ORIGINAL ARTICLE

Chromosomal anomalies and Y-microdeletions among Chinese subfertile men in Hong Kong

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Objective To report the type and frequency of chromosomal anomalies and Y-microdeletions among Hong Kong Chinese subfertile men with sperm concentrations lower than 5 million/mL.

Design Retrospective study.

Setting A reproductive centre in Hong Kong.

Participants A total of 295 Chinese subfertile men who underwent both karyotyping and Y-microdeletion studies from 2000 to 2007 were categorised as having non-obstructive azoospermia (n=71), very severe oligospermia (sperm concentration >0 and ≤2 million/mL, n=158), and severe oligospermia (sperm concentration >2 and <5 million/mL, n=66).

Main outcome measures Karyotyping and Y-microdeletion studies.

Results The prevalence of chromosomal anomalies and Y-microdeletions in the study population were 8.5% (25/295; 95% confidence interval, 5.6-12.3%) and 6.4% (19/295; 3.9-9.9%), respectively. The total prevalence of chromosomal anomalies and Y-microdeletions was 13.2% (39/295; 95% confidence interval, 9.6-17.6%) as five cases of non-obstructive azoospermia showed both Y structural alterations and AZFbc deletion. The corresponding figures for chromosomal anomalies in the groups with non-obstructive azoospermia, very severe oligospermia, and severe oligospermia were 21.1% (15/71; 95% confidence interval, 12.3-32.4%), 5.7% (9/158; 2.6-10.5%), and 1.5% (1/66; 0.0-8.2%). While for Y-microdeletions they were 8.5% (6/71; 3.2-17.5%), 8.2% (13/158; 4.5-13.7%) and 0% (0/66; 0.0-4.4%), respectively. The respective overall prevalence rates for chromosomal anomalies and Y-microdeletions in these groups were: 22.5% (16/71; 13.5-34.0%), 13.9% (22/158; 8.9-20.3%), and 1.5% (1/66; 0.0-8.2%).

Conclusions Our findings strongly support the recommendation for both karyotyping and Y-microdeletion analyses in subfertile men with sperm concentrations of 2 million/mL or lower before they undergo assisted reproduction treatment.

Key words
Chromosome deletion; Chromosome, human, Y; Infertility, male

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Introduction

Subfertility is defined by the World Health Organization (WHO) as failure to conceive over 12 months of unprotected frequent intercourse and affects approximately 15% of couples, and among these half are male-related.[1]

The use of intracytoplasmic sperm injection (ICSI) in assisted reproduction treatment has provided men with severe male-factor subfertility a chance to father their own children.[1-3] However, in subfertile men with genetic anomalies, the technique is associated with an increased risk of transmitting any genetic defect to their offspring.[3-6]

Numerous studies have demonstrated that men with azoospermia or severe oligospermia have a higher incidence of chromosomal anomalies[7,8] and Y-microdeletions.[4,8] Reports regarding the prevalence of chromosomal anomalies and Y-microdeletions in Chinese populations are few.[9-14] Tse et al[12,13] reported the rate of Y-microdeletions in Hong Kong Chinese to be 8.5 to 9.1% among men with non-obstructive azoospermia or severe oligospermia. Lin et al[11] found Y-microdeletions in 11.7% of Taiwan Chinese men with non-obstructive azoospermia. Chiang et al[10] reported the rate of chromosomal anomalies and Y-microdeletions in Taiwan Chinese infertile men to be 23.6%. A study in Mainland China showed that about 25% of Chinese infertile patients with azoospermia or severe

香港不孕症男性的染色體異常及Y染色體微缺失

目的 報告香港不孕症華籍男性（精子數目少於5 000 000/mL）的染色體異常的種類及發生率，和Y染色體微缺失的情況。

設計 回顧研究。

安排 香港一所生育中心。

參與者 2000至2007年期間接受染色體核型分析及Y染色體微缺失研究的295位不孕症華籍男性，病人分為三組：71人有非梗阻性無精子症、158人有極嚴重精子減少症（精子數目少於2 000 000/mL），以及66人有嚴重精子減少症（精子數目多於2 000 000/mL但仍少於5 000 000/mL）。

