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*·Original Article·*

# Y chromosome microdeletions in azoospermic patients with Klinefelter's syndrome

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**Abstract**

**Aim:** To study the occurrence of Y chromosome microdeletions in azoospermic patients with Klinefelter's syndrome (KFS). **Methods:** Blood and semen samples were collected from azoospermic patients with KFS ($n$ = 14) and a control group of men of proven fertility ($n$ = 13). Semen analysis was done according to World Health Organization (WHO) guidelines. Blood samples were processed for karyotyping, fluorescent *in situ* hybridization (FISH) and measurement of plasma follicle stimulating hormone (FSH) by radioimmunoassay. To determine Y chromosome microdeletions, polymerase chain reaction (PCR) of 16 sequence tagged sites (STS) and three genes (*DFFRY, XKRY* and *RBM1Y*) was performed on isolated genomic DNA. Testicular fine needle aspiration cytology (FNAC) was done in selected cases. **Results:** Y chromosome microdeletions spanning the azoospermia factor (*AZF*)a and *AZF*b loci were found in four of the 14 azoospermic patients with KFS. Karyotype and FISH analysis revealed that, of the four cases showing Y chromosome microdeletion, three cases had a 47,XXY/ 46,XY chromosomal pattern and one case had a 46,XY/ 47,XXY/ 48,XXXY/ 48,XXYY chromosomal pattern. The testicular FNAC of one sample with Y chromosome microdeletion revealed Sertoli cell-only type of morphology. However, no Y chromosome microdeletions were observed in any of the 13 fertile men. All patients with KFS had elevated plasma FSH levels. **Conclusion:** Patients with KFS may harbor Y chromosome microdeletions and screening for these should be a part of their diagnostic work-up, particularly in those considering assisted reproductive techniques. *(Asian J Androl 2006 Jan; 8: 81-88)*

**Keywords:** azoospermia; azoospermia factor; follicle stimulating hormone; Klinefelter's syndrome; Y chromosome; microdeletion

## 1 Introduction

In the last few years considerable progress has been made in understanding the pathogenesis of spermatogenic arrest and infertility. Infertility is often due to hypogonadism; Klinefelter's syndrome (KFS) is the commonest cause of hypogonadism and infertility [1]. KFS is the most common sex chromosomal abnormality in men, with an incidence of approximately 1 in 500 newborn phenotypic males [1]. Men with KFS have a 47,XXY chromosome complement and, of these, 15 % are classified as mosaic, with a 46, XY/ 47, XXY chromosomal

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complement. KFS is characterized by seminiferous tubular dysgenesis, azoospermia and elevated levels of serum gonadotropins.

Studies have shown that the deletion of azoospermia factor (*AZF*) loci on the long arm of Y chromosome results in spermatogenic failure and these loci are recurrently deleted in infertile males [2–6]. Previously, men with non-obstructive azoospermia had no therapeutic fertility options except anonymous donor insemination or adoption. The ability to extract spermatozoa from the testes of some men with non-obstructive azoospermia using the multi-biopsy technique of testicular sperm extraction followed by intracytoplasmic sperm injection (ICSI) offers an efficacious therapeutic approach [7]. The possibility of using ICSI with testicular sperm has also been proposed in KFS patients [8]. The widespread use of such procedures has raised some interesting and important genetic issues about the inheritance of genetic anomalies, such as Y chromosome microdeletions in the offspring [9].

There are conflicting reports on the occurrence of Y chromosome microdeletions in KFS patients [1, 10–13]. Tateno *et al*. [1] failed to find microdeletions of the deleted in azoospermia (*DAZ*) or the Y chromosome ribonucleic acid recognition motif (*YRRM*) genes in KFS patients. On the other hand, others have reported low incidence of Y chromosome microdeletions [11–13]. The objective of our study was to analyze Y chromosome microdeletions at 16 different sequence tagged sites (STS) and three genes by polymerase chain reaction (PCR) in Indian KFS patients attending the infertility clinic at a tertiary care hospital.

