of heterochromatin blocks, satellites, repeat sequence regions and inversions (Christofolini et al. 2012). The most common inversion variant is $inv(9)(p12q13)$ (Schaffer et al. 2013). The contradiction exists as to the consequences of the heterochromatin variations seen in the general population with respect to its inheritance.

Cytogenetic studies are essential for evaluating of chromosomal aberrations and heterochromatin variants. Karyotyping of infertile couples is important not only from a diagnostic viewpoint, but even more importantly, to gain the better understanding of gametogenic impairment associated with chromosomal abnormalities (Ananthapur et al. 2014).

Objectives

The aim of the study was to determine the frequency of chromosome aberrations and heterochromatin variants in the survey of infertile men in the Prešov region (Slovakia) over a period of years 1998-2014.

METHODOLOGY

Cytogenetic analyses using G-banding were performed in 948 infertile men (mean 36.50 ± 8.35 years) and 478 fertile controls (mean 33.05 ± 9.15 years). Written informed consent was obtained from each participant.

For cytogenetic analysis peripheral blood samples were prepared according to the standard laboratory protocol (Rooney and Czepulkowski 1992). The cultures were incubated for 72 hours at 37°C in RPMI-1640 medium. The cell divisions were arrested in the metaphases by adding colchicine 4.10^5 M for 30-40 minutes before harvesting the cultures. The cultures were treated with 0.4 percent potassium chloride hypotonic solution and fixed in 3:1 methanol-acetic acid mixtures. The banded slides were microscopically analyzed using G-banding and C-banding methods according to ISCN nomenclature (Mitelman 1995). Statistical analysis was performed with SPSS® version 17.0 statistical package (SPSS Inc., Chicago, IL, USA) for Windows®. Fisher's exact test was used to compute P-values and 95 percent confi-

dence intervals (CI) for the odds ratios (OR). A P-value <0.05 was considered statistically significant.

RESULTS

Cytogenetic analyses confirmed a normal 46, XY male karyotype in 97.4 percent of infertile men. Chromosome aberrations were detected in 2.6 percent of infertile men. Sex chromosomal abnormalities were detected in 68 percent from all abnormal karyotypes. Types of chromosomal aberrations detected in analysed survey of infertile men in the Prešov region (Slovakia) are presented in Table 1. The most common cytogenetic findings of chromosome aberrations represented karyotype 47, XXY. Mosaic forms of chromosome aberrations 47,XXY/46,XY and 46,XYY/46,XY were detected in 8 percent of infertile men. Detected autosomal abnormalities included 16 percent of chromosomal translocations and 12 percent of autosome chromosomes inversions. The frequency of sex chromosomal abnormalities in infertile men with azoospermia (11.5 %) was significantly higher than in men with severe oligozoospermia (1.0 %) ($P < 0.01$). There were no significant differences in the incidence of autosomal abnormalities between infertile men with azoospermia (3.2 %) and men with severe oligozoospermia (2.8 %) ($P > 0.05$).

The results of cytogenetic analyses of chromosome heteromorphism are summarized in Table 2. In analysed survey of infertile men in the Prešov region (Slovakia) 123 heterochromatin variants (13.0 %) of chromosomes 1, 9, 16 were detected. In the control group, there were 23 heterochromatin variants (4.8 %). The most frequent detected heterochromatin variants in the survey of infertile men in the Prešov region were Y chromosome heterochromatin variants (Yqh+/Yqh-

Table 1: Chromosomal findings in infertile men in the Prešov region (Slovakia) (1998-2014)

