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GENETICS

Molecular and cytogenetic studies of 101 infertile men with microdeletions of Y chromosome in 1,306 infertile Korean men

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Abstract
Objectives To determine the prevalence of Y chromosome microdeletions in infertile Korean men with abnormal sperm counts and to assess the clinical features and frequency of chromosomal abnormalities in Korean patients with microdeletions.
Methods A total of 1,306 infertile men were screened for Y chromosome microdeletions, and 101 of them had microdeletions. These 101 men were then retrospectively studied for cytogenetic evaluation, testicular biopsy and outcomes of IVF and ICSI.

Capsule A close relationship exists between microdeletions and spermatogenesis, although IVF outcome was not significantly affected by the presence of an AZFc microdeletion.

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Results The overall prevalence of Y chromosome microdeletions in infertile men was 7.7% (101/1,306). Most microdeletions were in the AZFc region (87.1%), including deletions of AZFbc (24.7%) and AZFabc (8.9%). All patients with AZFa, AZFbc and AZFabc deletions had azoospermia, whereas patients with an AZFc deletion usually had low levels of sperm in the ejaculate or in the testis tissues. Chromosomal studies were performed in 99 men with microdeletions, 36 (36.4%) of whom had chromosomal abnormalities. Among the infertile men with Y chromosome microdeletions in this study, the incidence of chromosomal abnormality was 48.6% in the azoospermic group and 3.7% in the oligozoospermic group. Among the 69 patients with microdeletions and available histological results, 100.0% of the azoospermic group and 85.7% of the oligozoospermic group had histological abnormalities. The frequency of both chromosomal abnormalities and histological abnormalities was higher in the azoospermic group compared to the oligozoospermic group. Thirty-four ICSI cycles with either testicular (n=14) or ejaculated spermatozoa (n=20) were performed in 23 couples with men with AZFc microdeletion. Thirteen clinical pregnancies (39.4%) were obtained, leading to the birth of 13 babies.
Conclusions The study results revealed a close relationship between microdeletions and spermatogenesis, although IVF outcome was not significantly affected by the presence of the AZFc microdeletion. Nevertheless, Y chromosome microdeletions have the potential risk of being transmitted from infertile fathers to their offspring by ICSI. Therefore, before using ICSI in infertile patients with severe spermatogenic defects, careful evaluations of chromosomal abnormalities and Y chromosome microdeletions screening should be performed and genetic counseling should be provided before IVF-ET.

Keywords Y chromosome microdeletion · Chromosomal abnormality · Azoospermia factor (AZF) · Intracytoplasmic sperm injection (ICSI)

Infertility affects approximately 15% of couples of reproductive age [1], with about half of the cases involving male factor infertility. Male infertility can be caused by a number of factors and therefore even the most comprehensive work-up including physical, serological, and hormonal examinations could fail to detect the etiology of reproductive disorders [2, 3].

Numerous studies have demonstrated that one or more genes located on the long arm of the Y chromosome are likely to be involved in the complex process of spermatogenesis. These genes or gene families are located in the distal Yq11 region, and are defined as the azoospermia factor (AZF). The AZF region can be divided into four non-overlapping sub-regions called AZFa (proximal), AZFb (middle), AZFc (distal) and AZFd (between AZFb and AZFc). The AZF region cannot be visualized on karyotypes and therefore must be discerned through molecular biology techniques. At present, it can be accurately diagnosed as either a chromosomal abnormality or a Y chromosome microdeletion, which is likely connected to spermatogenic failure [4–6]. These microdeletions are associated with either reduced sperm count (oligozoospermia) or complete absence of spermatozoa (azoospermia) [7].

