

Genetic Risk of Azoospermia Factor (AZF) Microdeletions in Idiopathic Cases of Azoospermia and Oligozoospermia in Central Indian Population

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ABSTRACT

Background: Genetic factors cause about 15% of male infertility. Azoospermia factors (AZFa, AZFb, and AZFc) present on Yq are most important for spermatogenesis. We have made an attempt to evaluate the frequencies of microdeletions of AZFa, AZFb, AZFc in idiopathic cases of azoospermia and oligozoospermia from central Indian population.

Materials and Methods: We have analyzed a total of 156 subjects (95 oligozoospermia and 61 azoospermia) & 50 control subjects. DNA samples were analyzed for microdeletions of Y chromosome by PCR-screening of 18 sequences-tagged-site (STS) markers from different region of the AZF on Yq and SRY on Yp.

Results: Out of 156 cases analyzed, 13 (8.33%) subjects (8 azoospermia and 5 oligozoospermia) showed partial deletion

of AZF regions, of which deletion in AZFc region was the most common (84.6%) followed by AZFb (15.4%) and AZFa (15.4%). The sites and sizes of deletions varied among patients. Histological study of the testicular tissue of the available subjects, who showed microdeletions of Y chromosome, showed spermatogenic arrest at different stages. The frequency of Y chromosome microdeletion in our subjects was 8.33%.

Conclusion: Some Indian studies reported low frequencies of microdeletions than that of our result. We suggest that the frequency of deletions may be affected by the involvement of different genetic factors, ethnic population and different geographical regions. PCR based Y chromosome screening for microdeletions will be useful and great help to infertility clinics for genetic counselling and assisted reproduction.

Keywords: Male Infertility, Y microdeletions, AZF factor

INTRODUCTION

Infertility is a major reproductive health problem. Worldwide reports suggest that infertility affects an estimated 10% to 15% of couples. Nearly 50% of these are accountable to the male partner [1]. Various causative factors have been identified for male infertility including varicocele, obstruction of spermatic duct, erectile dysfunction and failure of ejaculation, various conditions causing imbalance in levels of sex hormones Testosterone, dihydrotestosterone, Follicle stimulating hormone (FSH), Leutinizing hormone (LH), Androgen receptor and last but not the least genetic defects. Environmental factors have also been considered in causing a decline in male reproductive health. In more than 60% of cases of infertility the origin of altered testicular function is unknown [2]. Genetic factors have been found to play a role in about 10% of male infertility. In about 15% cases of male infertility no organic cause is traceable (idiopathic infertility). Male fertility depends on successful spermatogenesis, in which a number of genes participate. So, it is evident that deletions or mutations in genes controlling spermatogenesis would result in infertility [3].

The male sex-determining region (SRY) has been mapped on the short arm of Y chromosome at Yp11. The critical genes involved in the sequential cascade of process of spermatogenesis are located on the long arm of the Y chromosome (Yq11). Researchers reported azoospermic individuals with microscopic deletions of distal euchromatic part of long arm of the Y-chromosome and on the basis of these findings in azoospermic men [4], they proposed existence of a spermatogenesis gene complex called "azoospermia factor" (AZF) on Yq. Yq part mainly divided in important AZFa, AZFb and AZFc regions [5]. Most of the deletions occur de novo and in confinement of one or more of these non-overlapping regions, AZFc being the most frequently deleted. AZFa, AZFb and AZFc are considered to be the most crucial region of Yq concerned

with sperm production. Major candidate genes in AZFa region are USP9Y, DBY, UTY and TB4Y, in AZFb region there are EIF1AY, PRY, TTY2, RBMY genes and in region AZFc the genes are DAZ1, DAZ2, BPY2, PRY and CDY. USP9Y, RBMY and DAZ genes exist in multiple copies, of which only some are functional [6]. 'Deleted in azoospermia gene-DAZ' that exists in AZFc region, is expressed specifically in testis. DAZ is also the most frequently deleted site in non-obstructive severe or mild oligozoospermic infertile men [7,8]. Deletions in the AZFa and AZFb regions have been associated with azoospermia. The microdeletions in the AZF regions not only reflect as altered seminal parameters but correlate to varied testicular histological profile ranging from sertoli cell only syndrome (SCOS) to hypospermatogenesis [1].