主要結果測量 染色體核型分析及Y染色體微缺失研究。

結果 研究對象的染色體異常及Y染色體微缺失的現患率分別為8.5%（25/295；95%置信區間：5.6-12.3%）和6.4%（19/295；3.9-9.9%）。由於有5例非梗阻性無精子症同時有Y染色體結構改變和AZFbc缺失，染色體異常及Y染色體微缺失的總現患率為13.2%（39/295；95%置信區間：9.6-17.6%）。在非梗阻性無精子症、極嚴重精子減少症，以及嚴重精子減少症三組病人中，染色體異常的現患率分別為21.1%（15/71；12.3-32.4%）、5.7%（9/158；2.6-10.5%），以及1.5%（1/66；0.0-8.2%）；Y染色體微缺失的現患率則分別為8.5%（6/71；3.2-17.5%）、8.2%（13/158；4.5-13.7%），以及0%（0/66；0.0-4.4%）；而染色體異常及Y染色體微缺失的總現患率分別為22.5%（16/71；13.5-34.0%）、13.9%（22/158；8.9-20.3%），以及1.5%（1/66；0.0-8.2%）。

結論 本研究的結果顯示，精子數目少於2 000 000/mL的不孕症男性，在助孕治療前，應接受染色體核型分析及Y染色體微缺失測試。

oligospermia had chromosomal anomalies or Y-microdeletions.[14]

Screening for genetic defects for subfertile men, particularly those with azoospermia or severe oligospermia, is the basis for genetic counselling and risk assessment prior to initiation of assisted reproduction treatment. This involves karyotyping and detection of microdeletions of the AZF region in the Y chromosome. This study aimed to report the type and frequency of chromosomal anomalies and Y-microdeletions among Hong Kong Chinese subfertile men with non-obstructive azoospermia or sperm concentrations lower than 5 million/mL.

Methods

Participants

Chinese subfertile males who underwent both karyotyping and Y-microdeletion studies on peripheral blood lymphocytes over the period 2000 to 2007 were included in this retrospective analysis.

When the subfertile couples attended the subfertility clinic of the Department of Obstetrics and Gynaecology, Queen Mary Hospital, their partners were requested to submit two semen samples to the andrology laboratory. Semen analysis was performed according to WHO guidelines.[15] Men with azoospermia and severe oligospermia were further evaluated by a consultant urologist to confirm the non-obstructive cause of their azoospermia and uncorrectable nature of oligospermia. Those who had sperm concentrations lower than 5 million/mL were advised to undergo both karyotyping and Y-microdeletion studies on peripheral blood.

This study utilised the database of the Genetic Screening for Male Subfertility at Tsan Yuk Hospital. From 2000 to 2007, 326 Chinese men underwent both karyotyping and Y-microdeletion studies. All the cases were checked against the semen analysis database of Department of Obstetrics and Gynaecology at Queen Mary Hospital. Seventeen cases with sperm counts greater than or equal to 5 million/mL, seven cases with unknown sperm counts (attending private practitioners), and seven cases of men who suffered from obstructive azoospermia (including one with a non-specific congenital cause and one with congenital bilateral absence of vas deferens) were excluded. Therefore, the remaining 295 men were included in the final analysis.

Chromosome analysis

From each subject, peripheral blood was collected in a sodium heparin vacutainer. Culture and harvest of peripheral blood lymphocytes was performed according to the AGT cytogenetics laboratory manual.[16] Lymphocytes were cultured for 72 hours in RPMI-1640 with phytohaemagglutinin at 37°C. Colcemid was added before harvesting. 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). The fixed cell suspension was spread on glass slides. Metaphases were stained with Giemsa using the GTG technique. Chromosomal analysis was performed on Giemsa-banded metaphases, using a bright field microscope. At least 15 metaphases were routinely analysed from each participant. Whenever an anomaly was suspected, at least 30 cells were counted.

Y-microdeletion studies

Molecular analysis of the AZF region of Y chromosome was performed on DNA extracted from peripheral blood by polymerase chain reaction (PCR). Six Y-chromosome specific-sequence tagged site

