Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population

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Abstract

Purpose Yq microdeletions are the leading genetic cause of male infertility and its detection is clinically relevant for appropriate genetic counseling. We aimed to determine the prevalence and type of Yq microdeletions, the associated seminal phenotypes and the STS markers that are relevant for its testing in Indian population.

Methods Yq microdeletion analysis was carried out in 1,636 infertile cases in our centers. Additional data was collected from published studies in Indian population leading to a total of 3,647 cases.

Results In our cohort, 3.4 % (56/1,636) of infertile men had Yq microdeletions. Combining the data from other published

studies identified 215/3,647 (5.8 %) infertile individuals to harbor Yq microdeletions; with 6.4 % in azoospermia, 5.8 % in oligozoospermia and 3.2 % in oligoasthenozoospermia and teratozoospermia cases. No significant differences in the deletion frequencies were observed between idiopathic vs non idiopathic cases (5.8 vs 8.6 % respectively). Deletions of AZFc were at highest frequency (46.6 %) with double deletions most commonly observed in azoospermic subjects. With respect to the STS markers, screening with the six European Academy of Andrology (EAA) markers would miss 3.1 % of cases; additional non EAA markers that would contribute significantly to screening are sY746, sY82, sY121, sY128, sY130, sY143, sY145 & sY160.

Interpretations and conclusions The frequency of Yq microdeletions is lower in Indian population as compared to Western counterparts. There is no major association of Yq microdeletions with seminal parameters or cause of infertility. Clinically it will be necessary to offer Yq microdeletion testing to all the classes of infertile men. The EAA markers may not be adequate to detect microdeletions in Indian infertile men.

Capsule Yq microdeletion testing in male infertility: An Indian experience.

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Introduction

According to WHO estimates, one in every ten couples of reproductive age experience involuntary childlessness. In approximately half of the cases, the male partner usually has reduced or no sperm in the semen. Clinically referred to as male infertility, these men are either azoospermic (no sperm in the semen) or oligozoospermic ($<15 \times 10^6$ spermatozoa/mL of ejaculate). While cryptorchidism,

infections, alcohol abuse and radio/chemotherapy are known factors leading to oligo/azoospermia, a large number of cases remain unexplained and often referred to as idiopathic male infertility [16].

Cytogenetic and molecular analyses of the idiopathic infertile patients have identified submicroscopic deletions of the Y chromosome mainly on the q arm and are referred to as Yq microdeletions [13, 25]. Representing as one of the well recognized causes of spermatogenic failure, Yq microdeletions involve loss of one or more of three discrete loci termed as Azoospermia Factor (AZF) a, AZFb and AZFc. Since its first description in a subset of azoospermic men [25], Yq microdeletions have been described in a spectrum of infertile men, the incidence of Yq microdeletions is estimated to be 7–8 % with a wide variation across populations [13, 18].

Testing for Yq microdeletions in infertile men is considered to be clinically relevant as it helps in establishment of a correct diagnosis for the cause of infertility [12]. Further, the occurrence and type of microdeletion correlates with testicular phenotype and is a good predictor of sperm retrieval during Testicular Sperm Aspiration or TESA [15]. Also, oligozoospermic men with microdeletions have been reported to progress to azoospermia over time [1, 9, 13], higher incidence of poor quality embryos has been observed in couples where the male partner has microdeletions [35]. In addition, it is important to note that infertile men carrying Yq microdeletions always pass on the defect to their male offsprings born after Intracytoplasmic Sperm Injections (ICSI) thereby perpetuating infertility in the next generation [7]. Thus, Yq microdeletion testing is highly recommended for all the infertile men who opt for ICSI for biological parenthood [31]. Therefore, testing for Yq microdeletion is just not clinically relevant in terms of establishing the diagnosis but is also required for appropriate genetic counseling and clinical management of the infertile cases.