<i>Chromosomal findings</i>	<i>Frequency</i>
Normal	923 (97.4 %)
Chromosomal aberrations	25 (2.6 %)
Sex chromosome aberrations	17/948 (1.8 %)
	47,XXY [15]
	mos 46,XXY/46,XY [1]
	mos 46,XYY/46,XY [1]
	7/948 (0.7%)
Autosomal Abnormalities	
Translocations	45,XY,t(14;21)(q10;q10) [1]
	45,XY,t(13;15)(q10;q10) [1]
	46,XY,t(15;16)(p15;q13) [1]
Inversions	46,XY,inv(2)(p13;q21) [1]
	46,XY,inv(8)(p23;q31) [1]
	46,XY,inv(14)(q11;q23) [1]
Others	46,XY/46,XY,t(6;15)(q16;q12) [1]
	46,XX male [1]
	46,XY,inv(9)(p12;q12) [4]
	46,XY,inv(9)(p11;q12) [3]
	46,XY,inv(9)(p11;q13) [4]

(2.6 %). Heterochromatin variants of chromosome 1 (1.1 %) were the common variants in the control group. Statistically significant differences in the overall incidence of heterochromatin variants in the survey of infertile men and controls in the Prešov region were detected ($p < 0.0001$; 95 % CI 1.703-4.501)(Table 3).

The results of the study show that chromosomal polymorphisms are common among infertile men. These results highlighted the role of chromosome anomalies in the pathogenesis of human fertility.

DISCUSSION

Reproductive disorders are closely associated with cytogenetic abnormalities. The etiology

Table 2: Heterochromatin variants in infertile men and controls in the Prešov region (Slovakia) (1998-2014)

<i>Chromosome</i>	<i>Heterochromatin variants</i>	<i>Group</i>			
		<i>Infertile men (n = 948)</i>		<i>Controls (n = 478)</i>	
		<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>
1	1qh+/1qh-	10/9	2.00	3/2	1.05
9	9qh+/9qh- inv(9)	9/6/4	2.00	2/2/0	0.84
16	16qh+/16qh-	9/8	1.79	2/1	0.63
13 / 14 / 15	13s+/14s+/15s+	8/5/17	0.84/0.53/1.79	2/2/4	0.42/0.42/0.84
21 / 22	21s+/22s+	6/7	0.63/0.74	0/1	0/0.21
Y	Yqh+/Yqh-	2/23	2.64	1/1	0.42

Table 3: The results of testing statistical significance of heterochromatin variants in infertile men and controls in the Prešov region (Slovakia) (1998-2014)

	Chromosome									Total
	1	9	16	13	14	15	21	22	Y	
P	0.275	0.120	0.095	0.510	1.000	0.243	0.187	0.280	0.003**	p<0.0001
95 % CI	0.677- 5.950	0.774- 8.459	0.797- 12.455	0.399- 3.849	0.218- 9.411	0.681- 7.640	0.540- INF*	0.441- 76.988	1.477- 39.534	1.703- 4.501

*INF - infinite

** statistically significant

values are presented as p- value according to Fischer exact test (95 % CI for the OR)

of male infertility is unknown in approximately one third of patients. The unaccountable forms of male infertility may be caused by several factors, such as varicocele, cryptorchidism, spermatic duct obstruction, urogenital tract infections, antisperm antibodies, retrograde ejaculation, endocrine disturbances, systemic diseases, testicular malignancy and environmental factors (Warhaus 2007; Suganya et al. 2015).

Several studies have reported a wide range (2,1 % to 19,48 %) of chromosomal abnormalities in infertile men (Hofherr et al. 2011; Quan et al. 2013; Ananthapur et al. 2014; Kate et al. 2014). Reasons for this discrepancy might be in selection of patient groups, ethnic differences and the quantity of samples. Chromosomal abnormalities are confirmed as one of the frequent causes of male infertility, the incidence of which has been shown to be as high as 20 percent in azoospermic males, with the sex chromosomes more commonly involved (Ananthapur et al. 2014; Suganya et al. 2015). The severity of the semen parameters and the frequency of chromosomal abnormalities seem to be positively correlated (Ghorbel et al. 2012; Kim et al. 2012; Kate et al. 2014; Naasse et al. 2015).