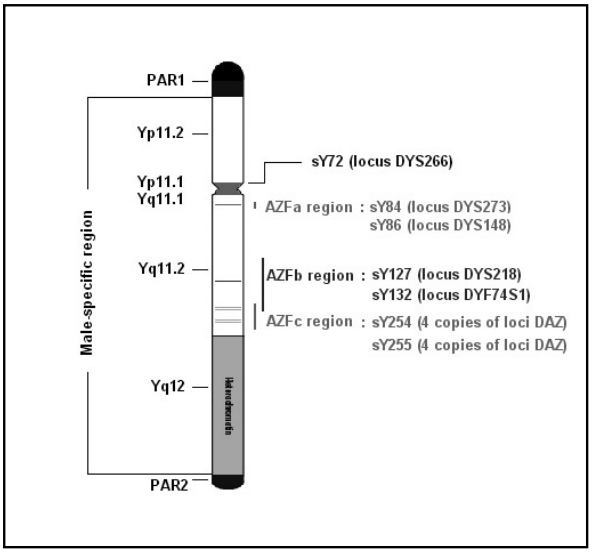

FIG. Location of the six specific-sequence tagged site markers and the internal control marker on the Y chromosome
sY72 was the internal control marker. sY84 and sY86 were mapped to the AZFa region. sY127 and sY132 were mapped to the AZFb region, while sY254 and sY255 were mapped to the AZFc region. AZFb and AZFc were overlapping according to the study of Repping et al.[20] The locus information was searched in the website of http://www.ncbi.nlm.nih.gov/. PAR1 and PAR2 were abbreviations of pseudoautosomal regions 1 and 2, respectively and showed sequence homology with that on both ends of chromosome X

(STS) markers were used according to Tse et al.[12,13] Locations of the latter are shown in the Figure and corresponded to: sY84, sY86 (AZFa region); sY127, sY132 (AZFb region); sY254, sY255 (AZFc region). For each participant, three sets of multiplex PCR reactions were carried out, each of which included an internal control marker for PCR amplification. The internal controls were either sY72 (located on chromosome Y, Fig) or the β globin gene (located on chromosome 11). Normal female DNA, normal male DNA, and water were run in parallel for each set of multiplex PCR tests. Female and male DNA acted as negative and positive controls respectively, and the reaction with water assured lack of DNA contamination.

Results

The present study only entailed men with non-obstructive subfertility. They included men with azoospermia (n=71), very severe oligospermia with sperm concentrations of 2 million/mL or lower (n=158), and severe oligospermia with sperm counts higher than 2 million/mL but lower than 5 million/mL (n=66).

Chromosome analysis

Table 1 shows findings pertaining to the 25 cases with chromosomal anomalies. The prevalence of chromosomal anomalies is summarised in Table 2 with 21.1% (15/71; 95% confidence interval [CI], 12.3-32.4%), 5.7% (9/158; 2.6-10.5%), and 1.5% (1/66; 0.0-8.2%) in the non-obstructive azoospermic group, the very severe oligospermic group, and the severe oligospermic group, respectively. The overall prevalence was 8.5% (25/295; 95% CI, 5.6-12.3%).

In the azoospermic group, there was a significant difference between the prevalence of sex chromosome and autosomal chromosomal anomalies (18.3%, 13/71 vs 2.8%, 2/71; P=0.006, Chi squared test; Table 2). The commonest chromosomal anomaly was 47,XXY (Klinefelter syndrome); five cases were pure types and three were mosaics. Five cases of Y structural alterations were also identified (case 324, 206, 242, 187 and 264; Table 1), all of whom had AZFbc deletions. Case 324 had a tiny ring Y chromosome. Case 206 and case 242 had mosaic isodicentric Y. Cases 187 and 264 had derivative Y chromosomes with duplication of the distal short arm (results not shown). Only two cases of autosomal chromosome anomalies were found; one consisted of a ring chromosome 21 and one was a mosaic supernumerary marker chromosome. The marker chromosome was smaller than chromosome 21 and with satellites at one end. The overall prevalence of chromosomal anomalies in the azoospermic group was 21.1% (15/71; 95% CI, 12.3-32.4%).

The very severe oligospermic group had a higher prevalence of chromosomal anomalies compared to the severe oligospermic group, but the difference was not statistically significant (5.7%, 9/158 vs 1.5%, 1/66; P=0.30, Chi squared test; Table 2). In the very severe oligospermic group, there were four cases of sex chromosome anomalies, three of reciprocal translocations (including a complex translocation; case 15, Table 1), and two autosomal aberrants (cases 292 and 317; Table 1). The four cases of sex chromosome anomalies included: one 47,XYY (case 137, Table 1), one mosaic 47,XXY (case 282, Table 1), one mosaic ring Y (case 299, Table 1) showing no deletion of the AZF region, and one Y aberrant (case 280, Table 1) with duplication of the Y-chromosome segment from Yp11.2 to Yq12 (Fig), which included the Y centromere and the AZF region. Regarding autosomal aberrants (Table 1), case 292 had additional chromosomal material near the centromeric region at the short arm of chromosome 5. The other (case 317) showed a decrease in the length of the long arm of chromosome 5 and an increase in length of chromosome 13, for which clarification of breakpoints needs further study. In the severe oligospermic group, there was only one case with a chromosomal anomaly, namely mosaic trisomy 21 (case 30, Table 1).