Phenotypes resulting from different microdeletions of the Y chromosome are variable. AZFa microdeletions are associated with Sertoli cell only (SCO) syndrome, where there are either no visible germ cells in any seminiferous tubules (SCO I) or germ cells are present in a minority of tubules. These cells arise from a failure to complete differentiation and maturation of spermatocytes and spermatids, leading to the degeneration of germ cells within most tubules (SCO II) [8]. AZFb deletions show more variable defects. Patients with large AZFb deletions are azoospermic and those harboring partial AZFb deletions present with a range of infertile phenotypes, including mild and severe oligozoospermia. Spermatogenic arrest is observed in about half of these AZFb deletions [9]. Deletions of the AZFc region quantitatively reduce sperm density and are associated with a wide range of phenotypical features ranging from azoospermia to mild to severe oligozoospermia [10]. Patients with microdeletions limited to AZFd may present with mild oligozoospermia or even normal sperm counts associated with abnormal sperm morphology [11]. Approximately 38% and 23% of men with infertility are diagnosed as azoospermia and severe oligozoospermia, respectively. Moreover, about 10% to 15% of idiopathic cases of azoospermia and severe oligozoospermia have microdeletions in AZF regions as the etiologic factor [12–14]. The frequency of Y chromosome microdeletions varies between 1% [15] and 55% [16] in the worldwide whereas the few studies performed in Asian male populations showed frequencies of 7.6% to 16.5% in Japan [17–22], 11.0% to 19.4% in China [23, 24], 10.6% to 11.7% in Taiwan [25, 26], 6.4% in Hong Kong [27] and 2.0% to 12.0% in India [12, 28, 29]. A recently published study reported that the cumulative frequency of Y chromosome microdeletions was 3.5% in infertile males [30]. It appears that a significant percentage of infertile males with microdeletions in AZF regions do not have children by natural means of reproduction.

Chromosomal abnormalities can also cause male infertility. In the general male population, the incidence of chromosomal abnormalities ranges from 0.7% to 1.0%, whereas it is approximately 10.6% among azoospermic and oligozoospermic men [31, 32]. The severity of the semen parameters and the frequency of chromosomal abnormalities seem to be positively correlated [30].

Microdeletions of the Y chromosome and various chromosomal abnormalities can cause male infertility; therefore genetic screening should be suggested to infertile men. Reports regarding the prevalence of chromosomal anomalies and Y chromosome microdeletions in Korean populations are rare. The aim of this study was to determine the prevalence of Y chromosome microdeletions in infertile Korean men with abnormal sperm counts and to assess the clinical features and frequency of chromosomal abnormalities in Korean patients with Y chromosome microdeletions. This study also investigated whether the presence of a Y chromosome microdeletion affected the outcome of *in vitro* fertilization (IVF) and the intracytoplasmic sperm injection (ICSI) program in a Korean population.

Materials and methods

1. Subjects

The molecular screening results of 1,306 infertile men with oligozoospermia or azoospermia who were referred to the Infertility Clinic at Cheil General Hospital and Women's Healthcare Center from September 1997 to April 2009 were analyzed retrospectively. The selection was based on primary infertility and on the presence of azoospermia or oligozoospermia (<20X10^6 sperm/ml) ejaculate with propulsive motility. All patients underwent semen analysis at least twice before a diagnosis of azoospermia and oligozoospermia. Obstructive azoospermic men were excluded. All subjects were of Korean ethnic origin. Of the 1,306 cases that underwent molecular screening, 101 patients with Y chromosome deletions were analyzed for detailed cytogenetic and histological examinations. The majority of these patients were azoospermia (n=73, 72.3%) and the rest had

severe oligozoospermia (n=28, 27.7%). Blood samples were collected for cytogenetic analysis (n=99) and testicular biopsy was performed in 69 patients to determine the cause of infertility. Written informed consent was obtained from each participant.

2. Semen analysis

Semen analysis was performed in all patients according to the guidelines of the World Health Organization [33]. Semen was collected at the laboratory after 3 to 5 days of sexual abstinence and semen samples were subjected to evaluation for total count, percent motility, and forward progression using computer assisted semen analysis (CASA). At least two abnormal semen analyses in at least two ejaculates were required before a diagnosis of oligozoospermia and azoospermia was verified by pellet analysis after centrifugation (1500 rpm, 10 min).

3. Microdeletions of the Y chromosome

Genomic DNA was extracted from the peripheral blood of each patient and five Y chromosome specific-sequence tagged site (STS) markers were used for the detection of Y chromosome microdeletions. These STSs included the following: sY14 (*SRY* gene as an internal control); sY84 (AZFa region); sY129 and sY134 (AZFb region); and sY254 and sY255 (within the *DAZ* gene, AZFc region). Deletions in the fourth region (between AZFb and AZFc), which used to be called AZFd, were not screened separately.