Detected frequency of chromosomal aberrations in our study (2.6 %) was within the range of the published data. Karyotypic abnormalities identified in our study included autosomal abnormalities and sex chromosome abnormalities. The most common sex chromosomal aberration was Klinefelter's syndrome. Autosomal abnormalities (involving chromosome 14 and 15) detected in 4.2 percent suggest that some genes present on these chromosomes might play an important role in spermatogenesis. The frequency of sex chromosomal abnormalities in infertile men with azoospermia (11.5 %) was significantly higher than in men with severe oligozoospermia (1.0 %) (P<0.01). Our findings support the previous notion that abnormalities of sex chromosomes are

primarily found in azoospermic patients, while balanced autosomal aberrations are the most frequent abnormalities in oligozoospermic males (Elghezal et al. 2006; Kim et al. 2012; Ocak et al. 2014; Zhang et al. 2015). An increased prevalence of chromosomal structural abnormalities has been documented in patients with recurrent implantation failure (Caseiro et al. 2015). Carriers of a balanced translocation can produce unbalanced gametes, which can cause fertilization failure, implantation failure or embryo loss (Mau-Holzmann 2005; Caseiro et al. 2015). These findings suggest the involvement of karyotype abnormalities in the pathogenesis of implantation failure.

Morphological variations of constitutive heterochromatin are frequently detected during routine cytogenetic analysis. Most often, chromosomes vary in size and position of heterochromatin in 1qh, 9qh, and 16qh regions. Although inherited variants haven't been reported to be associated with any risk for phenotypic abnormalities. Chromosomal heteromorphisms have been found to have a higher frequency relative to the normal population and have been regarded as abnormalities in some studies (Caglayan et al. 2010; Kate et al. 2014). The analyses of polymorphisms in genes involved in spermatogenesis represent one of the most exciting areas of research in genetics of male infertility. Polymorphisms in these genes are considered as potential risk factors that may contribute to the severity of spermatogenic failure (Plaseska-Karanfilska et al. 2012). In this study the researchers identified the presence of heterochromatin variants in 13 percent of infertile men. The most common detected heteromatin variants were polymorphisms of Y chromosome (2.6 %). Heteromorphisms of other chromosomes (short-arm regions of D and G group of chromosomes), heteromorphisms of chromosomes 1, 9, 16 and inv(9)) have also been described. Pericentric inversion of chromosome 9,

inv(9)(p11q12)/inv(9)(p11q13) is a common chromosomal rearrangement and some cytogeneticists consider it as a normal variant, generally without phenotypic effects (Mierla et al. 2014). Heterochromatin variants of chromosome 9 were detected in our study in 2 percent of infertile men. Other common chromosomal variants detected in infertile men in the Prešov region were heterochromatin variants of chromosome 1 (1qh+/1qh-). The results of this study confirm higher frequencies of heterochromatin variants in infertile men than in controls (13 % vs. 4.8 %), the differences were statistically significant ($p < 0.0001$). It is likely that polymorphisms associated with a specific genetic background and/or with environmental factors can lead to spermatogenic impairment. The exact mechanism by which chromosomal abnormalities induce infertility is still not completely known. Some authors suggest that presence of abnormal chromatin interferes with meiotic division and affects sperm production (Etem et al. 2009). Genetic abnormalities which affect spermatogenesis may cause abnormal embryonic development, which in turn can lead to recurrent miscarriages (Fu et al. 2012). Severe male infertility has been correlated with an increase in chromosomal anomalies (Ananthapur et al. 2014; Kate et al. 2014). The results of the present study confirmed the higher occurrence of chromosome anomalies in Slovak infertile men and it is absolutely reason for indication of cytogenetic examination.

CONCLUSION

The results of the study confirmed the higher frequency of chromosome abnormalities in infertile men. Study of human chromosomes plays a key role in diagnosis, prognosis and monitoring of chromosomal abnormalities. The results of the study highlighted the need of cytogenetic analysis and efficient molecular genetic testing in male infertility diagnosis.

RECOMMENDATIONS

Analysis of cytogenetics anomalies is highly recommended to identify the causes of infertility and to choose the appropriate assisted reproduction technique.

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