Y-microdeletion studies

Table 3 details 19 cases with deletions in the AZF

TABLE 1. Twenty-five men with chromosomal anomalies

Case No.	Karyotype	AZF region	Sperm concentration (million/mL)
Sex chromosome anomalies (17 cases)			
6	47,XXY	Present	0
93	47,XXY	Present	0
165	47,XXY	Present	0
168	47,XXY	Present	0
233	47,XXY	Present	0
81	47,XXY[1]/46,XY[29]	Present	0
282	47,XXY[1]/46,XY[60]	Present	0.1
295	47,XXY[29]/46,XY[1]	Present	0
312	47,XXY[29]/46,XY[1]	Present	0
137	47,XYY	Present	0.2
299	46,X,r(Y)[26]/45,X[4]	Present	0.1
324	46,X,r(Y)	AZFbc deletion	0
206	46,X,idic(Y)(q11.2)[10]/45,X[20]	AZFbc deletion	0
242	46,X,idic(Y)(q11.2)[18]/45,X[12]	AZFbc deletion	0
187	46,X,der(Y)[33]/45,X[1]	AZFbc deletion	0
264	46,X,der(Y)[27]/45,X[3]	AZFbc deletion	0
280	46,X,ins dup(Y)(pter->p11.2::q12->p11.2::p11.2->qter)	Present	0.4
Autosomal chromosome anomalies (8 cases)			
15	46,XY,t(8;12;11)(q22.3;q24.1;q14.2)	Present	0.4
217	46,XY,t(1;5)(p22;q35)	Present	0.2
291	46,XY,t(1;13)(q11;q11)	Present	1
271	47,XY,+mar[13]/46,XY[17]	Present	0
42	46,XY,r(21)	Present	0
30	47,XY,+21[1]/46,XY[29]	Present	4.4
292	46,XY,5p+	Present	1.1
317	46,XY,5q-,13q+	Present	0.2

region, including five cases with cytogenetically detectable structural changes in the Y chromosome. The prevalence of AZF deletion is summarised in Table 4, being 8.5% (6/71; 95% CI, 3.2-17.5%), 8.2% (13/158; 4.5-13.7%), and 0% (0/66; 0.0-4.4%) in the non-obstructive azoospermic, very severe oligospermic, and severe oligospermic groups, respectively. Two types of AZF deletions were identified: AZFc and AZFbc deletion. Deletion of AZFc was the major type of deletion (73.7%, 14/19). Of the 14 cases with AZFc deletion, 13 were in the very severe oligospermic group. All five cases of AZFbc deletions were associated with non-obstructive azoospermia. No significant difference was observed in the prevalence of AZF deletions between the non-obstructive azoospermic group and the very severe oligospermic group (8.5%, 6/71 vs 8.2%, 13/158). In the severe oligospermic group, the AZF region deletion was not detected. The overall prevalence of AZF deletion was 6.4% (19/295; 95% CI, 3.9-9.9%).

There were five cases (all with non-obstructive azoospermia) having both chromosomal anomalies and Y-microdeletions. The overall prevalence of both defects was 13.2% (39/295; 95% CI, 9.6-17.6%; Table 5). The prevalence of genetic defects showed a significant difference (P=0.001, Chi squared test): 22.5% (16/71; 95% CI, 13.5-34.0%), 13.9% (22/158; 8.9-20.3%), and 1.5% (1/66; 0.0-8.2%) in the non-obstructive azoospermic, very severe oligospermic, and severe oligospermic groups, respectively (Table 5). The very severe oligospermic group showed a significantly higher prevalence of genetic defects than the severe oligospermic group (P=0.009, Chi squared test).

Discussion

In the present study, the prevalence of chromosomal anomalies was 21% (15/71) in Hong Kong Chinese subfertile men with non-obstructive azoospermia. This figure is comparable to that reported in a Taiwan study[10] of 23% (31/134), but higher than the 14% (36/256) reported from Mainland China[14] and in European studies (13.7-15.0%).[7,8] However both the present study and the Taiwan study only included men suffering from non-obstructive azoospermia.

