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Review Article

Genetics Section

Current Status of Y Chromosome Microdeletions: Prevalence, Distribution, Implication and Association with Male Infertility in Indian Men- A Review

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ABSTRACT

Introduction: Infertility affects about 15% of couples attempting pregnancy and in approximately 50% of these cases, male factors are responsible. Male infertility is clinically characterised by azoospermia and oligozoospermia depending on the amount of loss of genetic material and the size of the affected region on the Y Chromosome Microdeletions (YCM). The majority of genes located in the Y chromosome are involved in male related functions such as spermatogenesis in human, in addition to other endocrine and physiological factors. These microdeletions are located on q arm of Y chromosome, specifically Azoospermia Factor (AZF) region, hence called Yq microdeletions. These deletions are in form of complete/incomplete, recombination; mutations and Copy Number Variations (CNV) and vary in frequency depending on region, ethnicity, lifestyles and other epigenetic factors. Hence, this study is well reviewed in Indian men with infertility caused by AZF a,b,c and other partial deletions. So, it is important to the one who is affected by these mutations and infertile couples who adopt Assisted Reproductive Technologies (ARTs) after counseling. It is further useful for prediction of testicular sperm retrieval chances.

Aim: To review the current status of Yq microdeletion frequency in infertile Indian men with the available data and their correlation with testicular phenotypes as well as other factors. These would also reckon as a supportive to other clinical findings for diagnosis of specific deletion of infertility to adopt ARTs to the infertile couple.

Materials and Methods: Various studies including our data were collected to European Molecular Genetics Quality Network (EMQN) as well as analyse these Yq microdeletions screened using specific Sequence Tagged Sites (STS) of available kit like European Academy of Andrology (EAA) and non-EAAs using Polymerase Chain Reaction (PCR) technology. Various researchers from various zones of India contributed to microdeletion screening of Y chromosome using various STS to AZF locus. These data from 30 study groups were compared to geographical areas/zones, Indian populations, environment, selection criteria and other factors in this review.

Results: The data on thousands of Y chromosome analysis confirmed that the frequency of microdeletions are affected by sample size, selection criteria of subjects, different geographical regions, ethnicity, Oxidative Stress (OS), Deoxyribonucleic Acid (DNA) fragmentation and food styles in addition to genetic defects. In Indian subcontinent, these deletions contribute to 8.33% from screening of 5435 Y chromosomes (453/5435). Lower percent (5.37%) of Yq microdeletions in Western India than other parts was observed, being highest in South East (20.52%) and North East zones (17.77%) as mentioned in the present study. These variations in Yq microdeletions are attributed to geographic region, foodstyle, other environmental factors and others. AZFc deletions were more prevalent and correlated to azoospermia (referred/selected, 66%; deleted 61%) from 30/15 citations respectively in present cohort over oligospermic and/or severe oligospermic men followed by b and a sub-regions including b+c, a+b and others in AZF locus. Amongst 30 study groups, 27 exhibited AZFc deletions at higher rate.

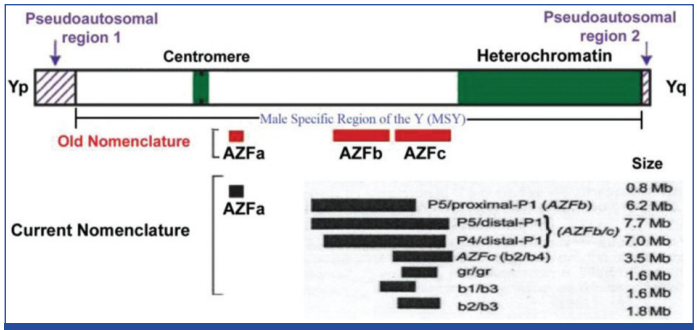
Conclusion: From these data in India, it was hence noticed that screening of Yq microdeletion is an important criterion and its correlation with spermeograms is very necessary to infer degree of infertility in men. Such cases are strongly suggested to undergo genetic counselling before adoption of ARTS as deletions increase risk of genetic anomalies, low birth weight and congenital malformations in New Births (NB) of Intracytoplasmic Sperm Injection and Testicular Sperm Ejaculates (ICSI/TESE) adopted cases. Thus, Y deletion evaluation reckons the diagnosis of type of male infertility and its prevention in the next generation propagation through ARTs adopting infertile couples after counselling.