In India, Yq microdeletion testing in diverse classes of infertile men has been reported by different groups and a very wide variation (0–28 %) in the prevalence of Yq microdeletions have been observed. Babu et al. [6] were the first to report the occurrence of Yq microdeletions in the Indian population and a prevalence of 5 % was observed. However, Sakthivel and Swaminathan [27] failed to detect any microdeletions; Nangevenkar et al. [21] observed microdeletions in only 1 % of their group of idiopathic infertile men. Our group [1] previously reported a prevalence of Yq microdeletions at a frequency of 3 % in a cohort of 200 azoospermic and oligozoospermic individuals. In contrast, a very high frequency of 24.7 % and 28.6 % of Yq microdeletions has been reported [2, 19]. Such wide variations have not been reported in frequency of Yq microdeletions and has not been observed in other population based studies. Several reasons can be attributed for such large variations observed in

the frequency of Yq microdeletions in different studies. Firstly, as India is an admixture of multiple unique races, there could be geographic and/or racial variations in the occurrence of Yq microdeletions amongst infertile men. Indeed, ethnic differences have been observed in the frequency of Yq microdeletions in infertile men [34]. Secondly, there is a wide deviation in the sample size across different studies (14–340 individuals) which could be a reason for such wide variation. Beyond sample size and ethnicity, the markers used for microdeletion testing also influence the frequency. Yq microdeletion testing by PCR involves use of several unique Sequence Tag Site (STS) markers which are a determinant of the sensitivity and specificity for detection of deletions [26, 28]. In the studies published from India, the numbers and types of STS markers used by different authors for detection of Yq microdeletions are not uniform, in some instances very limited and/or non conventional markers have been utilized [14, 17, 36] which could also be a possible reason for the observed variations. Considering these factors the true frequency of Yq microdeletions in infertile men from India is hence unknown.

To overcome some of these issues and have a consensus on the frequency of Yq microdeletions, we analyzed our cohort of 1,636 infertile men who were selected and screened using uniform criteria. We also analyzed the data published from different parts of India with the aim to estimate the prevalence of Yq microdeletions. In addition, the type of deletions prevalent in the population and the association of these deletions with seminal parameters was determined. Since different studies across the country have used different STS PCR markers for analysis, we also sought to identify the markers that may be of diagnostic significance for Yq microdeletion testing in Indian population.

Materials and methods

The Yq microdeletion testing is a part of the routine clinical workup of infertile cases. A total of 1,636 infertile subjects with no known cause of azoospermia or oligozoospermia have been tested at our centers for the presence of Yq microdeletions. 30 normozoospermic cases of unexplained infertility have also been screened. The criteria for selection of patients and controls have been detailed previously [1, 9, 10]. For data analysis, the patients were grouped based on their seminal parameters viz severe oligozoospermia (SOAS, $\leq 5 \times 10^6$ million spermatozoa/mL of ejaculate), oligozoospermia ($< 15 \times 10^6$ million spermatozoa/mL of ejaculate), azoospermia (no spermatozoa in ejaculate). Cases with asthenozoospermia (< 40 % motile spermatozoa in ejaculate), teratozoospermia (< 4 % normal forms of spermatozoa in ejaculate), or oligoasthenozoospermia (reduced

motility and count) were pooled and grouped as OligoAsthenoteratozoospermia (OAT).

For Yq microdeletion analysis DNA was isolated from peripheral blood and 6 pairs of STS primers spanning the AZF a, b and c regions were used for PCR. Biplex PCR for SRY (PCR positive control) along with one pair of each STS primer was performed essentially as described previously [1, 9, 10]. All reactions included a fertile male control sample, a female sample and a negative (water) control. All PCR products were analyzed on 2 % agarose gels stained with ethidium bromide.

Collection of Indian data

Literature was retrieved using PubMed/Google using the following keywords: Yq microdeletion, Y chromosome, male infertility, India. Data published in the form of full length articles with detailed information were included. Data published only as abstracts were excluded. Information from these articles were manually retrieved for the number of cases screened, number of cases showing deletion, types of deletion, clinical information, seminal parameters, inclusion and exclusion criteria, type and number of STS markers used. Data on numbers of fertile individuals screened and reported by the individual authors was also recorded. Data was curated to exclude any duplicate/overlapping information published by the same authors. Only those papers that had uniform and clear inclusion and exclusion criteria defined and classified according to the criteria as listed above were included. Data from papers where clear clinical information regarding the seminal parameters was not provided were considered as unclassified data. The papers included for the data analysis are listed in Table 1.

All the information was classified for the prevalence of microdeletions by the region, by seminal parameters, type of deletions, types of deletions for different seminal parameter, STS markers and deletion frequency using different STS markers. All statistical analysis was done manually and also using the SPSS software (Version-16.00).

