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Review Article

STS markers of Y chromosome in infertility cases in Indian population: A review

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ABSTRACT

Among infertile couples, thirty percent males seek advice for further work up of oligozoospermia and azoospermia. In these subsets of patients, Y chromosome microdeletion is a second line of investigation of male infertility work up. It is a relatively little-known in Indian population. Sequence tagged sites are important in determination of Y Chromosome microdeletion. For the purpose of screening of a disease, one should always use those markers that have less probability of missing any true case. As the prescribed markers used by European Academy of Andrology (EAA) have a tendency to miss some cases, proposed markers need to be revised from the initial screening. The aim of the study is to compare the prevalence of Y chromosome microdeletion in different part of India by using different STS markers. This study complied all studies from India who performed Y chromosome microdeletion test using various markers, both EAA and non-EAA markers. Yq microdeletion in Indian population ranges from 0- 15%. On review of various studies in Indian population, current study arrived at the conclusion that the investigator should use four, six, and four markers for AZFa, AZFb and AZFc respectively for initial screening of Y chromosome microdeletion. These markers have least probability of missing the potential cases of male infertility.

Key message: Yq microdeletion is the second most common cause of male infertility worldwide. Therefore, it must be evaluated in every patient with both EAA and non-EAA markers. Patient and their off springs are definitely benefitted by microdeletion test.

1. Introduction

The problem of infertility is now increasing progressively even in the affluent society due to stress related to jobs, career, etc. Infertility is defined as the inability to conceive even after one year after unprotected sexual intercourse. One in ten couples of reproductive age experience involuntary childlessness. Any health issue in man that prevent pregnancy in fertile female is known as male infertility. All available studies are of the opinion that both male and female factors are equally responsible for this distressing situation. The female factor carries a good prognosis, while the male factor carries relatively bad prognosis.[1] Y chromosome microdeletion is the second commonest cause of male fertility, the first being Klinefelter syndrome. In India, the overall prevalence of Y chromosome microdeletions (AZF deletions) was 8% as reported by the European Academy of Andrology (EAA).[1] Although SRY gene, is located on p arm of the Y chromosome, which is responsible for the differentiation of the gonad into either male and female gonad, azoospermia factor gene located on q arm of Y chromosome, is responsible for spermatogenesis. There are three azoospermia factors located largely as nonoverlapping regions. These are AZFa, AZFb and AZFc (AZFb and AZFc are partially overlapping).

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Table 1: Different population of India and incidence of microdeletion with different STS markers

S.No	Authors	Population	Sample type and size	Markers used	Deletions	Remark
1	Babu et al[2] (2002)	South India	20 Infertile subjects	AZFa:sY84, AZFb:sY127, AZFc: sY254	Total deletion- 3 (15%) – 1 Azoospermic 1 & 2 Oligozoospermic	
2	Ambasudhan et al (2003) Abstrac[3]	Varansi	177- Oligozoospermic & Azoospermic	sY84, sY83; sY153, sY159, sY17, sY161, sY254,sY255	Total deletion- 5% Azoospermic- 8 Oligozoospermic- 1	Testicular biopsy in 50 cases showed diverse stages of spermatogenic arrest with no specific correlation with the genotype
3	Dada et al (2003)[4]	Delhi	83 Infertile men And 25 Fertile men	AZFa: sY84, sY86; AZFb: sY127, sY134; AZFc: sY254, sY255	Total deletion 8/83 (8.5%) (Azoospermic – 9.58% Oligozoospermic- 10%, AZFa; AZFb; AZFc:: 0/8;1/8; 4/8 AZFab:: 3/8	Cytogenetic + PCR analysis Additional 29 subjects were excluded
4	Thangaraj K, Nalini G (2003)[5]	Kolkata	570 men to- 340 Azoospermic men, 230 Normal controls	30 STS markers few important are- AZFa: sY746, sY741, DFFRY, sY742, sY615; AZFb: sY100, sY113, sY127, sY134, sY149, sY146; AZFc: sY255, sY254, sY158, sY160, DYZ	Total deletion in azoospermic 29/340 (8.5%), AZFa; AZFb; AZFc:: 24.2%; 55.2%; 82.8% AZFac; AZFbc; AZFasbc:: 3.5%; 51.7%; 3.5%	Analysis of DNASequence at breakpoint
5	Athalye et al (2004)[6]	Mumbai, Maharashtra	100 Total Infertility cases - Azoospermic-27, Oligoasthenozoospermic- 56 Oligoasthenot-eratozoospermic- 7	"Promega version 1.1 kit" for 18 loci AZFa: sY81, sY182; AZFb: sY121, sYPR3, sY124, sY127, sY128, sY130, sY133; AZFd: sY145, sY153, sY152; AZFc: sY242, sY239, sY208, sY254, sY255, sY257	Total deletion – 12 % Azoospermia 8/27 (29.63%), Oligoasthenozoospermic 3/56 (5.35%), Oligoasthenot-eratozoospermic 1/7 (14.28%)	Two azoospermic men have maximum no of loci to be deleted (16/ 18); DAZ gene is most commonly involve in infertility