## 2 Materials and methods

Male subjects with primary infertility attending the Infertility Clinic in the Urology Department of the All India Institute of Medical Sciences (New Delhi, India) were enrolled in the present study. All experiments using human samples were carried out with informed consent following clearance from the Institutional Bio-safety and Ethical Committee. Based on cytogenetic analysis, 14 azoospermic KFS patients and 13 fertile males (with a child of less than 2 years; sperm count > 20 million/mL) having a normal karyotype were included in this study. The KFS patients were enrolled over a period of two years. Each patient was carefully examined to rule out other causes of infertility and a detailed family, occupational and reproductive history was collected using a predesigned proforma. The semen analysis was done according to World Health Organization (WHO) guidelines [14]. The plasma values of follicle stimulating hormone (FSH) were determined by radioimmunoassay. Whenever possible, testicular fine needle aspiration cytology (FNAC) was performed, as testicular biopsy was ethically not possible in these cases.

### *2.1 Karyotyping and fluorescent in situ hybridization (FISH)*

Peripheral blood cultures were set up for chromosomal analysis in all the cases and five well-spread G-banded metaphases were karyotyped using automated karyotyping software (Cytovision 2.81, Applied Imaging Corp., San Jose, CA, USA) [15]. In certain Klinefelter mosaic (KFM) cases the percentage of mosaicism was re-analyzed using FISH. This analysis was performed on metaphase spreads and interphase nuclei using commercially available fluorescent-labeled chromosome enumeration X and Y probes (CEP X/CEP Y; Vysis, Downers Grove, IL, USA) as described previously [16]. The slides prepared from fixed cell suspension obtained during cytogenetic analysis were denatured in 70 % formamide/ 2 × SSC at 73 ºC for 2–5 min. Images were captured using a Charged Coupled Devise (CCD) camera (Cohu Inc, Poway, CA, USA) attached to a Zeiss Axiophot microscope (Carl Zeiss AG, Konigsallee, Gottingen, Germany) using a triple band pass filter (rhodamine, fluorescein-isothiocyanate, and 4',6'-diamidino-2-phenylindole dihydrochloride) and analyzed using Quips Smart Capture FISH imaging software 2.81 (Vysis, Illinois, USA). A minimum of 200 interphase cells and 20 metaphase spreads were scored in each case. Only those signals well embedded in the nucleus were included for scoring. Clumped and overlapping nuclei and those with low fluorescence intensity or high background intensity were excluded. Patchy and diffused signals were included in the evaluation only if they were well separated. The criteria for defining a sample as "mosaic" were those in which 20 % of the nuclei/metaphase spreads had a variation in signal number from the majority or showed a signal pattern other than the normal sex chromosomal XY and XX signals. The cut-off value was calculated from the mean ± SD of results obtained from a group of normal control samples.

### *2.2 Analysis of Y chromosome microdeletions by PCR*

Genomic DNA was isolated by a Genomic DNA Isolation Kit (Promega, Madison, WI, USA) using the manufacturer's protocol. The concentration of the isolated genomic DNA was determined by spectrophotometric analysis at 260 nm.

All the DNA samples were processed for Yq microdeletions analysis using PCR. Each of these samples was analyzed using 19 sets of primers. DNA from fertile male subjects was taken as the positive control and the DNA from a female subject as the negative control in each reaction.

The STS and genes used were as follows:

*AZF*a: sY746, sY84, sY86, DFFRY

*AZF*b: XKRY, sY118, sY113, sY127, sY134, sY143, RBM1Y

*AZF*c: sY153, sY148, sY157, sY158, sY254, sY255, sY160 (heterochromatin region)

The sex-determining region of Y (SRY, i.e. STS sY14) was included as the internal control. Detailed sequences of the primers were given in Table 1.

The samples were subjected to PCR amplification using initial denaturation for 5 min at 94 ºC and 35 cycles of 94 ºC for 1 min, with the annealing temperature varying from 50 ºC to 64 ºC for 1 min and extension at 72 ºC for 1 min. The final extension time was 7 min at 72 ºC. The PCR products were analyzed on a 2 % agarose gel containing ethidium bromide (0.5 μg/mL). An STS or gene was considered absent only after at least three amplification failures in the presence of both the internal control (SRY) and the positive control. In addition, deletions with respect to various STS markers were reconfirmed by performing temperature gradient PCR in the absence or presence of 5 % dimethyl sulfoxide.