Polymerase chain reaction (PCR) was performed in a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). The PCR program was set at 96°C for 2 min, followed by 35 cycles at 94°C for 40 s, 62°C for 30 s and 72°C for 40 s, and a final extension step at 72°C for 10 min. The PCR products were analyzed on a 2% agarose gel containing ethidium bromide and visualized under UV exposure. An STS marker was considered to be deleted only after at least two failed PCR amplification attempts.

4. Chromosome analysis

Peripheral blood samples were collected from each patient in sodium heparin tubes for karyotyping. Lymphocytes were cultured for 72 h in RPMI-1640 with phytohemaglutinin at 37°C and colcemid was added before harvest. The cultured lymphocytes were treated with hypotonic solution (0.075 M potassium chloride) and then fixed in Carnoy's fixative (methanol:acetic acid=3:1 v/v). Chromosome analysis was performed with GTG banding using the peripheral blood lymphocyte technique. At least 30 metaphases were analyzed for each patient. All chromosomal abnormalities were reported in accordance with the current standard international nomenclature.

5. Hormone analysis

Reproductive hormone levels were assessed in a certified laboratory using validated assays. Testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were assessed by radioimmunoassay. Normal ranges were 1–14 mIU/ml FSH, 1.5–9.2 mIU/ml LH and 1.3–8.13 ng/ml testosterone.

Results

This study performed molecular screening tests for Y chromosome microdeletions in 1,306 infertile patients. Y chromosome microdeletions were detected in 101 of the 1,306 patients and the overall prevalence of Y chromosome microdeletions was 7.7% (101/1,306). A high incidence of microdeletions was found in the AZFc region (54.4%), with a low incidence in AZFa (5.0%) and AZFb (7.9%). AZFc was the most frequently deleted region, including deletions of AZFbc and AZFabc (87.1%). Larger microdeletions involving the AZFbc and AZFabc regions were detected in 23.8% and 8.9% of patients, respectively. All patients with AZFa, AZFbc and AZFabc deletions had azoospermia, whereas patients with an AZFc deletion (38.4% in the azoospermia group and 96.4% in the oligozoospermia group) usually had low levels of sperm in the ejaculate or in the testis tissues. Out of 27 men with AZFc deletions in the oligozoospermia group, 23 patients (85.2%) had severe oligozoospermia ($<1X10^6$ sperm/ml), whereas three (11.1%) had moderate oligozoospermia ($1–5X10^6$ sperm/ml) and one (3.7%) had mild oligozoospermia ($5–20X10^6$ sperm/ml). These results indicate that AZFc deletion, which is the most common deleted form in oligozoospermia, may not always cause spermatogenesis defects (Table 1).

A chromosomal study of the male infertile patients with Y chromosome microdeletions revealed that 36 of 99 patients (36.4%) had chromosomal abnormalities. Klinefelter's

Table 1 Incidence and types of Y chromosome microdeletions in infertile men with azoospermia or oligozoospermia

	Azoospermic (n=73)	Oligozoospermic (n=28)			Total (n=101)
		severe	moderate	mild	
AZFa	5 (6.8)	0 (0.0)	0 (0.0)	0 (0.0)	5 (5.0%)
AZFb	7 (9.6)	1 (3.6)	0 (0.0)	0 (0.0)	8 (7.9%)
AZFc	28 (38.4)	23 (85.2)	3 (11.1)	1 (3.7)	55 (54.4%)
AZFabc	9 (12.3)	0 (0.0)	0 (0.0)	0 (0.0)	9 (8.9%)
AZFbc	24 (32.9)	0 (0.0)	0 (0.0)	0 (0.0)	24 (23.8%)

*severe oligozoospermia: $<1X10^6$ sperm/ml semen; moderate oligozoospermia: $1–5X10^6$ sperm/ml semen; mild oligozoospermia: $5–20X10^6$ sperm/ml semen

syndrome, 47, XXY was detected in two patients (5.6%); deletions in nine patients (25.0%); dicentric isochromosome in eight patients (22.2%); 46, XX, male in four patients (11.1%); marker and mosaic chromosomes in five (13.9%) and six patients (16.6%), respectively; and derivative and inversion chromosomes in one patient each (2.8%). The remaining 63 patients (63.6%) had a normal 46, XY, male karyotype. Thirty-six patients had both chromosomal abnormalities and Y chromosome microdeletions. Of them, 18 patients had a deletion in the AZFbc region (50.0%), nine patients in AZFc (25.0%), eight patients in AZFabc (22.2%) and one patient in AZFa (2.8%). Two patients with Klinefelter's syndrome (47, XXY) had partial deletions in the AZFa and AZFc regions of the Y chromosome (Table 2). A chromosomal study was not performed in two of the patients with microdeletions.