Results

Frequency of Yq microdeletion in Indian population

In the present study, 3.4 % (56/1,636) of cases with male factor infertility had Yq microdeletions. Amongst these, deletions were only observed in cases with azoospermia and severe oligozoospermia (3.4 & 4.1 % respectively); no deletions were observed in the other categories (Table 2). In contrast to the present cohort, the frequency

of Yq microdeletions was found to be 7.9 % in the cumulative data published from India (Table 1).

The geographic variations in prevalence of Yq microdeletion is presented in Fig. 1. The lowest frequency of Yq microdeletions was observed in Mumbai (3.4 %) and the highest in Tamil Nadu (13.4 %). In other states the frequency ranged from 5 to 8 % and not significantly different from the overall frequency (Fig. 1).

Since the patients included in our cohort are predominantly from the northern and western part of India, and as there are geographic differences in the prevalence of Yq microdeletions in different parts of the country, we pooled the data from the papers published from India (Table 1) and estimated the overall frequency. To this end information on 3,647 infertile individuals and 1,245 normozoospermic controls was available, 215 infertile men screened had Yq microdeletions resulting in a prevalence of 5.8 %. No deletions were reported in fertile controls (Fig. 2).

Yq microdeletions and seminal parameters

Of the 3,647 individuals, 3,243 cases could be classified based on seminal parameters. The remaining 404 cases were designated as unclassified. Microdeletions were observed in 6.4 % of cases with azoospermia, 5.2 % with severe oligozoospermia, 5.8 % with oligozoospermia, and 3.2 % with OAT (Fig. 2). In the unclassified group that had mixed data, the frequency of the deletion was 5.9 %. Microdeletions were not observed in normozoospermic infertile or fertile cases (Fig. 2). The differences in frequency of Yq microdeletion observed in the different groups were not statistically significant ($p>0.05$). No difference was observed in the overall frequency when the unclassified data was removed (5.9 vs 5.8 %).

AZF deletions predominantly found in Indian population

Of the 215 cases harboring Yq microdeletion, information about the type of deletion was available for 189 cases (Table 3). Amongst the single locus microdeletion, AZFc deletions were found at the highest frequency (46.6 %). Of the double locus deletion AZFb,c were found in approximately 20 % of cases. Deletions of all 3 AZF loci were observed in 6 cases (3.2 %).

Comparison of Yq microdeletions in idiopathic and non-idiopathic infertility

The prevalence of Yq microdeletions was compared in cases with known (non-idiopathic) and unknown (idiopathic) causes of infertility. Based on the information provided, the cases were classified as idiopathic infertile and non idiopathic infertile. The non-idiopathic group

Table 1 Frequency of Yq microdeletions reported by different authors from India

Author	Numbers screened	Numbers deleted	Percentage
Abilash et al. 2010 [2]	89	22	24.7
Ambasudhan et al. 2003 [3]	172	9	5
Athalye et al. 2004 [5]	100	12	12
Babu et al. 2002 [6]	20	3	15
Khan et al. 2010 [14]	50	10	20
Mahanta et al. 2011 [17]	100	5	5
Mitra et al. 2006 [19]	14	4	28.6
Mittal et al. 2004 [20]	79	5	6.3
Nagvenkar et al. 2005 [21]	88	1	1.1
Pandey et al. 2010 [22]	64	2	3.3
Rao et al. 2004 [23]	251	10	3.9
Sachdeva et al. 2011 [26]	200	21	10.5
Sakthivel and Swaminathan 2008 [27]	74	0	0
Singh and Raman 2005 [30]	270	13	4.8
Swarna et al. 2004 [32]	70	9	12.8
Thangaraj et al. 2003 [33]	340	29	8.5
Viswambharan et al. 2007 [36]	30	4	13.3
Total	2,011	159	7.9

comprised of 92 individuals that included cases with unilateral/bilateral cryptorchidism, chromosomal abnormalities and varicocele. 3,555 individuals were classified in the idiopathic group. The frequency of Yq microdeletions was 8.6 % in the non-idiopathic group and 5.8 % in the idiopathic group; the difference is not statistically significant ($p>0.05$).