## 3 Results

The karyotype analysis revealed seven cases with a 47,XXY chromosomal pattern (KFS) and seven KFM cases. Of the latter, five had a 47,XXY/ 46,XY chromosomal pattern and two had a 46,XY/ 47,XXY/ 48,XXXY/ 48,XXYY chromosomal pattern (Table 2). The fertile male subjects had a karyotype of 46,XY chromosomes (data not shown).

The clinical parameters of the patients with KFS were summarized in Table 2. The age of the KFS patients ranged from 18 years to 32 years (Table 2), whereas the mean age of the normal samples was 31 years. All cases presented with primary infertility. Except for one patient having aspermia, all other cases showed the absence of sperm in the semen. The FSH levels in the KFS cases were found to be very high ([39.56 ± 20.73] mIU/mL). The samples taken from fertile men had mean 5 mIU/mL of FSH level (range [2–7] mIU/mL).

Four of the 14 (28.6 %) KFS patients showed deletions in both the *AZF*a and *AZF*b regions (Table 2, Figures 1 and 2). Deletions in STS represented by sY86, sY746 and sY127 were present in all four patients (Table 2, Figure 1). Two of these four patients also had a deletion in STS sY84 of the *AZF*a region (Figure 1). In addition, deletions in the *AZF*b regions were also found in STS sY134 in one subject (Table 2), and STS sY113 (Figure 2B) and sY118 (Table 2) in another. In comparison, using similar conditions of PCR, none of the fertile men showed any Y chromosome microdeletions. FNAC was carried out in two patients with KFS. Case 240/03 with multiple cell lines (47,XXY [87 %]/46,XY [7 %]/ 48,XXYY [3 %]/48, XXXY [3 %]) and *AZF*a and *AZF*b microdeletions had Sertoli cell only type II syndrome (SCO II, isolated foci of spermatogenesis along with Sertoli cells) and case 288/03 with a 47,XXY chromosomal complement had Sertoli cell-only type I syndrome (SCO I, complete absence of germ cells in the seminiferous tubules and presence of only Sertoli cells, see Table 2). The latter case had no *AZF* microdeletions.

## 4 Discussion

The role of Y chromosome microdeletions in male infertility has been well established. Microdeletions on the long arm of human Y chromosome, postulated as the *AZF* factor, are associated with spermatogenic failure [2–6]. The reported frequency of Y chromosome microdeletions varies from 1 % [3] to 55 % [2], which is largely related to different inclusion criteria [13].

Cytogenetic abnormalities are known factors for spermatogenic failure, most of which are numerical or structural. KFS is a numerical chromosomal aberration, in which most patients have a 47,XXY chromosomal component or are mosaic (46,XY and 47,XXY), or are mosaic variant cases with additional cell lines (48,XXYY and 48,XXXY). Surprisingly, in the present study we observed a very high incidence of KFM (seven out of 14), of which two were variants. An incidence of KFM or mosaic variant of approximately 15 % in KFS patients has been reported previously [17]. Conventional karyotyping of metaphase spreads of peripheral blood lymphocytes,

Table 1. Nucleotide sequence of 19 selected sets of primers. *AZF*, azoospermia factor; NRY, non-recombining region of Y-chromosome; Sl. No., serial number; STS, sequence tagged site.