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Table 1 continued

6	Rao et al (2004)[7]	Hydrabad	251 infertile male (varicocele-57 idiopathic infertility-194) 25 control	24 markers AZFa: sY86, sY87, sY610 DBY, sY620, USP9Y; AZFb: sY127, sY134, sY143, sY634; AZFc: sY153, sY205, sY232, sY254, sY255, sY277, sY283, sY624; and sY158, sY160 (heterochromatic distal Yq region UTY (AZFa), SMCY, EIF1AY, CDY2 from the AZFb region and CDY1 (AZFc) were also used. Additional STS for SRY gene (sY14)	Total deletions 15.13%, 24.56% (out of 57 varicocele), 12.37% (out of 194 idiopathic infertility)	Deleted STS are sY153, sY158, sY205, sY232, sY254, sY255, sY277, sY283, sY624
7	Mittal et al 2004[8]	Lucknow, Uttar Pradesh	79 Infertile cases (54 Azoospermic, 25 0ligozoospermic	AZFa: sY81, sY84; AZFb: sY124, sY128, sY133; AZFc: sY254, sY255; SRY (sY14)	Azoospermic- 3.7% (2/54) Oligozoospermic- 12%(3/25) AZFa; AZFb; AZFc; AZFbc:: 0: 2/79:2/79: 1/79	Patient with the deletion of AZFc region presented with decline in sperm count
8	Swarna M & Babu SR 2004 Abstract[9]	Hyderabad, Andhra Pradesh	70 Idiopathic infertile	sY84, sY87, sY127, sY254, sY158	Total deletion12.8%, (9/70) Azoospermic – 4/9 =44.4%, Sever Oligozoospermic – 4/9 = 44.4%, Oligoasthenzoospermic – 1/9 = 11.1%	
9	Nagvenkar et al (2005)[10]	Mumbai	88 – 42 men with Azoospermic, 46 men with sever Oligozoospermia	AZFa: sY84, sY86; AZFb: sY127, sY134; AZFc: sY254, sY255; sY160, SRY and ZFX	Total deletion- 1/88 AZFc region+ sY160	Cytogenetic + PCR analysis
10	Singh & Raman (2005)[11]	Varansi	270 males form various genetic disorders associated with infertile phenotype	40 sets of marker AZFa-sY84, AZFb-sY117, AZFc-sY152	Total deletion 13/ 270 AZFc; AZFbc:: 11/13; 2/13	
11	Dada et al (2006)[12]	New Delhi	Azoospermic 140 Fertile control 50	AZFa: sY84, sY86; AZFb: sY127, sY134; AZFc: sY254, sY255	Total: 6.01%	