Testicular biopsy results were available in 69 out of the 101 men with Y chromosome microdeletions. Thirty-three patients (47.8%) had SCO syndrome, whereas 18 (26.1%) had maturation arrest. In addition, testis biopsies revealed severe hypospermatogenesis or hypospermatogenesis in 12 patients (17.4%), Leydig cell hyperplasia in two patients (2.9%) and other pathological histology in two patients (2.9%). Notably, all four patients with a deletion in the AZFa region had SCO syndrome, which is characterized by the absence of germ cell phenotypes (Table 3). Testicular biopsies were not taken at all in 32 patients with Y chromosome microdeletions.

Among the infertile men with Y chromosome microdeletions in this study, the incidence of chromosomal abnormality was 48.6% (35/72) in the azoospermic group and 3.7% (1/27) in the oligozoospermic group. Among the 69 patients with microdeletion and available histological

Table 2 Distribution of normal and abnormal karyotypes in men with Y chromosome microdeletions

Karyotypes	AZF deletions					
	a	b	c	abc	bc	Total
Normal (46,XY)	4	8	45	1	5	63 (64.0%)
Abnormal	1	0	9	8	18	36 (36.4%)
Klinefelter's syndrome (47,XXY)	1		1			
46,XX,male				4		
46,XY,inv9			1			
46,X,der(Y)/45X			1			
46,X,del(Y)(q11.23)			1		1	
46,X,del(Y)(q11.2)			2		1	
46,X,?del(Y)(q12)					2	
46,X,?del(Yq)/45,X					1	
46,X/46,X,del(Y)(q11.2)					1	
46,X,idic(Y)(q11.2)[58]/46,X,i(Y)(p10)[45]			1			
46,X,?i(Y)(p10).ish idic(Y)(q11.21)(DYZ3++,DYZ1-,SRY++)				1		
46,X,?idic(Y)(q11.2)					1	
46,X,?idic(Y)(q11.2)/45,X					1	
46,X,idic(Y)(q11.2)					1	
46,X,idic(Yq)/45,X					1	
46,X,idic(Y)(q?11.2)[67]/45,X[23]/46,XY[10]					1	
45,X[4]/46,X,idic(Y)(q11.222)[113]					1	
46,X,+mar/45,X				1		
46X,mar				1		
46,X,+mar(90)/45,X(19)					1	
46,X,+mar1(69)/45,X(30)/46,X,+mar2(4)					1	
46,X,+mar1/45X/46,X,+mar2					1	
mos 46,X,+mar1/45,X/46,X,+mar2			1			
mos 46,X,del(Y)(q12)/45,X			1			
mos 45,X/46,XY			1		1	
mos 46,X,+mar/45,X				1		
mos 45,X/46,Xder(Y).ish der(Y)					1	
Total	5	8	54	9	23	99

Table 3 Histological abnormalities of infertile men with Y chromosome microdeletions

Testicular histology	AZF deletions					
	a	b	c	abc	bc	Total
Normal		1	1			2 (2.9)
Sertoli cell only syndrome (SCO)	4		16	3	10	33 (47.8)
Maturation arrest		5	6		7	18 (26.1)
Severe hypo- or hypospermatogenesis		1	10		1	12 (17.4)
Leydig cell hyperplasia			1	1		2 (2.9)
Other pathological histology (atrophy, hematoma)		1			1	2 (2.9)
Total	4	8	34	4	19	69

results, histological abnormalities were found in 100.0% (55/55) of the azoospermic group and 85.7% (12/14) of the oligozoospermic group. According to the severity of sperm counts in the oligozoospermia group, in more detail, chromosomal abnormalities (3.7%, 1/27) and histological abnormalities (85.7%, 12/14) were found only in the severe oligozoospermia subgroup. The frequency of both chromosomal abnormalities and histological abnormalities was higher in the azoospermic group compared to the oligozoospermic group (Table 4).