TABLE 2. Type and frequency of chromosomal anomalies in 295 Chinese subfertile men with non-obstructive azoospermia, very severe oligospermia, and severe oligospermia encountered from 2000 to 2007

	% (No. of men/total No.)			
	Non-obstructive azoospermia	**Very severe oligospermia counts >0 and ≤2 million/mL**	**Severe oligospermia counts >2 and <5 million/mL**	**Overall all those with counts <5 million/mL**
Sex chromosome anomalies				
47,XXY	7.0 (5/71)	0.0 (0/158)	0.0 (0/66)	1.7 (5/295)
Mosaic 47,XXY	4.2 (3/71)	0.6 (1/158)	0.0 (0/66)	1.4 (4/295)
47,XYY	0.0 (0/71)	0.6 (1/158)	0.0 (0/66)	0.3 (1/295)
Ring Y	1.4 (1/71)	0.6 (1/158)	0.0 (0/66)	0.7 (2/295)
Mosaic isodicentric Y	2.8 (2/71)	0.0 (0/158)	0.0 (0/66)	0.7 (2/295)
Other Y aberrants	2.8 (2/71)	0.6 (1/158)	0.0 (0/66)	1.0 (3/295)
Subtotal	18.3 (13/71)	2.5 (4/158)	0.0 (0/66)	5.8 (17/295)
95% CI*	10.1-29.3%	0.7-6.4%	0.0-4.4%	3.4-9.1%
Autosomal chromosome anomalies				
Reciprocal translocation	0.0 (0/71)	1.9 (3/158)	0.0 (0/66)	1.0 (3/295)
Supernumerary marker chromosome	1.4 (1/71)	0.0 (0/158)	0.0 (0/66)	0.3 (1/295)
Ring chromosome 21	1.4 (1/71)	0.0 (0/158)	0.0 (0/66)	0.3 (1/295)
Mosaic trisomy 21	0.0 (0/71)	0.0 (0/158)	1.5 (1/66)	0.3 (1/295)
Other autosomal aberrants	0.0 (0/71)	1.3 (2/158)	0.0 (0/66)	0.7 (2/295)
Subtotal	2.8 (2/71)	3.2 (5/158)	1.5 (1/66)	2.7 (8/295)
95% CI	0.3-9.8%	1.0-7.2%	0.0-8.2%	1.2-5.3%
Total	21.1 (15/71)	5.7 (9/158)	1.5 (1/66)	8.5 (25/295)
95% CI	12.3-32.4%	2.6-10.5%	0.0-8.2%	5.6-12.3%

* CI denotes confidence interval

TABLE 3. Men with Y-microdeletions (n=19)

Case No.	**AZF region**	**Karyotype**	**Sperm concentration (million/mL)**
AZFc deletion (14 cases)			
37	AZFc deletion	46,XY	1.2
90	AZFc deletion	46,XY	1.0
128	AZFc deletion	46,XY	0.8
151	AZFc deletion	46,XY	0
173	AZFc deletion	46,XY	0.4
207	AZFc deletion	46,XY	0.2
212	AZFc deletion	46,XY	1.0
235	AZFc deletion	46,XY	0.4
237	AZFc deletion	46,XY	0.1
255	AZFc deletion	46,XY	0.4
256	AZFc deletion	46,XY	1.0
267	AZFc deletion	46,XY	1.4
277	AZFc deletion	46,XY	0.2
286	AZFc deletion	46,XY	0.2
AZFbc deletion (5 cases)			
187	AZFbc deletion	46,X,der(Y)[33]/45,X[1]	0
206	AZFbc deletion	46,X,idic(Y)(q11.2)[10]/45,X[20]	0
242	AZFbc deletion	46,X,idic(Y)(q11.2)[18]/45,X[12]	0
264	AZFbc deletion	46,X,der(Y)[27]/45,X[3]	0
324	AZFbc deletion	46,X,r(Y)	0

TABLE 4. Type and frequency of Y-microdeletions in 295 Chinese subfertile men with non-obstructive azoospermia, very severe oligospermia, and severe oligospermia encountered from 2000 to 2007