| Sl. No. | STS/gene | Primer sequence | Product size (bp) | Region |
|---|---|---|---|---|
| **1** | SRY-F | 5' - GAA TAT TCC CGC TCT CCG GA - 3' | 472 | NRY |
| | SRY-R | 5' - GCT GGT GCT CCA TTC TTG AG - 3' | | |
| **2** | sY746-F | 5' - TTG ACT GCT TAT TCT ACA CAA GGC - 3' | 216 | *AZF*a |
| | sY746-R | 5' - CAG GGG AAA TTG GGT TTT- 3' | | |
| **3** | sY84-F | 5' - AGA AGG GTC TGA AAG CAG GT - 3' | 326 | *AZF*a |
| | sY84-R | 5' - GCC TAC TAC CTG GAG GCT TC - 3' | | |
| **4** | sY86-F | 5' - GTG ACA CAC AGA CTA TGC TTC - 3' | 320 | *AZF*a |
| | sY86-R | 5' - ACA CAC AGA GGG ACA ACC CT - 3' | | |
| **5** | DFFRY-F | 5' - GAG CCC ATC TTT GTC AGT TTA C- 3' | 111 | *AZF*a |
| | DFFRY-R | 5' - CTG CCA ATT TTC CAC ATC AAC C - 3' | | |
| **6** | XKRY-F | 5' - CAC TCA TGG AGA AGG GTA GG - 3' | 94 | *AZF*b |
| | XKRY-R | 5' - GTC ACA CTC AGC CTC TTT AC - 3' | | |
| **7** | sY118-F | 5' - GTA CCT CTG CAG GCA CTG AT- 3' | 217 | *AZF*b |
| | sY118-R | 5' - ACA CAA TCC AAC CTG GCT AA- 3' | | |
| **8** | sY113-F | 5' - GTT CTT TCC ACA GCC CAT AG - 3' | 290 | *AZF*b |
| | sY113-R | 5' - TGG AAC ACA ATC CAA AAT TG - 3' | | |
| **9** | sY127-F | 5' - GGC TCA CAA ACG AAA AGA AA - 3' | 274 | *AZF*b |
| | sY127-R | 5' - CTG CAG GCA GTA ATA AGG GA - 3' | | |
| **10** | sY134-F | 5' - GTC TGC CTC ACC ATA AAA CG - 3' | 301 | *AZF*b |
| | sY134-R | 5' - ACC ACT GCC AAA ACT TTC AA - 3' | | |
| **11** | sY143-F | 5' - GCA GGA TGA GAA GCA GGT AG - 3' | 311 | *AZF*b |
| | sY143-R | 5' - CCG TGT GCT GGA GAC TAA TC - 3' | | |
| **12** | RBM1Y-F | 5' - CTT TGA AAA CAA TTC CTT TTC C - 3' | 800 | *AZF*a |
| | RBM1Y-R | 5' - TGC ACT TCA GAG ATA CGG - 3' | | |
| **13** | sY153-F | 5' - GCA TCC TCA TTT TAT GTC CA - 3' | 139 | *AZF*c |
| | sY153-R | 5' - CAA CCC AAA AGC ACT GAG TA - 3' | | |
| **14** | sY148-F | 5' - AAA TGA AAA AAG ATA CGA AAC TCG - 3' | 202 | *AZF*c |
| | sY148-R | 5' - GAA TCC CAC CCA AGA ATC TG - 3' | | |
| **15** | sY254-F | 5' - GGG TGT TAC CAG AAG GCA AA - 3' | 400 | *AZF*c |
| | sY254-R | 5' - GAA CCG TAT CTA CCA AAG CAG C - 3' | | |
| **16** | sY225-F | 5'- GTT ACA GGA TTC GGC GTG AT - 3' | 126 | *AZF*c |
| | sY225-F | 5'- CTC GTC ATG TGC AGC CAC - 3' | | |
| **17** | sY158-F | 5' - CTC AGA AGT CCT CCT AAT AGT TCC - 3' | 231 | *AZF*c |
| | sY158-R | 5' - ACA GTG GTT TGT AGC GGG TA - 3' | | |
| **18** | sY157-F | 5' - CTT AGG AAA AAG TGA AGC CG - 3’ | 285 | *AZF*c |
| | sY157-R | 5' - CCT GCT GTC AGC AAG ATA CA - 3' | | |
| **19** | sY160-F | 5'- TAC GGG TCT CGA ATG GAA TA - 3' | 236 | *AZF*c |
| | sY160-R | 5'- TCA TTG CAT TCC TTT CCA TT - 3' | | |

when up to 20 cells are counted, may miss low-grade mosaicism. In a recent study, 18 KFS patients with a 47,XXY chromosomal complement, as per conventional karyotyping, were further analyzed by FISH [18]. Scoring of 400 interphase and 40 metaphase lymphocyte nuclei per patient revealed low-grade mosiacism in this group [18]. No significant differences were observed between interphase nuclei and metaphase spreads, suggesting that FISH

Table 2. Clinical details of 14 patients with Klinefelter's syndrome(KFS). *AZF*, azoospermia factor; FNAC, fine needle aspiration cytology; FSH, follicle stimulating hormone; Lab. No., laboratory number; SCO I, Sertoli cell only type I syndrome; SCO II, Sertoli cell only type II syndrome; Sl. No., serial number; STS, sequence tagged site; Nil, No deletions detected in these samples.