According to global estimate about 10% cases of idiopathic azoospermia and oligozoospermia occur due to be deletions in AZF region. Therefore AZF deletions are amongst most common causes of spermatogenic failure in man identifiable by molecular genetics tool [9]. The aim of this study was to identify the microdeletions in AZFa, AZFb and AZFc regions of human Y chromosome by STS markers and to correlate the deletion-patterns with clinical phenotypic profile such as seminal parameters, hormonal status and testicular histology. Attempts to be made to find out any site specific microdeletions of Y chromosome in idiopathic azoospermic and oligozoospermic cases from central India population.

MATERIALS AND METHODS

Selection of patients

We included 179 infertile men in present study, belonging to the age group of 25-45 years (32.18 ± 4.61) as cases and 50 age matched fertile men as controls. Infertile men were referred to our human molecular division of cytogenetics lab from institutional reproductive biology unit. All the subjects enrolled as cases had primary infertility

for more than one year. With a 3-days period of sexual abstinence, semen sample was obtained from each infertile subject. Complete semen analysis was performed including semen volume, pH, sperm concentration, motility and morphology. On the basis of seminal profile, infertile subjects were categorized as azoospermia (no sperm count), severe oligozoospermia (sperm count below 5 million per ml) and oligozoospermia (5-20 million sperm per ml) [10]. Pre structured questionnaire was completed by all the subjects. Among these subjects having no gonadal abnormalities, varicocele or a history of cryptorchidism, mumps, orchitis, diabetes or testicular injury were selected.

We also recorded the history of habits like alcoholism, smoking, tobacco chewing and made a note of any major illness in the family. In azoospermic men trans-rectal sonography was performed to know the status of ejaculatory duct, seminal vesicle abnormalities etc. The scrotal ultra-sonography was done to assess the testicular volume and abnormalities. The serum level of Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) were recorded in each subject by radioimmunoassay (RIA). Nineteen (19) azoospermic and severe oligozoospermic infertile subjects were excluded from the study because of abnormal hormones profile of FSH, LH, Free Testosterone. In a few selective cases testicular biopsy was taken for study of histology. Informed consent forms were obtained from each infertile male before inclusion in study. The Institutional ethical committee had approved this study.

Chromosomal Analysis was done on phytohemagglutinin-M stimulated whole peripheral blood culture following standard protocol of our lab. Those infertile subjects with a normal 46,XY karyotype as shown by GTG banding were included in present study.

Sequence-tagged site (STS) PCR used for defining microdeletions

Human genomic DNAs were extracted from peripheral blood lymphocytes of 160 non-obstructive idiopathic infertile men, 50 normal healthy proven fertile subjects as positive controls and 5 normal women as negative controls using a phenol-chloroform Proteinase-K standard protocol [8,11].

Different STS primers were used for 18 AZF loci, four primers: sY746, sY86, sY84 & DFFRY for AZFa; 7 primers: sY113, sY118, sY127, RBMIY, XKRY, sY134, & sY143 for AZFb and 7 primers: sY153, sY148, sY157, sY255, sY254, sY158, sY160 for AZFc. All STSs were used in series of order and the localization of the sequences proposed by Vollarth 1992. The internal control was STS primer sY14 for sex determining region of the Y (SRY). 50-100 ng DNA samples were subjected to PCR amplification reactions with 1 X PCR assay buffer, 1.5 mmol/l MgCl₂, 200 μmol/l deoxy-nucleotidetriphosphate (dNTPs), 1 μmol/μl of each primer pair and 0.5 U Taq-DNA polymerase (Merck Biosciences, Bangalore, India). The reactions were performed in a Veriti 96 well thermal cycler (Applied Biosystem, CA, USA) for 30 cycles using standard protocol. Initial denaturation was done at 94°C for 3 min. & final extension at 72°C for 7 minutes in the last cycle. The subsequent denaturations were set at 94°C for 60 second, and extension at 72°C for 60 second and same for all the samples.