Twenty-three couples in which the male had an AZF microdeletion attempted IVF/ICSI or frozen-thawed embryo transfer cycles. In the 34 fresh cycles, the mean age of female partners was 31.7±3.9 years and mean fertilization rate was 65.1% (Table 5). The rate of good embryos was 53.9%. Thirteen pregnancies resulted from the 33 fresh embryo transfer cycles (39.4%). Six male and seven female offspring were born. Because no spermatozoa were obtained in men with complete AZFa, AZFb, AZFbc or AZFabc microdeletions, all clinical pregnancies occurred among patients with AZFc microdeletions after ICSI using ejaculated sperm (seven couples) or testicular sperm (three couples). Unfortunately, ten additional transfers of frozen-thawed embryos led to two clinical pregnancies that both resulted in miscarriage.

Discussion

The frequency of microdeletions (7.7%) detected in the present study is within the range reported worldwide (5.7–21.0%) [15] and lower than other reports of Asian populations (9.6–19.4%) [34]. This wide variation may be due to ethnic differences, selection of different patient groups, genetic background and the STS marker sets used [30, 35]. Microdeletions of the Y chromosome are associated with either a reduced sperm count or the complete absence of spermatozoa, and are more common among azoospermic than oligozoospermic patients [7]. Most of the patients with microdeletions had deletions in the AZFc region (87.1%), including AZFbc and AZFabc. This result supports the concept that deleted in azoospermia (DAZ) has a significant role in male fertility and is consistent with previous reports indicating that the AZFc region is the most frequently deleted region in males with low sperm counts [30].

Numerous studies have shown a high incidence of chromosome abnormalities in infertile men, ranging from 2.2% to 14.3% [36, 37]. To date, the frequency of chromosomal abnormalities in men with Y chromosome microdeletions has been poorly evaluated, either because the study population was too small or the criteria of inclusion excluded an abnormal karyotype. In the present study, however, chromosomal analysis results were available for 99 of the 101 patients with Y chromosome microdeletions. Of them, 36 patients (36.4%) had chromosomal abnormalities. The prevalence of patients with both defects was slightly higher than that reported by Ng et al. (2009) in Hong Kong (26.3%) [27] and Kumtepe et al. (2009) in Turkey (21.0%) [34]. This variability is probably related to differences in the selection of patient groups, ethnic differences, and sample size. Recently, Vicdan et al. [38] reported that frequency of

Table 4 Frequency of chromosomal and histological abnormalities in azoospermic and oligozoospermic men with Y chromosome microdeletions

		Azoospermic (n=73)	Oligozoospermic (n=28)			Total (n=101)
			severe	moderate	mild	
Chromosomal abnormality	(+)	35 (48.6)	1 (3.7)	0 (0.0)	0 (0.0)	99
	(−)	37 (51.4)	22 (81.5)	3 (11.1)	1 (3.7)	
Histological abnormality	(+)	55 (100.0)	12 (85.7)	0 (0.0)	0 (0.0)	69
	(−)	0 (0.0)	2 (14.3)	0 (0.0)	0 (0.0)	

*severe oligozoospermia: <1X10^6 sperm/ml semen; moderate oligozoospermia: 1–5X10^6 sperm/ml semen; mild oligozoospermia: 5–20X10^6 sperm/ml semen

Table 5 Clinical outcomes of patients with Y chromosome microdeletions

	TESE	Ejaculation	Total
No. of cycles	14	20	34
No. of patients	14	9	23
Female age (y), mean±SD	30.4±2.9	32.6±4.2	31.7±3.9
No. of retrieved oocytes, mean±SD	18.3±7.3	19.0±9.3	18.7±8.5
Maturation rate of oocytes, n (%)	179/256 (69.9)	293/380 (77.1)	472/636 (74.2)
Fertilization rate, n (%)	116/189 (61.3)	202/299 (68.0)	318/488 (65.1)
No. of cultured embryos, mean±SD	6.9±2.3	6.0±2.0	6.4±2.2
Good embryo rate, n (%)	45/97 (46.4)	72/120 (60.0)	117/217 (53.9)
No. of transferred embryos, mean±SD	3.7±0.5	3.4±1.1	3.5±0.9
β-hCG positive rate, n (%)	7/14 (50.0)	9/19 (47.3)	16/33 (48.4)
Clinical pregnancy rate/ET, n (%)	6/14 (43.0)	7/19 (36.8)	13/33 (39.4)
Live birth rate/ET, n (%)	3/14 (21.4)	7/19 (36.8)	10/33 (30.3)
Children born, n	4	9	13