| Sl. No. | Lab. No. | Age (years) | Semen analysis | FNAC | FSH (mIU/mL) | Karyotype | STS deleted | *AZF* deleted |
|---|---|---|---|---|---|---|---|---|
| 1 | 210 / 03 | 24 | Azoospermic | - | 47.58 | 46,XY (60 %) / 47,XXY (40 %) | sY86, sY746, sY113, sY118, sY127 | *AZF*a *AZF*b |
| 2 | 213 / 03 | 20 | Azoospermic | - | 27.00 | 47,XXY | Nil | Nil |
| 3 | 215 / 03 | 18 | No emission | - | 20.70 | 47,XXY (87 %) / 46,XY (7 %) / 48,XXYY (3 %) / 48,XXXY (3 %) | Nil | Nil |
| 4 | 240 / 03 | 28 | Azoospermic | SCO II | 24.22 | 47,XXY (87 %) / 46,XY (7 %) / 48,XXYY (3 %) / 48,XXXY (3 %) | sY84, sY86, sY746, sY127 | *AZF*a, *AZF*b |
| 5 | 288 / 03 | 28 | Azoospermic | SCO I | 41.47 | 47,XXY | Nil | Nil |
| 6 | 290 / 03 | 20 | Azoospermic | - | 28.00 | 47,XXY | Nil | Nil |
| 7 | 01 / 04 | 32 | Azoospermic | - | 20.00 | 47,XXY | Nil | Nil |
| 8 | 26 / 04 | 26 | Azoospermic | - | 25.04 | 47,XXY (80 %) / 46,XY (20 %) | sY86, sY746, sY127 | *AZF*a *AZF*b |
| 9 | 30 / 04 | 20 | Azoospermic | - | 50.10 | 47,XXY (85 %) / 46,XY (15 %) | sY84, sY86, sY746, sY127, sY134 | *AZF*a *AZF*b |
| 10 | 52 / 04 | 30 | Azoospermic | - | 61.9 | 47,XXY (90 %) / 46,XY (10 %) | Nil | Nil |
| 11 | 99 / 04 | 28 | Azoospermic | - | 20.00 | 47,XXY | Nil | Nil |
| 12 | 132 / 04 | 23 | Azoospermic | - | 92.31 | 47,XXY | Nil | Nil |
| 13 | 179 / 04 | 22 | Azoospermic | - | 55.92 | 47,XXY (80 %) / 46,XY (20 %) | Nil | Nil |
| 14 | 190 / 04 | 25 | Azoospermic | - | Not Available | 47,XXY | Nil | Nil |

on interphase nuclei can be routinely used to screen aneuploidy status. Interestingly, two KFS patients with a high prevalence of normal 46,XY lymphocytes had sperm in their ejaculate [18]. However, in the present study, FISH and cytogenetic analysis did not reveal any differences in the percentage of mosaic cell lines. Increased gonosomal aneuploidy frequencies in spermatozoa of KFS patients has also been reported [19]. This knowledge may help in the genetic counseling of KFS patients prior to ICSI treatment, because a low level of mosaicism in their lymphocytes may also be reflected in the germ cells. It may just be a chance phenomenon that we observed an incidence of 50 % of KFM or its variants in our study of KFS patients. A larger series might determine the true incidence of such cases. It might be stated that, being a tertiary care referral hospital with full diagnostic facilities, we are able to identify KFS cases with the slightest phenotypic abnormality.

Though the studies on microdeletions in Y chromosomes have been very common in cases of idiopathic infertility, there have been very few studies on Y chromosome microdeletions in KFS patients. Tateno *et al*. [1] investigated deletions of Yq interval 6, *DAZ* and *YRRM* genes in 21 KFS patients with ($n$ = 1) and without ($n$ = 20) spermatogenesis. Their findings suggested no deletions of Yq interval 6 in these patients. Similarly, in another study of combined cytogenetic and Y chromosome microdeletion screening, no deletions in the Y chromosome were found in KFS patients [10]. In a clinical study of 42 infertile men positive for *AZF*c deletion, it was