Association of AZF deletion type with seminal parameters

Figure 3 illustrates the variation of AZF deletion type with the different seminal parameters. The highest frequency of deletion was observed in AZFc locus irrespective of the cause of infertility. In azoospermic cases 35 % had only AZFc deletions, 29 % had both AZFb and c deleted. In case of severe oligozoospermia, AZFc locus alone was deleted in 81 % of cases followed by AZFa in 10 % and AZFb in 6 % cases. In the oligozoospermic patients AZFc region was

deleted in 58 %, AZFa in 17 % and AZFb in 13 % of the cases. For OAT group, 40 % had deletion of AZFc locus followed by 20 % in AZFa locus.

Table 2 Frequency of Yq microdeletion observed in the present study

Seminal parameters	Numbers screened	Numbers deleted	Percentage
Azoospermia	992	34	3.4
Severe oligozoospermia	525	22	4.1
Oligozoospermia	75	0	0
Oligoasthenoteratozoospermia	14	0	0
Infertile normozoospermia	30	0	0
Total infertile	1,636	56	3.4
Fertile controls	431	0	0

Fig. 1 Geographic variations in frequency of Yq microdeletions in India. Numbers in bracket indicate the number of cases with deletions/total number of cases studied

Fig. 2 Frequency of Yq microdeletion and its association with seminal parameters

Variation of Yq microdeletion frequency with number of STS markers

Analysis of Yq microdeletion involves use of multiple STS markers spanning various AZF loci. Authors have used as low as 4 to as high as 30 STS markers in different studies. Figure 4 shows the association of Yq microdeletion deletion with the number of STS markers used. With a correlation coefficient of 0.01 and slope of 0.09 no significant association between number of STS markers used and deletion frequency was observed.

Variation in Yq deletion frequency with the use of EAA and non-EAA markers

To test if the markers prescribed by European Academy of Andrology (EAA) are adequate to detect the Yq microdeletion profiles in Indian patients, the data set was analyzed based on the prevalence of the deletions in cases screened only with EAA markers along with non-EAA STS markers.

Table 3 Prevalence of the deletions of various AZF loci in infertile men ($n=189$)

Deletion type	Numbers deleted	%
AZF _a	22	11.6
AZF _b	19	10.1
AZF _c	88	46.6
AZF _{a,b}	11	5.8
AZF _{b,c}	37	19.6
AZF _{a,c}	6	3.2
AZF _{a,b,c}	6	3.2

Out of the 3,647 infertile cases, 628 were screened using both EAA and non-EAA markers (Table 4). The frequency of deletions was significantly higher in the group where other markers along with EAA markers were used for testing (8.5 vs 5.4 %). Based on this analysis, approximately 3.1 % (CL 95 %, 1.7–4.5) of patients would be missed out if only EAA markers were used for screening.

Selection of EAA and non-EAA markers significantly contributing to Yq microdeletion screening

We determined the markers that are most often deleted in infertile patients. The details of all the non-EAA STS markers, the number of cases with deletion identified and their statistical significance is presented in supplementary Table 1. A synopsis of significant markers is shown in Table 5. Five of the six EAA markers significantly contributed towards the Yq microdeletion testing (Table 5). The AZF_b marker sY134 had the lowest confidence limits (CL 95 %, −0.17 to 1.97) and did not contribute significantly.

A total of 33, 21 and 27 non-EAA STS markers have been reported to be used for screening of the AZF_a, AZF_b and AZF_c respectively. In case of AZF_a, 12/33 markers detected the deletion atleast once. Amongst these, sY746 and sY82 were falling within the predetermined margin of statistical equivalence (95 % CL). In case of AZF_b, 16/21 non-EAA markers were found to be deleted with sY121, sY128, sY130 and sY143 had CL in the range of 95 %. In case of AZF_c, 20/27 markers reportedly were deleted atleast once with sY145 and sY160 had CL falling within the predetermined margin of statistical equivalence (Table 5).

Fig. 3 Association of the deletions of various AZF loci with seminal parameters

Discussion

Yq microdeletions, in the last decennia have emerged as one of the major genetic factors contributing to male infertility. Analysis of large datasets internationally has demonstrated the prevalence of Yq microdeletions in the range of 7–10 % [18, 29]. The diagnosis of Yq microdeletions is considered to be clinically relevant for appropriate counseling and management of male infertility and is highly recommended for cases undertaking ICSI as a fertility treatment option owing to 100 % transmission of the genetic defect to the male offspring from fathers carrying the microdeletions.