*ET: embryo transfer; Good embryo: grade I and II (≥6 cells & ≤25% fragmentation) on day 3

chromosomal abnormalities increases with severity of semen parameters; the incidence of chromosomal abnormality among oligozoospermic and azoospermic groups was reported to be 4.6% and 13.7%, respectively. Considering this, it is not surprising that in the present study the frequency of chromosomal abnormalities was higher in azoospermic men with microdeletions (48.6%) than in oligozoospermic men with microdeletions (3.7%) (Table 4).

Large deletions including AZFabc and AZFbc appeared to be associated with higher chromosomal abnormalities than other deletions. In this study, the incidence of chromosomal abnormality was 50.0% (18/36) and 22.2% (8/36) in the AZFbc and AZFabc regions, respectively. The incidence of chromosomal deletions in the AZFa, AZFb and AZFc regions was 2.8%, 0.0% and 25.0%, respectively (Table 2). Data from this study show that the incidence of chromosomal abnormality increased with larger deletions. All these abnormalities involved the sex chromosome, with a majority of mosaicism with isodicentric Y chromosome, 45,XO/ marker chromosome, or Yq deletion. An association between Y chromosome microdeletions and 45,X0/46,XY chromosomal mosaicism or isodicentric Y chromosome has been previously proposed [39–42]. Moreover, it was suggested that microdeletions in the Y chromosome might be associated with Y chromosomal instability leading to mitotic loss of the Y chromosome. The preimplantation genetic diagnosis (PGD) could be considered a treatment option for these cases because there is a risk of transmitting this unstable Y chromosome, which may lead to gonadal dysgenesis in the offspring, though PGD is not allowed in these cases in Korea.

In terms of overall testicular histology, the predominant trait was SCO syndrome (47.8%). All four patients with deletions in the AZFa region and 3 out of 4 patients with AZFabc deletions had SCO syndrome, but different phenotypes were found in other subsets. Krausz et al. [43] reported that deletions of the AZFa, AZFb and AZFc regions correlate with SCO syndrome, maturation arrest, and hypospermatogenesis and SCO syndrome type II, respectively. Nevertheless, it is very difficult to draw a conclusion about genotype/phenotype correlations in this study as well as in other studies [44].

Many studies have reported that mature spermatozoa were obtained in 50% of patients with AZFc deletions, despite reduced fertilization rates and embryo scores after ICSI [45]. In addition, when testicular sperm extracted from AZFc-deleted patients was used for an ICSI cycle, a reduction in fertilization and per-cycle pregnancy rates was observed [46, 47]. In the present study, all men of couples who achieved successful pregnancies through IVF/ICSI cycles had an AZFc deletion, a normal karyotype, and oligozoospermia. Fertilization rates (65%) in the Y chromosome microdeletion group were significantly lower than in the control group (data not shown). However, this fertilization rate was higher than previous studies (53% for Patrat et al. [39] and 55% for Stouffs et al. [48]). In addition, the fertilization rate was slightly higher with ejaculated spermatozoa (68%) compared to testicular spermatozoa (61%) in men with Y chromosome microdeletions [39, 48]. IVF outcome by ICSI using testicular or ejaculated sperm did not seem to be significantly affected by the AZFc deletion in this study and in previous studies [27, 39, 49]. However, ICSI treatment might lead to vertical transmission, expansion and de novo Yq microdeletions in male fetuses [50]. Therefore, careful evaluations of chromosomal abnormalities and Y chromosome microdeletions in infertile men with azoospermia and severe oligozoospermia should be considered before they undergo assisted reproduction techniques (ART) such as ICSI. This would make it possible to offer genetic counseling and to predict the chances of finding spermatozoa when performing testicular sperm extraction (TESE).