	% (No. of men/total No.)			
	Non-obstructive azoospermia	Very severe oligospermia >0 and ≤2 million/mL	Severe oligospermia >2 and <5 million/mL	Overall all those with counts <5 million/mL
AZFa deletion	0.0 (0/71)	0.0 (0/158)	0.0 (0/66)	0.0 (0/295)
AZFb deletion	0.0 (0/71)	0.0 (0/158)	0.0 (0/66)	0.0 (0/295)
AZFc deletion	1.4 (1/71)	8.2 (13/158)	0.0 (0/66)	4.8 (14/295)
AZFbc deletion	7.0 (5/71)	0.0 (0/158)	0.0 (0/66)	1.7 (5/295)
AZFabc deletion	0.0 (0/71)	0.0 (0/158)	0.0 (0/66)	0.0 (0/295)
Total	8.5 (6/71)	8.2 (13/158)	0.0 (0/66)	6.4 (19/295)
95% Confidence interval	3.2-17.5%	4.5-13.7%	0.0-4.4%	3.9-9.9%

TABLE 5. Incidence of chromosomal anomalies and Y-microdeletions in 295 Chinese subfertile men with non-obstructive azoospermia, very severe oligospermia, and severe oligospermia encountered from 2000 to 2007

	% (No. of men/total No.)			
	Non-obstructive azoospermia	Very severe oligospermia >0 and ≤2 million/mL	Severe oligospermia >2 and <5 million/mL	Overall all those with counts <5 million/mL
Sex chromosome anomalies	18.3 (13/71)	2.5 (4/158)	0.0 (0/66)	5.8 (17/295)
Autosome anomalies	2.8 (2/71)	3.2 (5/158)	1.5 (1/66)	2.7 (8/295)
AZFc deletion	1.4 (1/71)	8.2 (13/158)	0.0 (0/66)	4.8 (14/295)
AZFbc deletion	7.0 (5/71)	0.0 (0/158)	0.0 (0/66)	1.7 (5/295)
Total	22.5 (16/71)*	13.9 (22/158)	1.5 (1/66)	13.2 (39/295)*
95% Confidence interval	13.5-34.0%	8.9-20.3%	0.0-8.2%	9.6-17.6%

* Five men with azoospermia had both chromosomal anomalies and AZFbc deletion

Nevertheless, we could not rule out the possibility of ethnic difference in the prevalence rates reported in different studies.

In the azoospermic group, 47,XXY (Klinefelter syndrome) was the commonest type of sex chromosome anomaly with a prevalence of 11% (8/71; five pure and three mosaic karyotypes), while Chiang et al[10] found it to be 16% (21/134) and Zhou-Cun et al[14] reported 11% (27/256) for the Taiwan and Mainland China populations, respectively. Nevertheless, some XXY individuals can become fathers through ICSI,[3] using sperm retrieved directly from larger testicular tubules.

Although data on the genetic risk of offspring conceived by ICSI of sperm from 47,XXY males were scarce, Tachdjian et al[17] summarised 36 successful pregnancies in the literature and their own experience of a twin pregnancy of karyotypically normal neonates 46,XX and 46,XY. The 36 pregnancies produced 32 karyotypically normal neonates, two karyotypically normal pregnancy losses, one healthy unkaryotyped neonate, and one 47,XXY prenatally diagnosed foetus which was reduced in the triple pregnancy. The remaining two foetuses (46,XX and 46,XY) were born uneventfully.[17] Thus, though the genetic risk in the offspring of 47,XXY individuals remains unknown, it is presumed to be low.

The prevalence of sex chromosome anomalies showed a significant difference between the azoospermia and oligospermic groups (18.3% vs 0-2.5%, P<0.001; Table 2). The difference in autosomal chromosome anomalies was not distinct (2.8% vs 1.5%-3.2%; Table 2). Both findings were consistent with previous reports.[10,14]

The association of ring chromosome 21 with non-obstructive azoospermia (case 42, Table 1) was also reported in the study of Chiang et al.[10] An earlier report showed that three males with ring 21 were azoospermic, while eight healthy females with ring 21 were fertile.[18] However, female carriers were at risk of Down's syndrome and spontaneous abortions.[18] The mechanism for the impaired spermatogenesis remains unclear, but may involve the interference due to unpaired ring chromosome 21 or the normal homolog on the XY bivalent during male meiosis.[18]

Reciprocal translocations were found in the oligospermic group (2%, 3/158). Preimplantation genetic diagnosis is recommended, as translocation carriers bear a high risk of unbalanced embryos from sperm with imbalanced outcomes associated with the different modes of chromosome segregation in meiosis (except for the alternate 2:2 mode of segregation).[3]