Fig. 4 Association of the frequency of microdeletions with numbers of STS markers

Internationally, microdeletion testing is available in most andrology centers and the laboratory guidelines for the same have been issued [28].

Previous studies reported from India have revealed a wide variation (0–28 %) in the frequency of Yq microdeletions (Table 1), with the average being 7.9 % (159/2,011 cases). In contrast, a significantly lower frequency (3.4 %) of Yq microdeletions was observed in our study, which to the best of our knowledge is the largest cohort reported from the Indian subcontinent. The difference in the prevalence of Yq microdeletions in this cohort with that observed for the rest of the country prompted us to believe that there may be population specific differences in the frequency of Yq microdeletions. Indeed, significant differences in the frequency of Yq microdeletions

Table 4 Comparison of the performance of the EAA and non-EAA STS markers for detection of Yq microdeletions

	Numbers screened	Numbers deleted	%
Deleted with EAA & non EAA STS	628	54	8.5
Deleted with EAA STS	628	34	5.4
Deleted with non EAA STS	628	20	3.1

Table 5 STS markers significantly contributing to Yq microdeletion screening

	STS	Screened	Deleted	%	95 % CL
AZFa EAA markers	sY84	1,149	11	0.9	0.38–1.53
	sY86	905	4	0.4	0.00–0.88
AZFb EAA markers	sY127	464	7	1.5	0.37–2.63
	sY134	314	3	0.9	–0.17–1.97
AZFc EAA markers	sY254	482	17	3.5	1.83–5.17
	sY255	482	19	3.9	2.14–5.66
AZFa non EAA markers	sY746	881	8	0.9	0.27–1.55
	sY82	89	5	5.6	0.74–10.50
AZFb Non EAA markers	sY121	100	4	4	0.08–7.92
	sY128	179	8	4.4	1.33–7.47
	sY130	300	5	1.6	0.15–3.05
	sY143	314	7	2.2	0.54–3.84
AZFc non EAA markers	sY145	189	5	2.6	0.28–4.92
	sY160	303	9	2.9	0.97–4.83

in infertile males were observed when the data was segregated on the geographic basis. Highest deletion frequency of Yq microdeletion (13.4 %) was observed in patients from South India (Tamil Nadu); the lowest (3.4 %) was observed in patients from West (Maharashtra; Mumbai). However it should be noted that the number of individuals screened in Tamil Nadu is relatively small (193 infertile) as compared to other regions. Intriguingly though, compiling all the data from the two Southern states (Tamil Nadu and Hyderabad) the frequency of deletion is still higher (8.7 %) as compared to Western (3.4 %), Eastern (7.7 %) and Northern (5.4 %) states. These findings indicate that there are population specific variations in incidence of Yq microdeletions within India. Indeed, the south Indian population is known to be genotypically distinct from the northern and western counterparts and historically is believed to have evolved independently [24]. Thus it is important to systematically survey Indian groups to identify those that are genetically predisposed to infertility.

Beyond ethnicity, the selection criteria, sample size and the markers used for microdeletion testing also influence the frequency. While our cohort is large and uniform in terms of selection criteria and markers used for testing, the population is mainly from the western and northern regions and not a complete representation of the entire country. Thus, to nullify the multiple confounding variables including the ethnicity and determine the factual frequency of Yq microdeletions in the Indian population, we combined the data from our cohort and those published previously with uniform patient selection criteria. The results revealed an overall frequency of Yq microdeletions to be 5.8 % (215/3,647) in the infertile individuals. This frequency is lower than those reported in meta-analysis (7.4–8.2 %) performed on world literature [13, 18]. These observations imply that although the Yq microdeletions contribute towards male

infertility in the Indian population, the association appears somewhat less than that observed in the Western counterparts. A similar lower frequency of Yq microdeletions is observed in German and Scandinavian countries as compared to other Western countries [29]. An influence of Y chromosome background (e.g. haplogroups) has been suggested as a possible cause in propensity of the Yq deletions [4]. Whether Y chromosome haplogroups contribute to the lower microdeletion frequency observed in the Indian infertile men needs to be investigated.