Different annealing temperatures that were used (56°C -60°C for 30 second- 50 second), depending on different types of STS markers. PCR product was analyzed on a 2 % agarose gel electrophoresis containing Ethidium bromide (0.5 mg/ml). In the event of detecting a microdeletion of a Y-specific sequence tagged sites (STSs) with a primer, the PCR assay was repeated thrice for confirmation.

STATISTICAL ANALYSIS

The clinical findings of infertile men are given as mean ± SD. All computations were carried out with the SPSS statistical analysis software. p<0.05 was considered significant.

RESULTS

Chromosomal analysis was done on 160 idiopathic infertile men, among these 96 (60%) had oligozoospermia and 64 (40%) had azoospermia. However 3 subjects (P-54, P129 & P35) were 47,XXY Klinefelter's Syndrome (KFS) and one was (p-35) 47,XYY male and no other structural chromosomal arrangement or Y chromosomal deletion was detected in GTG banding preparation. All infertile men were subjected for chromosomal analysis and Y deletion mapping simultaneously. However, Klinefelter's syndrome and 47,XYY male were excluded from category of idiopathic infertile men. STS-PCR analysis was done in 156 cytogenetically normal infertile men and 50 fertile controls.

In PCR Yq microdeletion analysis, 13 infertile men (8.33%) with normal cytogenetic profile were detected with the presence of either one or more deleted STS loci. All deletions were recorded in interstitial region of Yq11 in the AZF locus of five oligozoospermic and eight azoospermic infertile men [Table/Fig-1]. No deletions were identified in any of the 50 fertile men. In normal fertile women, negative control, STS failed to amplify. The STS-PCR data of the 13 infertile subjects with deletion at Yq11 is presented in [Table/Fig-2]. Histopathology of testis biopsy in subjects P27 (azoospermia) and P132 (severe oligozoospermia) had shown the germ cell maturation arrest [Table/Fig-3a] with AZFc loci microdeletions and patient P103 (azoospermic) had germ cell development arrest at spermatid stage [Table/Fig-3b] with AZFc deletion of sY153, sY148, sY157 STS markers.

Andrological examinations

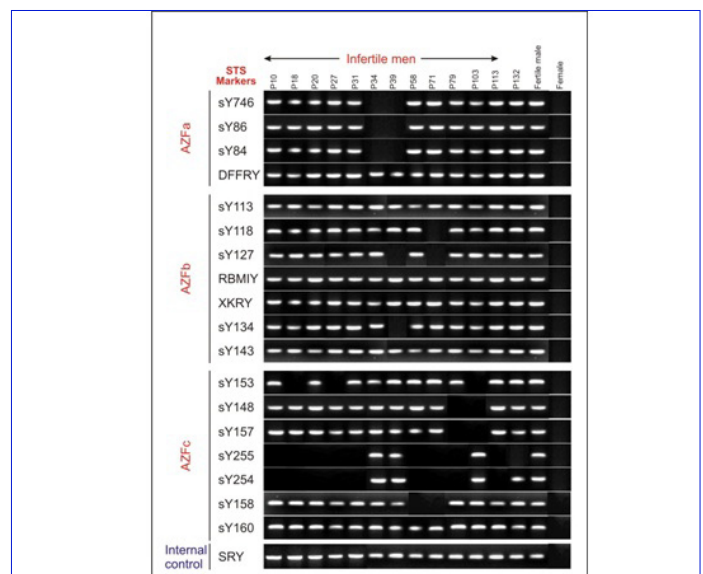
The mean volume of left and right testis were 9.28 ±2.17 ml and 10.57 ±1.90 ml respectively in 13 infertile males with microdeletions compared to 9.76 ±1.85 ml and 11.20 ±1.92 ml in 143 males without Y chromosome deletions. In males with deletions the values were slightly below as compared to those without deletions, but results were insignificant (t-test).