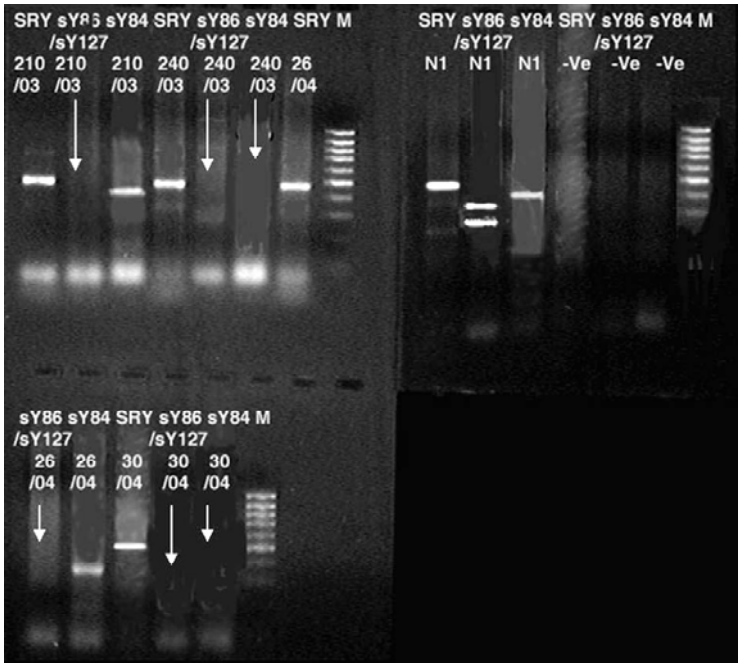


Figure 1. Gel electrophoresis profile of four Klinefelter's syndrome (KFS) samples of the SRY, sY86, sY127 and sY84 sequence tagged site (STS) regions. The STS SRY (472 bp), sY86 (326 bp) and sY127 (274 bp) (through multiplex polymerase chain reaction [PCR]) and sY84 (326 bp) were PCR amplified using the genomic DNA isolated from the KFS subjects 210/03, 240/03, 26/04 and 30/04, one fertile man (N1) and one woman (–Ve control) as the template DNA. All PCR amplified products were analyzed by 2 % agarose gel electrophoresis. The marker (M) represents the 100 bp DNA ladder and the arrow denotes the deletion in the particular STS region.

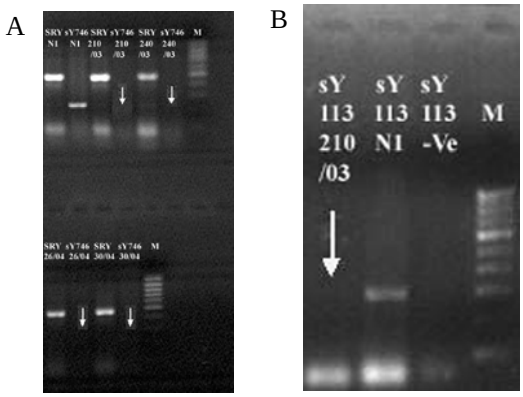


Figure 2. Gel electrophoresis profile of Klinefelter's syndrome (KFS) samples of the sY746 and sY113 sequence tagged site (STS) regions. The STS SRY (472 bp), sY746 (216 bp) and sY113 (290 bp) were polymerase chain reaction (PCR) amplified using the genomic DNA isolated from the KFS patients. (A): the PCR amplified product profile for SRY and sY746, resolved by 2 % agarose gel electrophoresis of patients 210/03, 240/03, 26/04 and 30/04 and a normal fertile man (N1). (B): the PCR amplified product profile of patient 210/03, one fertile man (N1) and one woman (–Ve). The marker (M) represents the 100 bp DNA ladder and the arrow denotes the deletion in the particular STS region.