The Yq microdeletions arise out of deletions of one or more of the three AZF loci viz AZFa, AZFb and AZFc. Of the information available in 189 deleted individuals, in accordance with most published studies, the prevalence of single AZFc microdeletion was found to be the highest in all the groups' of infertile men (~47 %). However, this frequency is lower than that reported internationally where the AZFc deletions are observed in 60–70 % of infertile men [18]. Intriguingly, the deletion of single AZFb is lower in the Indian as compared to the Western population (10 vs 15 %) [13, 18]; the frequency of AZFa deletion is almost double (11 vs 5 %). Further, the frequency of double deletions (AZF a+b, b+c) is also higher in the Indian population as opposed to world literature. Beyond these differences, in 6/189 (3.2 %) cases, AZFa+c deletion have been observed leaving AZFb intact. This is an unusual combination and not reported earlier [13, 18, 29]. These observations together suggest that not just the frequency but the type of Yq microdeletions also differ in Indian subjects. It is possible that the causes and/or the mechanisms that lead to AZF deletions in the Indian population may be different and warrants more detailed investigations.

We next analyzed the clinical manifestations mainly semen parameters associated with Yq microdeletions and male infertility. Most studies have assessed azoospermic or severe

oligospermic individuals, some studies have also evaluated other forms like asthenozoospermia and teratozoospermia. Of the 3,647 cases, information on seminal parameters was available for 3,243 individuals; the others were grouped as unclassified cases. The results revealed no significant variations in the frequency of Yq microdeletions amongst different groups, except for the oligoasthenoteratozoospermia group that had the lowest frequency of 3.2 %. This is in contrast to international reports where high frequency of Yq microdeletions is observed in azoospermic men as compared to oligozoospermic individuals [13, 18, 29]. It is possible that the selection criteria in different studies included herein may not be very stringent thereby diluting small the underlying differences. Indeed in some studies cases with chromosomal defects, varicocele and cryptorchidism have also been investigated along with idiopathic cases [8, 11, 19, 21, 23]. It has been observed that the frequency of Yq microdeletion is higher in idiopathic infertile cases as opposed to a mixed population [13]. Thus we segregated the patients based on idiopathic ($n=3,555$) and non-idiopathic causes ($n=92$) which included chromosomal abnormalities, cryptorchidism and varicocele. The results revealed no major differences in the deletion frequency between these two subgroups (5.8 % idiopathic vs 8.6 non-idiopathic). This observation suggests that in the Indian population, there does not seem to be an association with Yq microdeletions atleast in terms of seminal parameters or cause of infertility and clinically it will be necessary to offer Yq microdeletion testing to all the classes of infertile men.

There are few groups who have reported the association of a particular type of Yq microdeletion with the clinical phenotype. In general, it has been suggested that deletions of AZFa and b are associated with azoospermia whereas deletions of AZFc are associated with oligozoospermia [13]. While in this study highest frequency of AZFc deletions were observed in severe oligozoospermia (81 %) and oligozoospermia (58 %) cases; however, AZFa and b deletions were found at comparable frequencies in azoospermic and oligozoospermic individuals. Interestingly, in the azoospermic group, the frequency of double deletions was found to be higher (37 %) as opposed to SOAS and oligozoospermic groups (3 and 12 % respectively). These observations together imply that it is possible that not only the loci but the length of deletion (involving multiple loci) may contribute to the severity of the phenotype. It is known that oligozoospermic individuals with Yq deletions progress to azoospermia over time [1, 29]. Thus, it would be clinically relevant to closely follow-up the oligozoospermic patients especially those have double deletions for their possible rapid progression to azoospermia and counsel them. This information will be necessary as such patients will need to be appropriately counseled and managed for sperm cryopreservation for fertility options using ART.