Hormones

The mean serum FSH and LH were 12.68 ±2.58 mIU/ml and 7.41 ±1.28mIU/ml respectively in 13 infertile men with Yq microdeletions compared with 11.39 ±1.83 mIU/ml and 7.26 ±1.41 mIU/ml in 143 infertile men without Yq microdeletions.

DISCUSSION

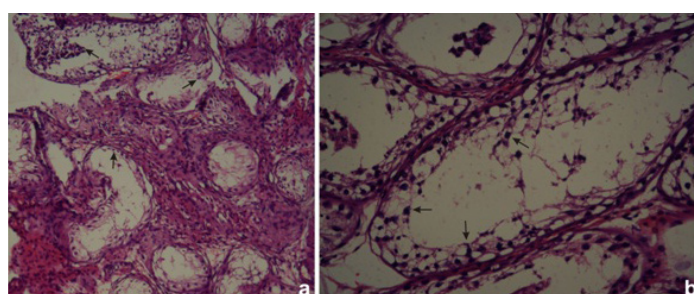
In present study we assessed the prevalence of Y chromosome microdeletions in male infertility and made an attempt to find out the relationship between microdeletions and male infertility from



[Table/Fig-1]: Gel illustration showing amplified products of sequence tagged sites (STS) markers corresponding AZFa, AZFb, AZFc regions

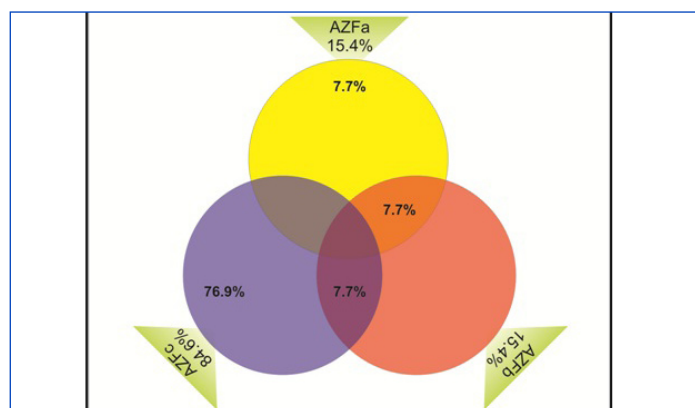
	Patients												
	P10	P18	P20	P27	P31	P34	P39	P58	P71	P79	P103	P113	P132
Left/Right testicular Volume (ml)	10.6/ 12.1	9.32/ 7.4	10.31/ 6.19	12.3/ 7.52	11.6/ 7.93	10.8/ 12.43	10.71/ 11.5	6.47/ 8.61	9.30/ 8.42	13.7/ 10.5	12.5/ 11.91	8.37/ 6.73	11.48/ 9.51
Sperm Concentrations (cell x 10 ⁶ /ml)	0	2	2.5	0	4	0	0	0	0	0	0	3.5	0.1
Serum FSH/LH (mIU/ml)	9.33/ 8.29	12.36/ 6.81	10.05/ 4.66	15.1/ 7.92	12.7/ 8.2	18.34/ 9.62	12.54/ 7.16	10.39/ 6.72	10.8/ 7.48	14.6/ 9.2	12.4/ 6.91	15.47/ 7.21	10.72/ 6.25
Testicular Histology	NP	NP	NP	Maturation Arrest	NP	NP	NP	NP	NP	NP	Development Arrest at spermatid stage	Maturation Arrest	
Karyotype	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY	46,XY
Deleted AZF regions	c	c	c	c	c	a	a, b	c	b, c	c	c	c	c