In this study, five azoospermic men had altered

Y chromosome structure and AZFbc deletion. Cytogenetic detection of the deletion of the distal long arm of the Y chromosome (Yq) was first described in six azoospermic men by Tiepolo and Zuffardi in 1976.[19] The authors proposed the presence of factors controlling spermatogenesis in the Yq region.[19] The putative genes were subsequently mapped to three subregions of AZFa, AZFb and AZFc in the Yq11.2 region and further research[20] clarified the deletion junctions (boundaries) of the AZFb, AZFc and AZFbc deletions (Fig). Essentially, the AZFc region covers 3.5 Mb of genetic material including the *DAZ* gene (deleted in azoospermia).[20] The AZFbc deletion of 7.0-7.7Mb[20] DNA loses both the *DAZ* gene and *RBMY* gene (RNA-Binding Motif Y-Linked), which are believed to play a role in male germ cell development. A report of the ESHRE Capri Workshop Group[3] stated that sperms have not been recovered in men with AZFb or AZFbc deletions.

The differences in Y-microdeletion type and their frequency in different reports may reflect variations in the sample group and selection of STS markers.[21] In this study, the prevalence of Y-microdeletions in Hong Kong Chinese subfertile men who suffered from non-obstructive azoospermia or oligospermia (sperm counts lower than 5 million/mL) was 6.4% (19/295) while Tse et al[12,13] reported an incidence of 8.5 to 9.1%. In the present report only AZFc and AZFbc deletions were identified, whilst AZFa and AZFb deletions were not. Tse et al[13] detected one case of AZFb deletion. The studied populations of Tse et al[12] contained a higher proportion of azoospermia cases. In the first study, the latter comprised 35 non-obstructive azoospermic subjects and nine with severe oligospermia (<1 million/mL). While in the second study, there were 59 who had non-obstructive azoospermia and 47 had oligospermia (<5 million/mL).[13]

In the present study, the prevalence of Y-microdeletions in the non-obstructive azoospermic men was 8.5% (6/71), consistent with the findings of Tse et al[12,13] but lower than that reported by Lin et al[11] (11.7%, 11/94). Regarding Y-microdeletions, the present study and the two by Tse et al[12,13] reported only absence of amplification of the six STS markers of sY84, sY86, sY127, sY132, sY254, or sY255. However, Lin et al[11] used a panel of 27 STS markers and two of the 11 cases showed only isolated absence of amplification of sY243 and sY269 (also in the AZFc region) distal to the *DAZ* genes. According to best practice guidelines[5] for Y-microdeletion studies, when both markers of sY254 and sY255 were deleted, a diagnosis of complete deletion of the AZFc region could be made. The clinical significance of isolated absence of amplification of sY243 and sY269 is unclear.

AZFc deletion was the predominant type of Y-microdeletion (74%, 14/19) detected, which was the most frequent type of microdeletion associated with very severe sperm deficiency or azoospermia in men from many populations.[4,9-14,22] Other deletion types of AZFa, AZFb, AZFbc, and AZFabc showed varying frequencies, depending on the composition of the study population. The Y chromosome has the least number of genes but the highest copy numbers of repetitive sequences. Sporadic AZFc deletion may arise from non-allelic homologous recombination of the highly repetitive DNA sequence around the *DAZ* gene during male meiosis.[23] With the development of assisted reproductive technologies (particularly ICSI), these men can now father offspring, with vertical transmission of the deletion to the male offspring.[3,6]

No Y-microdeletion and only one chromosome anomaly were detected in the severe oligospermic group. Although the study population was small (n=66), this finding implies that clinically relevant genetic defects are rare in patients with sperm concentration exceeding 2 million/mL.

In conclusion, the overall prevalence of chromosomal anomalies and Y-microdeletions were 22.5% (16/71; 95% CI, 13.5-34.0%), 13.9% (22/158; 8.9-20.3%), and 1.5% (1/66; 0.0-8.2%) in the non-obstructive azoospermic, very severe oligospermic, and the severe oligospermic groups, respectively. Most genetic defects were found in patients with azoospermia or sperm concentrations of 2 million/mL or lower. Our findings strongly support the recommendation to perform both karyotyping and Y-microdeletion analyses for subfertile men with sperm concentrations of 2 million/mL or lower, prior to offering them assisted reproduction treatment.

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