Keywords: Polymerase chain reaction-sequence-tagged site markers, Regional distribution, Spermeograms, Yq microdeletion screening

INTRODUCTION

Cytogenetically, human 'Y' chromosome is an acrocentric type composed of two Pseudoautosomal Regions (PAR1 and PAR2), a short arm (Yp) and long arm (Yq), that are separated by a centromere [1]. PARs and short arm Yp are euchromatic while a large portion of the long qarm is heterochromatic except around centromere which is euchromatic in nature. The non-recombining region (NRY) is the locus beyond the PARs which does not recombine with X-Chromosome during meotic paring [2]. This region has heterochromatic and euchromatic loci. The heterochromatic locus of Yq comprises distal Yq containing two highly repetitive sequences DYZ1 and DYZ2 [1,3]. Variation in this region of the long arm of Y has been observed [2], whose significance is much unknown [Table/Fig-1] [4].

The euchromatic region encompasses the pericentromeric region and the short and long arms of Y which refer to the male specific region on Y (MSY; [Table/Fig-1]). This region contains genes for sex determination and regulation of brain functions [1,4,5].



[Table/Fig-1]: Old and new classification of Y chromosome in relation to AZF with modification [4].

These genes are critical in development of gonadal differentiation, development and function like Sex-Determining Region on Y Chromosome (SRY), Testis-Specific Protein on Y chromosome (TSPY), while the longarmed (Yq) has 12 genes and gene families mainly [1,5,6]. After this discovery, the Y chromosome is well studied in various species and several functional genes (approximately 70) are detected [7]. Basically these genes fall in two categories viz., housekeeping genes homologous to X and gene families expressed specific to testes, the candidate genes. The involvement of non-recombining region of Y(NRY) locus related to Yq in male infertility was coined in 1997, almost 23-year-ago. However, in 1976, Italian researchers identified detectable deletions at distal end of band Yq11 in 6 infertile males (6/1170). Their investigation revealed that deletions are de novo and could be related to azoospermia [8]. Based on this, the authors proposed aspermatogenesis factor called the AZF on the Yq locus. With the advent of molecular maps of the 'Y', the studies in AZF region and male infertility were extensively elaborated [9-11]. Using a panel of markers, infertile males who had deletions in the Yq 11.23 were identified [1,12,13]. These studies disclosed in this region, as three sub regions; proximal, middle and distal defined as AZFa, AZFb and AZFc, respectively [14]. The Deleted in Azoospermia (DAZ) gene in AZFc locus was considered as a strong candidate for male infertility, little is known about other genes in this locus [12]. This region has the size 0.8 Mb (AZFa), 6.2 Mb (AZFb/P5/Proximal-P1), 7.7 Mb (AZFb/c; P5/distal P1 and P4/distal P1), 3.5 Mb (AZFc (b2/b4), 1.6 Mb (gr/gr), 1.6 Mb (b1/b3) and 1.8 Mb (b2/b3) as mentioned in [Table/Fig-1] [1,15,16].

AZFa encodes only single copy genes of four mapped. These genes are X-homologous genes that escape during inactivation. These are ubiquitin peptidase 9 Y-linked (USPY9) whose function is beyond regulation of sperm production. Dead Box on Y Chromosome (DBY) deletion leads to Sertoli Cell Only Syndrome (SCOS) and involves also early stages of development Ubiquitously Transcribed Tetratricopeptide Repeat on Y Chromosome (UTY) gene status in male infertility is unknown. Same is for thymosine beta 4 Y linked (Tβ4Y). Thus, these candidate genes contribute 5% of deletion [16-18]. AZFb genes contribute about 16% q deletions in infertile males. Deletions bring about azoospermia, oligospermia. It also contains admixture of AZFc locus [Table/Fig-1]. It encodes 5 single copy of genes and others are more than single copy. These are Cyorf15, RPS4Y2, E1F1AY, SMCY, XKRY, HSFY, PRY and RBMY. The RBMY is the most important genes of AZFb involved in spermatogenesis. Deletion of PRY and RBMY lead to hypospermatogenesis/complete loss of spermatogenesis [19-23]. Especially, RBMY protein is involved in all stages of sperm production and male cancers [12]. AZFc locus contributes to 60% of deletions. AZFa and AZFb are essential for initiating spermatogenesis, while AZFc is necessary for its completion. The AZFc in most infertile men lead to severe oligospermia (<5 X 10^6/mL) to azoospermia [24,25]. It spans 4.5Mb and codes for 2% candidate genes and in families of transcription units expressed in testis [26,27]. Thus, it has DAZ, basic protein Y2, Chromo Domain on Y (CDY1), Golgi autoantigen, golgin subfamily a 2 likely chondroitin sulfate proteoglycan 4 like Y, Testes Specific Transcript Y (TTTY)-linked-3, TTTY4 and TTTY17 [28]. Non-Allelic Homologous Recombination (NAHR) occurs between amplicons which include deletion, duplication and both leading to Copy Number Variation (CNV) in this sub region [29]. Hence, this region is more susceptible for microdeletions. Four partial deletions are identified viz., b2/b4, gr/gr, b2/b3 and b1/b3 having about 1.6 Mb [29-31]. Amongst these, gr/gr is the common deletion occurring due to recombination [32]. The b1/b3 differs from others, as it also encompasses the part of AZFb and results in loss of RBMY1 and PRY leading to loss of spermatogenesis in high frequency [27]. However, these partial microdeletions encompassing AZFc and AZFb regions are important in causing spermatogenic alterations in male fertility; their importance needs to be answered further. AZF deletions and semen phenotypes correlate with Yq microdeletions so, it is appropriate to consider Y deletions as a cause of testicular semen pathologies. Hence, about 25-55% of males with gonadal pathologies are related to hypospermatogenesis, arrest of sperm maturation and SCOS. Amongst, about 5-25% males are with severe oligospermia to azoospermia [32,33] harboring YCMs. But depending upon the deletion on AZF locus deleted, the phenotypic alteration varies [1,16,34]. Accordingly, the patients need to be suggested for ARTs like Intracytoplasmic Sperm Injection (ICSI) and Testicular Sperm Ejaculates (TESE).