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Table 1 continued

12	Viswambharan et al (2007)[13]	Coimbatore Tamilnadu	30 Infertile men(17 Azoospermic+13 severly Oligozoospermic) 20 Controls	AZFa: sY84, DBY AZFb: sY127, RBM1 AZFc: sY254, BPY2	Total deletion 29/340 (8.5%) AZFa; AZFb; AZFc:: 24.2%; 55.2%; 82.8%	
13	Mitra et al 2008[14]	New Delhi	170 infertile men (51 Oligozoospermic, 119 Azoospermic) 101 fertile control	19 markers: some important are AZFa: sY746, sY86, DFFRY; AZFb: XKRY sY118, sY113, sY127, sY134, sY143, RBMIY; AZFc: sY153, sY148, sY157, sY158, sY254, sY255, sY160	Total – 5.29% Azoospermic- 5.29%, Oligozoospermic – 0%, AZFa; AZFb; AZFc:: 2/9:1/9:3/9 AZFbc:: 3/9	In PCR reactions, sY153 primer was used which was outside the DAZ region.
9	Sakthivel and Swaminathan (2008)[15]	Tamilnadu Erode Nilgiri	287 men – 147 Cases, 140 controls; infertile men (45 blood sample, 72 semen sample; 30 paired sample) Control (90 blood sample, 30 semen sample; 10 paired sample)	AZFa: sY740, sY86, sY741, sY84, sY745; AZFb: sY99, sY100, sY109, sY127, sY129, sY133, sY134, sY138, sY143; AZFc: sY152, sY146, sY156, sY255, sY254, sY158	Total-12.9% (19/147) AZFa; AZFb; AZFc:: 10.5%; 5.3%; 68.4% AZFab: AZFbc:: 5.3%: 10.5%	Emphasized necessity to analyse sperm DNA than blood DNA, Spermiogram of men showed asthenozoodspermia, oligozoospermia, insufficient production of mature sperm enable reproduction in infertile men with deletion AZFb and AZFc
11	Suganthi et al (2009)[16]	Tamilnadu	215 Infertile 120 Sever Oligozoospermic and 95 Azoospermic	AZFa: sY84, sY85, DFFRY, DBY AZFb: sY127, sY143, sY134 AZFc: sY158, sY157, sY254, sY255, sY145, sY152	Total deletion- 11.1% Azoospermic 7.4% AZFa; AZFb; AZFc:: 4.16%; 20.8%; 45.83%, AZFac; AZFbc; AZFabc:: 8.3%; 12.5%; 8.3%	Used drop of blood on blotting paper, confirmed that AZFc deletion is more often seen in sever hypospermatogenesis rather than SCOS Deletion of AZFac could be in sever oligozoospermia & these cases can be considered for sperm retrival.