observed that one case could be categorized as KFM [11]. In an independent study of the prevalence of Y chromosome microdeletions in 186 oligospermic and azoospermic males opting for ICSI, it was observed that only one male belonged to the KFM category and also had *AZF*c microdeletions [12]. In another screening study for Y chromosome microdeletions in 226 Slovenian sub-fertile men, it was observed that five patients had low-level mosaicism (abnormal karyotype < 2.5 %) and of these five patients only one had *AZF*c microdeletion [13]. These studies describe very weak mosaicism in comparison to ours, where the mosaicism found in the cells is as high as 87 % in some cases (Table 2). Our study is the first detailed one on Y chromosome microdeletions in KFS patients with 19 sets of primers. This study illustrated deletions in the *AZF*a and *AZF*b regions, in contrast to the deletions in the *AZF*c region observed in KFS patients by others [11–13]. The deletions were further confirmed by running temperature gradient PCR. The *AZF*a and *AZF*b combined deletions were found in 28.6 % of the patients. The high incidence of Y chromosome microdeletions observed by our group may be a chance

phenomenon. In parallel studies carried out by our group, using the same sets of PCR primers, idiopathic infertile azoospermic men ($n$ = 67) showed an incidence of Y chromosome microdeletions in 19.4 % (unpublished observations). Of the 13 patients who were positive for Y chromosome microdeletions, three had deletions only in *AZF*a, three only in *AZF*b, one in *AZF*a and *AZF*b, one in *AZF*b and *AZF*c, and five in *AZF*c alone. At this stage, the reasons for the high incidence of Y chromosome microdeletions observed in *AZF*a and *AZF*b loci in the Indian population is not clear. An independent study of Y chromosome deletions in 340 azoospermic Indian men, which used 30 STS markers, revealed an overall deletion of 8.5 % [4]. Those showing deletions had *AZF*c deletion in 82.8 %, *AZF*b in 55.2 % and *AZF*a in 24.1 %. However, the authors failed to see any deletions in the sY86 and sY84 STS, commonly recommended for analysis. On the other hand, the missing STS were sY746, sY741, sY742, sY615 and the gene DFFRY, which are inter-spread between or around sY86 and sY84. Hence, the chances of finding a deletion is higher if more sets of primers are used, as suggested by Thangaraj *et al*. [4]. But this contrasts with the recommendations of the European Academy of Andrology guidelines [20], which suggest that over 90 % of microdeletions can be detected with the use of only two STS markers for each *AZF* loci.

Although the nature of the histological changes at the testicular level still needs to be defined, patients with KFS are known to have progressive deterioration of their testicular architecture. After the onset of puberty, their testes usually shrink and become firm. Observed high levels of FSH in our study was in agreement with very low levels of inhibin-B and high levels of FSH reported in KFS patients [21]. The KFS case finding by Oates *et al*. [11] shows SCO II syndrome, which is in agreement with the prevailing notion. Though it was not possible to take the FNAC of all the patients in our study as it is clinically not recommended, the FNAC report from two patients showed an SCO II type of morphology in one case and SCO I in another. The findings of Kamp *et al*. [22] suggest a high frequency of *AZF*a deletions in men with SCO syndrome. It can also be interpreted that deletions in *AZF*a give rise to more severe phenotypes such as SCO syndrome, which is in agreement with our findings. In this study we found that case 240/03 with *AZF*a and *AZF*b deletions had few spermatogenesis loci in the testes (SCO type II). Determination of telomerase activity in therapeutic testicular biopsies of KFS patients have revealed that those with high telomerase activity (> 39.0 units/μg protein) have a high probability of the presence of sperm [23]. In a study of 24 non-mosaic KFS patients, it was observed that men who had spermatozoa in their testicular tissues (50 %), were positive for both 46,XY and 47,XXY spermatogonia in their seminiferous tubules [24]. In contrast, KFS patients without spermatozoa in their testicular tissues were positive only for 47,XXY spermatogonia.

It has been shown that sperm can be retrieved from pure and mosaic KFS patients for use in ICSI, leading to successful pregnancy [8, 25] and in such cases the consequences of vertical transmission of deletions may be further studied. Follow-up of 42 infertile men with deletion in the *AZF*c region and 18 children conceived through the use of ICSI revealed that though the offspring were healthy, the sons inherited the *AZF*c deletion with no increase in length [11]. In cases where sperm could be retrieved, the presence of Y chromosome microdeletions had no obvious impact on fertilization or pregnancy rate.

Due to observed Y chromosome microdeletions in KFS patients, the analysis may be imperative in routine clinical follow-up of such cases, followed by genetic counseling with respect to the risk of transmitting Y chromosome microdeletions to the male progeny, if the patients opts for assisted reproductive techniques.

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