Detection of Yq microdeletions utilizes amplification of genomic DNA using primer pairs that specifically span the sequences in the AZF loci. Known as Sequence Tagged Sites (STS), these primer pairs are considered unique and specific to the locus of interest. Based on the physical map of the Y chromosome and deletion maps of the AZF loci in infertile men, several STS markers have been identified and utilized for Yq deletion testing. However, in many instances these markers have been found to be repetitive, nonspecific that span multiple loci and/or are highly polymorphic in nature, leading to false positive and false negative results. To circumvent these problems, European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) recommended use of six STS markers that are considered most robust, non-polymorphic and specific, that correctly identify the microdeletions in more than 99 % of cases with no false negativity [28]. Often referred to as EAA markers, these six sets of STS markers have been extensively utilized internationally for Yq microdeletion testing in various clinical settings. However, there have been growing concerns regarding the use of only these six markers considering the heterogeneity in the Yq sequences in different populations. Indeed, studies have shown high frequency of false negativity when only the six EAA markers have been used for analysis [26]. While no association was observed between the number of STS markers used for testing and the frequency of microdeletions, to test if only the EAA markers are sufficient in detecting the microdeletions in the Indian population, we compiled the data from studies were along with EAA, other markers (now on referred to as non-EAA markers) were used for detection of Yq microdeletions. Analysis obtained from 628 individuals revealed that the mean Yq microdeletions frequency is 5.4 % if only the EAA markers are used for detection, the frequency increases to 8.5 % when other STS markers along with EAA are used. These results imply ~3.1 % of the cases would be missed if only the six marker EAA screening strategy would be used. This analysis clearly necessitates the use of non-EAA markers in conjunction with EAA markers for correct detection of Yq microdeletions in our population.

Several STS markers have been identified to be unique for the Yq region and all of these can be potentially used for screening. However, including all of these markers would be beyond the reach of practical feasibility especially for clinical use as a routine screening program. Since many Indian studies have used a variety of both EAA and non-EAA markers in their screening program, compiling the information obtained for each of the marker would logically permit us to identify the STS markers which may be of clinical utility. In the present dataset, along with six STS markers information on 81 non-EAA STS markers that span the three AZF loci was available. Amongst the six EAA markers, our analysis demonstrated that five markers

contributed significantly towards microdeletion testing and had high confidence limits; the AZFb EAA marker sY134 however had the lowest deletion frequency and had CL below 95 %. In case of non-EAA STS markers, for the AZFa locus sY746 and sY82; for the AZFb, sY121, sY128, sY130 and sY143 and for AZFc sY145 and sY160 had CL within 95 %. These markers are not non repetitive and not included in the ‘abandoned’ category [28] and thus could be used in conjunction with EAA markers for future screening programs. While these are statistically relevant markers emerged out of analysis of the Indian data and could be used with high confidence, there are several points that must be considered before these (or any others) be utilized clinically. In general, STS-PCR based screening strategy is associated with a degree of false positivity [28]. According to international guidelines for STS-PCR screening, isolated single STS deletions are unconventional and should be considered in the polymorphic category unless confirmed by an independent technique like Southern blotting [13, 28, 29]. Secondly, considering the issues of allele dropouts in multiplex PCR and failure of amplification in simplex PCR, repeat independent testing or biplex PCR using an internal housekeeping control must be utilized especially when deletions are detected with unconventional markers [18, 28]. However, in the data compiled herein there are several instances of isolated STS deletions, in most studies only single STS PCR strategy has been used. Except for one study [3], none of the deletions identified using the unconventional markers have been validated by Southern blotting or independent PCR technique. Thus there is a possibility that some of the deletions reported herein may be false positives. Also it is noteworthy that the entire AZF loci, especially the AZFc is highly polymorphic and several interstitial deletions are found in fertile/normozoospermic men and hence it is suggested that some of the reported deletions could be polymorphisms and may not be pathogenic deletions [13, 18, 29]. Indeed, in our hands several AZFc non-EAA markers have shown a deletion in normozoospermic fertile controls (our unpublished data) implying their non pathogenic nature. Thus very stringent criteria must be used for selection of markers and screening strategy while offering Yq microdeletion testing clinically. .

In summary, the data derived in the present analysis suggest a lower frequency of Yq microdeletions (5.8 %) in Indian population as compared to the Western counterparts, differences also exist in the type of deletions and the clinical manifestations associated with the deletions. Furthermore, the data suggest that the EAA markers may not be adequate to detect microdeletions in Indian infertile men. This information will be of clinical utility to aid the health care providers for diagnosis, management and counseling of infertile couples especially those who opt for assisted conception techniques for biologic parenthood.

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