In conclusion, this study describes one of the largest studies of male infertility caused by Y chromosome microdeletions, especially in an Asian population. As such it is a significant contribution to the study of male infertility. The frequency of Y chromosome microdeletions in infertile men from Korea was comparable to the frequency reported in other countries and regions. However, there was a slightly higher incidence of chromosomal abnormalities in infertile men who had microdeletions when compared to other studies. Data from the present study revealed a close relationship between microdeletions and spermatogenesis; however, IVF outcome was not significantly affected by the presence of an AZFc microdeletion. Nevertheless, Y chromosome microdeletions carry the potential risk of transmittance from infertile fathers to their offspring by ICSI. Therefore, before attempting ICSI in infertile patients with severe spermatogenic defects, screening for microdeletions and chromosomal abnormalities should be performed and genetic counseling should be provided before IVF-ET and ART.

Conflict of Interest None to disclose

References

1. Iammarrone E, et al. Male infertility. Best Pract Res Clin Obstet Gynaecol. 2003;17(2):211–29.
2. Pasqualotto FF. Investigation and assisted reproduction in the treatment of male infertility. Rev Bras Ginecol Obstet. 2007;29:103–12.
3. Crosignani PG, et al. Recommendations of the ESHRE workshop on unexplained infertility. Hum Reprod. 1993;8(6):977–80.
4. Vogt P, et al. Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. Hum Genet. 1992;89(5):491–6.
5. Ferlin A, et al. Molecular and clinical characterization of Y chromosome microdeletions in infertile men: a 10-year experience in Italy. J Clin Endocrinol Metab. 2007;92:762–70.
6. Chandley AC. Chromosome anomalies and Y chromosome microdeletions as causal factors in male infertility. Hum Reprod. 1998;13(1):45–50.
7. Vogt PH, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum Mol Genet. 1996;5 (7):933–43.
8. Poongothai J, Gopenath TS, Manonayaki S. Genetics of human male infertility. Singapore Med J. 2009;50(4):336–47.
9. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. Endocr Rev. 2001;22:226–39.
10. Reijo R, et al. Diverse spermatogenic defects in human caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nature Gen. 1995;10:383–93.
11. 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.
12. Athalye AS, et al. A study of Y chromosome microdeletions in infertile Indian males. Int J Hum Genet. 2004;4:179–85.
13. Dada R, Gupta NP, Kucheria K. Yq microdeletions—azoospermia factor candidate genes and spermatogenic arrest. J Biomol Tech. 2004;15(3):176–83.
14. Raicu F, et al. Screening for microdeletions in human Y chromosome—AZF candidate genes and male infertility. J Cell Mol Med. 2003;7(1):43–8.
15. van der Ven K, et al. Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. Mol Hum Reprod. 1997;3:699–704.
16. Foresta C, et al. High frequency of well-defined Y-chromosome deletions in idiopathic Sertoli cell-only syndrome. Hum Reprod. 1998;13:302–7.
17. Kobayashi K, et al. PCR analysis of the Y chromosome long arm in azoospermic patients: Evidence for a second locus required for spermatogenesis. Hum Mol Genet. 1994;3:1965–7.
18. Nakahori Y, et al. The Y chromosome region essential for spermatogenesis. Horm Res. 1996;46 Suppl 1:20–3.
19. Kato H, et al. Screening for deletions in interval D16–22 of the Y chromosome in azoospermic and oligozoospermic Japanese men. J Hum Genet. 2001;46:110–4.
20. Ewis AA, et al. Microdeletions of a Y-specific marker, Yfm1, and implications for a role in spermatogenesis. J Hum Genet. 2002;47:257–61.
21. Sawai H, et al. Molecular analysis of the Y chromosome AZFc region in Japanese infertile males with spermatogenic defects. J Reprod Immunol. 2002;53:37–44.
22. Kihaile PE, et al. Embryo outcome in Y-chromosome microdeleted infertile males after ICSI. Mol Reprod Dev. 2004;68:176–81.
23. Fu J, Li L, Lu G. Relationship between microdeletion on Y chromosome and patients with idiopathic azoospermia and severe oligozoospermia in the Chinese. Chin Med J. 2002;115:72–5.
24. Song NH, et al. Screening for Y chromosome microdeletions in idiopathic and nonidiopathic infertile men with varicocele and cryptorchidism. Chin Med J. 2005;118:1462–7.
25. Lin YM, et al. Y-chromosome microdeletion and its effect on reproductive decisions in Taiwanese patients presenting with nonobstructive azoospermia. Urology. 2000;56:1041–6.
26. Lin YM, et al. Gene-based screening for Y chromosome deletions in Taiwanese men presenting with spermatogenic failure. Fertil Steril. 2002;77:897–903.
27. Ng PP, et al. Chromosomal anomalies and Y-microdeletions among Chinese subfertile men in Hong Kong. Hong Kong Med J. 2009;15(1):31–8.
28. Dada R, Gupta NP, Kucheria K. Molecular screening for Yq microdeletion in men with idiopathic oligozoospermia and azoospermia. Journal of Bioscience. 2003;28:163–8.
29. Abid S, et al. Clinical and laboratory evaluation of idiopathic male infertility in a secondary referral center in India. J Clin Lab Anal. 2008;22:29–38.
30. Hofherr SE, et al. Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience. J Assist Reprod Genet. 2011;28:1091–8.
31. Lange R, Engel W. Chromosomal aberrations as a cause of male subfertility. J Fertil Reprod. 1991;9:41–4.
32. Dohle GR, et al. Genetic risk factors in infertile men with severe oligozoospermia and azoospermia. Hum Reprod. 2002;17(1):13–6.
33. WHO. World Health Organization Laboratory Manual for the Examination of Human Semen and Sperm and Sperm-Cervical Mucus Interaction. UK: Cambridge University Press; 1999.
34. Kumtepe Y, et al. A genetic survey of 1935 Turkish men with severe male factor infertility. Reprod Biomed Online. 2009;18 (4):465–74.
35. Vutyavanich T, et al. Frequency of Y chromosome microdeletions and chromosomal abnormalities in infertile Thai men with oligozoospermia and azoospermia. Asian J Androl. 2007;9:68–75.