[Table/Fig-2]: Findings of 13 infertile men with Yq deletions
 Mean \pm SD: FHS= 12.68 \pm 2.58 mIU/ml; LH= 7.41 \pm 1.28 mIU/ml; Right testicular vol.= 10.57 \pm 1.90ml; Left testicular vol.= 9.28 \pm 2.17 ml; AZF= Azoospermia Factor



[Table/Fig-3a]: (a): Photomicrographs showing primary spermatogenic arrest (arrows)

[Table/Fig-3b] (b) absence of mature sperms in seminiferous tubules (arrows)



[Table/Fig-4]: Vendiagram shows percentage of microdeletions in AZF regions. The AZFa region was involved in 15.4%, of which AZFa alone was involved in 7.7% where as AZFa & AZFb were involved in 7.7%. AZFb region was also involved in 15.4% deletions. Deletion of AZFc found 84.6%, of which deletion of AZFb&c were 7.7% and deletion of AZFc alone was 76.9%

central India population. Tiepolo & Zuffardi (1976) proposes the location of spermatogenesis controlling factors on nonfluorescent euchromatic portion of 'q' arm of Y chromosome. Comprehensive Y-chromosomal mapping studies comprising of large number of severely infertile men with identified phenotypes, revealed common deletion pattern in azoospermic males [12]. The frequently deleted region was located in AZFc. Y chromosome deletions mostly arose de novo and these deletions were the cause of spermatogenic failure observed in infertile subjects. DAZ genes cluster has frequently deleted and frequencies of deletions increase with the severity of spermatogenic defects in infertile subjects [13].

In the present study, thirteen infertile azoospermic & oligozoospermic men (8.33%) who were karyotypically normal showed microdeletions of Y chromosome spanning over the AZF a, AZFb, AZFc loci. Efforts have been made to determine the incidences of Yq microdeletions and to correlate the size and

site of the Yq deletion with the infertile phenotype. Regionwise percentage of microdeletions are shown in [Table/Fig-4] and deletion mapping of the total 13 subjects of AZF regions demonstrated that the AZFc was having the highest number of microdeletions in 11(84.6%) infertile men and more commonly microdeletions were detected by STS primers sY254 & sY255 which cover the DAZ gene [Table/Fig-1]. Two infertile azoospermic males P34 and P39 were identified with AZFa and AZFa+b large deletions respectively, while subject P71 was found with isolated AZFb+c deletion.

Frequency of Yq microdeletions observed in both oligozoospermic and azoospermic men is compared with the reports from different parts of world and other regions in India [8,14]. In south European population (Italy) the average frequency is around 15% while in north European populations (Germany, France, The Netherlands etc.) the frequency of Y chromosome deletion in infertile cases is low (1-4%) [1]. The data available from Australia, New Zealand, China, Japan and south East Asian countries the reported frequencies were around 10% [15]. However, the prevalence differs in a wide range, from 1% to 55%. Majority of the reported cases in Asian population deletions noted in AZFc region and occasionally in AZFa and AZFb regions [16]. The reported frequencies of microdeletions range from 2% to 10% in India. Deletion in AZFc region is more frequently observed as compared to AZFa and AZFb loci invariably in all studies [17]. Among these studies different loci of STS, including European Andrology Associations (EAA) recommended markers had been used for detection of Y chromosome microdeletion of infertile male population [9]. Thangaraj et al., found overall 8.5% deletions on AZF region in azoospermic men. Dada et al., had documented in 9.63% subjects showing deletion in AZF region in idiopathic infertile men [18]. Ambasadhan et al., showed around 5% deletions [19] and Mitra et al., reported around 5.29% deletions in oligozoospermic and azoospermic subjects [20]. Singh and Raman et al., studied microdeletion of Y chromosome. They stated AZFc to be the most frequently deleted region in azoospermic / oligozoospermic men [21]. These differences in deletion frequency among these reports from India may be due to selection bias of subjects and the choice of STS markers. The different geographical regions, environmental factor, occupational exposures and many ethnic populations have also been stated to be influencing deletion frequency of Y chromosome in infertile males. All these conditions might be necessary to determine the variation in deletion pattern and frequency in different ethnic and environmental influences worldwide populations [2,17].