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Table 1 continued

10	Abilash et al (2010)[17]	Chennai, South India	34 Azoospermia 55 Oligozoospermia	AZFa: sY82, sY84; AZFb: sY164; AZFc: sY158, sY160, sY240, sY254, sY255, sY277, CDY; AZFd: sY145, sY152	AZFa: AZFb: AZFc: AZFd: Azoo-27%, 4%, 56,% 13% Oligo-33%, 7%, 48%, 12%	AZFd Deletion present in boundaries of AZFb and AZFc Higher frequency of deletion when compared with other European ethnic population
12	Pandey et al (2010)[18]	Varansi (U.P.)	64 Infertile cases	sY156, SPGY, sY254, DAZLA3, 92R7	No deletion In AZFa, AZFb, Deletion in AZFc region -3.33%	no direct relationship exists between Y-microdeletions with hormone profile changes
13	Sachdeva et al (2011)[19]	Delhi	200 infertile males	EAA markers: Non EAA markers:	3 % in EAA markers 7.5% in Non EAA markers	
14	Sen et al 2012[20]	Mumbai	1636 infertile (Oligozoospermic and Azoospermia) 30 normozoospermic	(AZFa) sY84, sY86, sY746, sY82 (AZFb) sY127, sY134, sY121, sY128, sY130, sY143, (AZFc) sY254, sY255, sY189, sY303 sY145, sY160	Total deletion 3.4% Azoospermia – 3.4%, Sever Oligozoospermic – 4.1% AZFa: AZFb: AZFc:: 11.6%:10.1%:46.6% AZFab: AZFbc: AZFac: AZFabc:: 5.8: 19.6: 3.2: 3.2%	Cohort study from different regions of India, emphasized Non EAA markers sY 746, sY82, sY121, sY128, sY130, sY143, sY145, sY160 are important for Indian population
15	Vijaylakshmi et al (2013)[21]	Tamilnadu	Cases-175 Controls-110	AZFa: sY84, sY86; AZFb: sY127, sY134 ; (AZFc) sY254, sY255	Total –12.56%, Azoospermic- 9.14% Oligozoospermic – 3.42%, Azoo- AZFa; AZFb; AZFc:: 1.14: 2.28: 5.72, Oligo- AZFa; AZFb; AZFc:: 0: 1.14:2.28	AZfc region showed higher frequency of deletion compared to AZFa& AZFb
16	Ray et al (2014)[22]	Kolkata, West Bengal	Urban (40) –Oligozoospermic 31& Azoospermic 9 Rural (30) -Oligozoospermic & 10 Azoospermic	AZFa : sY85, sY95, sY746; AZFb: sY131, sY130, sY143, sY142: AZFc: sY164, sY153, sY148, sY157	Total deletion 5%, Deletion in Oligozoospermic- 1.64%, Deletion in Azoospermic -15.79%	No significant urban rural variation

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Table 1 continued

19	Vijesh et al 2015[23]	Coimbatore	120 patients with Non obstructive Azoospermia, 109 with oligozoospermia, and 125 normal male controls	AZFc: DAZ and CDY genes	21 (9.17%) patients with classical AZF deletion
20	Prafulla & Pande S (2017)[24]	Nagpur	160 Infertility cases(90 Oligozoospermic and 70 Azoospermic 50 controls	AZFa: sY746, sY84, sY86, DFFRY; AZFb: sY113, sY118, sY127, RBMIY, XKRY, sY143, sY134; AZFc: sY153, sY148, sY157, sY255, sY254, sY158, sY160 and SRY	Total-10.6 % (17/160), AZFa;AZFb; AZFc:: 5.88%; 11.76%; 58.88% AZFac; AZFbc:: 5.88%: 17.64 %
21	Nailwal M et al 2017[25]	Gujrat	141 infertile men (41 Azoospermic+100 oligozoospermic) 159 fertile control men	AZFa: sY84, sY86; AZFb: sY121, sY127, sY134; AZFc: sY153, sY254, sY255, sY1191, sY1197, sY1291 SRY	Total – 24.11% Azoospermic – 13/41 =31.41%, Sever Oligozoospermic – 21/100 = 21% AZFa; AZFb; AZFc:: 6.38%; 9.22%; 17.2%
22	Pande et al 2018[26]	Mumbai	763	16markers AZFa: sY81, sY84, sY86, sY182; AZFb: sY121, sY124, sY127, sY130, sY134; AZFc: sY153, sY157, sY254, sY255, sY145, sY152	Total- 3.9 % (30/763) AZFa, AZFb, AZFc 0%, 0.8%, 2.6% AZFabc; AZFbc:: 0.1%: 0.4 %

Earlier association between deletion of AZF region on Y chromosome and impaired or abnormal spermatogenesis was established by Tiepolo L and Zuffardi O in 1976.[27] In India, this association was first established by Babu et al.[2] using Y chromosomes microdeletion.