36. Retief A, Van Zyl J, Menkveld M. Chromosome studies in 496 infertile males with a sperm count below 10 million per ml. Hum Genet. 1984;66:162–4.
37. Matsuda T, et al. Chromosomal survey of 1001 subfertile males: incidence and clinical features of males with chromosomal anomalies. Acta Urol Jpn. 1992;38:803–9.
38. Vicdan et al. Genetic aspects of human male infertility: the frequency of chromosomal abnormalities and Y chromosome microdeletions in severe male factor infertility. Eur J Obstet Gynecol Reprod Biol. 2004;117:289–92.
39. Patrat C, et al. Clinical data and parenthood of 63 infertile and Y-microdeleted men. Fertil Steril. 2010;93:822–32.
40. Patsalis P, et al. Effects of transmission of Y chromosome AZFc deletions. Lancet. 2002;360:1222–4.
41. Willis M, et al. Natural history of prenatally diagnosed 46, X, isodicentric Y. Prenat Diagn. 2006;26:134–7.
42. Alvarez-Nava F, et al. High incidence of Y-chromosome microdeletions in gonadal tissues from patients with 45, X/46, XY gonadal dysgenesis. Fertil Steril. 2008;89:458–60.
43. Krausz C, Quintana-Murci L, McElreavey K. Prognostic value of Y deletion analysis: what is the clinical prognostic value of Y chromosome microdeletion analysis? Hum Reprod. 2000;15:1431–4.
44. Krausz C, et al. Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. J Clin Endocrinol Metab. 2001;86:2638–42.
45. van Golde RJT, et al. Decreased fertilization rate and embryo quality after ICSI in oligozoospermic men with microdeletions in the azoospermia factor c region of the Y chromosome. Hum Reprod. 2001;16:289–92.
46. Oates RD, et al. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Hum Reprod. 2002;17:2813–24.
47. Choi NM, et al. Effects of Y Chromosome Microdeletion on the Outcome of in vitro Fertilization. Kor J Fertil Steril. 2007;34(1):41–8.
48. Stouffs K, et al. The choice and outcome of the fertility treatment of 38 couples in whom the male partner has a Yq microdeletion. Hum Reprod. 2005;20:1887–96.
49. Choi JM, et al. AZF microdeletions of the Y chromosome and in vitro fertilization outcome. Fertil Steril. 2004;81:337–41.
50. Lee SH, et al. Intracytoplasmic sperm injection may lead to vertical transmission, expansion, and de novo occurrence of Y-chromosome microdeletions in male fetuses. Fertil Steril. 2006;85(5):1512–5.