The testicular volume, FSH and LH levels in AZF deleted males seem to vary in different studies. In present study, testicular volume did not differ significantly between infertile men with and without deletion. Similarly, insignificant differences in the mean of testicular volume were found in some other studies [21]. Serum FSH and LH

level were recorded significantly changes between infertile men and normal fertile control subjects groups.

On correlating the deletion pattern with testicular histology the deletion of AZFa was found to be associated with complete absence of germ cells and presence of sertoli cells in the seminiferous tubules. The deletion of AZFb was associated with developmental arrest of germ cells at pachytene stage. The deletion of AZFc was variably associated with sertoli-cell-only syndrome (SCOS), developmental arrest of germ cells at spermatid stage and maturation arrest. The genes at each locus act at a particular stage of germ cells differentiation and deletion of a particular AZF locus results in a characteristics phenotype [21,22]. Our study testicular biopsy was obtained in three infertile men, two with azoospermia one with severe oligozoospermia. Histopathological picture in all the three subjects comprised of developmental arrest of germ cells and maturation arrest [Table/Fig-1], [Table/Fig-3a], [Table/Fig-3b]. These subjects were having microdeletions in the AZFc region. So, our findings are in agreement that deletion of AZFc is associated with the developmental arrest of germ cells at spermatid stage and maturation arrest [1]. Further, identification of gene products and their biological actions is necessary to observe the influence of AZF gene deletions on formation of mature spermatozoa. Some studies have shown that genetic basis of infertility should be addressed after a vivid consideration of different genetic components like X chromosome mutation [23] and mitochondrial DNA mutations [24].

The finding of 8.33% incidences of microdeletions of Y chromosome confined to AZF a, b and c regions in azoospermic and severe oligozoospermic infertile subjects, supports the views that this specified regions of Y chromosome is essential for regulation of spermatogenesis. Deletions in the crucial AZF regions of Y chromosome are adversely affecting the seminal profile leading to infertility. The relevance of the present study was the higher percentage of deletions detected in AZFc region. These results suggest that genes in the Yq euchromatic segment may be strongly associated with testicular germline population. Idiopathic male infertility, a multifactorial disorder, is governed by environmental factors and genetic components. The etiology to male infertility may differ between ethnic populations. Therefore, it is very important to take ethnic-environmental-geographical axis into consideration in the genetic basis of male infertility. However, Y chromosome microdeletions cannot be predicted clinically, on the basis of semen analysis or by karyotyping. Our data will be useful for infertility clinics for genetic counselling and to adopt appropriate methods for assisted reproduction. Thus PCR based Y chromosome screening for microdeletions are the appropriate approach for diagnosis, management and counselling of the infertile men.