The aim of the study is to compare the incidences of Y chromosome microdeletion in the different part of India by using different STS markers. This study compiled all studies from India which were based Y chromosome microdeletion test using various markers, both EAA and non-EAA markers. These studies were with and without cytogenetic test. Hence this review is intended to highlight the importance of non EAA markers in screening of male infertility cases in Indian population.

2. Materials and Methods

After searching literature in Pubmed, google scholar, MEDLINE and EMBASE upto June1 2019 using mesh terms " Yq microdeletion", "male infertility", "Azoospermia factor" and STS marker of male infertility, the relevant data was compiled. The unrelated studies were excluded based on their abstracts. This review included only those Indian studies conducted on infertile men, which used markers for three AZF regions, and which used only conventional PCR for analysis. Full text articles were collected from the respective journals and from the authors via email. A total of twenty two studies were included for the study. Data obtained were categorized manually. The markers, a number of subjects, type of subjects (infertile man or couple, man with oligozoospermia or azoospermia), regions/population and remarks or highlights of the studies were tabulated. Author included only Indian study for review to know prevalence specifically to India. Then author analysed the data of Indian studies which performed Yq microdeletion test by using different markers.

3. Discussion

In Indian scenario, various markers have been used by different researchers to evaluate microdeletion. Therefore, there is a wide variability in presentation of results from various parts of India. In south India, highest number of cases have been found using this microdeletion due to the use of greater number of markers (Table 1). Table 1 depicts the different studies conducted in different parts of India with the markers used and their results. For this reason, European Academy of Andrology (EAA) has recommended to use six markers to the extent of the deletion and one marker for positive internal control. For further extension other markers can be used.

Screening test means the test is capable of detecting the disease present even in minor, form which means it has high sensitivity and low specificity. Therefore, these markers which are proposed by EAA are revised for the Indian population for screening. Because screening test is generally chosen towards high sensitivity not to miss potential disease, any positive result indicates suspicion of disease that warrants confirmation. In the cohort study, Sen et al proposed to screen for few non EAA markers that contributed significantly towards microdeletion; sY746, sY82, for AZFa, sY121, sY128, sY130, sY143 for AZFb, sY145and sY160 for AZFc region along with EAA markers for Indian population.

A study by Sakthivel and Swaminathan, 2008[15] suggested that sperm/germ cell DNA shows more deletion as compared to blood DNA therefore it is better to analyse germ cell DNA. Though better option for screening is sperm germ cells, blood is easy for screening purpose. Sachdeva et al (2011)[19] found in their study that non EAA markers are further deleted even if EAA markers were not deleted.

Varicocele is also an important cause of infertility. In an Indian study on varicocele cases with infertility, sY153, sY158, sY254 and CDY1 gene markers were found deleted.[7] Therefore, these may be important markers to be tested in varicocele cases.

For extension analysis of deletion and duplication in AZFc region, five STS markers were developed; sY1161, sY1191, sY1201, sY1206, sY1291.[28–30] For extension analysis of deletion of AZFb region, sY105, sY1224, sY121, sY143, sY1192 and sY153 were developed.[31] For extension analysis of AZFa region sY82, sY83, sY1064, sY1065, sY1182, sY88 have been developed.[1]

Our review design has few limitations. Firstly, only Indian studies have been included which too do not represent the entire India. Therefore, the actual prevalence of Yq microdeletion in India cannot be commented upon. Secondly, environmental factors might have contributed to deletion which the author has not taken into account.

4. Conclusion

Present study concluded that Yq microdeletion in Indian population ranges from 0-15%. This study also gives comparison of different markers in different population of India. It also suggests that for initial screening, four markers for AZFa, six markers for AZFb, and four markers for the AZFc region should be tested in Indian population. Thereafter, if needed, one should proceed for further extension analysis. Yq microdeletion is a second line of investigation for male infertility therefore screening should be done with those markers that have least probability of missing the potential cases of male infertility. This evaluation may help in avoiding hormonal assessment in infertile couples.

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6. Conflicts of Interest

No conflicts of interest.

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