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REFERENCES

- [1] Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocrine Rev.* 2001; 22: 226-39.
- [2] Pal A, Waghmare J, Tarnekar A, Rawlani S, Ingole I. Genetic aspect of human infertility. *Pres. Cyto. & Genetics AICCG.* 2007; 13: 106-14.
- [3] Reijo R, Lee TY, Salo P, Alagappan R, Brown LG, Rosenberg M, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat Genet.* 1995; 10: 383-93.
- [4] Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum Genet.* 1976; 34: 119-24.
- [5] Vogt PH, Edelmann A, Krisch S, Henegariu O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet.* 1996; 5: 233-43.
- [6] Foresta C, Ferlin A, Moro E. Deletion and expression analysis of AZFa genes on the Human Y-chromosome revealed a major role for DBY in male infertility. *Hum Mol Genet.* 2000; 9: 1161-69.
- [7] Lahn BT, Page DC. Functional coherence of the human Y chromosome. *Science.* 1997; 278: 675-79.
- [8] Thangraj K, Gupta JN, Pavan K, Reddy AG, Subramanian S, et al. Y chromosome deletion in azoospermic men in India. *J Androl.* 2003; 24: 588-97.
- [9] Simoni M, Bakker E, Eurlings MCM, Matthijs G, Moro E, et al. Laboratory guidance for molecular diagnosis of Y chromosomal microdeletions. *J Androl.* 1999; 22: 292-99.
- [10] World Health Organization (WHO). Laboratory manual for the examination of human semen and sperm - cervical mucus interaction. IV Edition 2000; Cambridge University Press, Cambridge U.K.
- [11] Sambrook J, Russell DW. Molecular cloning: a laboratory manual 2000;(New York: Cold Spring Harbour Press).
- [12] Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, et al. The Human Y chromosome: A 43-interval map based on naturally occurring deletions. *Science.* 1992; 258: 52-9.
- [13] Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum Reprod.* 1999; 14: 1722-26.
- [14] Sen S, Pasi AR, Dada R, Shamsi MB, Modi M. Prevalence, phenotypes and screening markers for the Indian population. *J Assist Reprod Genet.* 2013; 324: 413-22.
- [15] Tse JY, Yeung WS, Lau EY, Ng EH, So WW, et al. Deletions within the azoospermia factor subregions of the Y-chromosome in Hong Kong Chinese men with severe male-factor infertility: controlled clinical study. *Hong Kong Med J.* 2000; 6: 143-46.
- [16] Wettasinghe TK, Jayasekara RW, Dissanayake VHW. The low frequency of Y chromosome microdeletions in subfertile males in a Sinhalese population of Sri Lanka. *Ind J Hum Genet.* 2012; 18(3): 320-25.
- [17] Sachdeva K, Saxena R, Majumdar A, Chadda S, Verma IC. Use of ethnicity-specific sequences tag site markers for Y chromosome microdeletion studies. *Genet Test & Mol Biomarkers.* 2011; 15: 451-59.
- [18] Dada R, Gupta NP, Kucheria K. Molecular screening for Yq microdeletion in men with idiopathic oligozoospermia and azoospermia. *J Biosci.* 2003; 28: 163-68.
- [19] Ambasudhan R, Singh K, Agarwal JK, Singh SK, Khanna A, et al. Idiopathic cases of male infertility from a region in India show low incidence of Y-chromosome microdeletion. *J Biosci.* 2003; 28: 605-12.
- [20] Mitra A, Dada R, Kumar R, Gupta NP, Kucheria K, et al. Screening for Y chromosome microdeletions in infertile Indian males: Utility of simplified multiplex PCR. *Ind J Med Res.* 2008; 127: 124-32.
- [21] Singh K, Raman R. Male infertility: Y chromosome deletion and testicular aetiology in cases of azoospermia. *Indian J Exp Bio.* 2005; 43: 1088-92.
- [22] Geoffroy-Siraudin C, Aknin-Seiffer I, Metzler-Guillemain C, Ghalamoun-Slaïmi R, Bonzi MF, et al. Meiotic abnormalities in patients bearing complete AZFc deletion of Y chromosome. *Hum Reprod.* 2007; 22: 1567-72.
- [23] Stouffs K, Tournaye H, Liebaers I, Lissens W. Male infertility and the involvement of the X chromosome. *Hum Reprod Update.* 2009; 15: 623-37.
- [24] Nakada K, Sato A, Yoshida K, Morita T, Tanaka H, et al. Mitochondrial-related male infertility. *Proc Natl Acad Sci USA.* 2006; 103: 15148-53.

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