Mechanisms of AZF Deletions

AZFa deletions are due to homologus intrachromosomal recombinations between two Human Endogenous Retroviral (HERV) sequences. AZFb, c deletions result recombination between palindromes (P5, P1 and P3, P1), respectively. Peculiar structural organisation of AZFc makes it more prone to structural rearrangements. Partial deletions occur as a result of recombination between subamplicons in AZFc. CNV occurs due to various mechanisms like gene dosage, mutation, deletion or duplication in AZF locus [1,29,35].

Yq Microdeletions Analysis

Y chromosome deletions are dynamic in addition to other genetic factors. These microdeletions involve deletions, duplications and both. Clinically, the Y chromosome changes can be categorised AZF deletions, partial AZFc deletions and the gene CNVs second to chromosomal anamolies.

MATERIALS AND METHODS

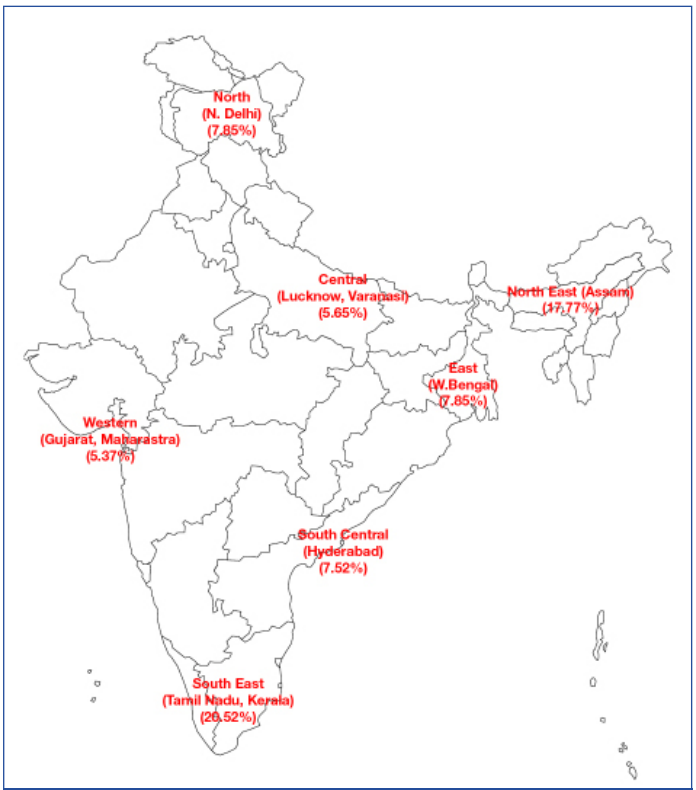
Genomic DNA was used for analysing micro deletions with Sequence Tagged Sites (STS) based on Polymerase Chain Reaction (PCR) technologies. Thus, these AZF deletions were described by adopting specific markers of EAA and non EAA STS as suggested by Sen S et al., [36]. About 18 STS were essentially used for proper identification of deletions in AZF locus of infertile cases. These include SY746, SY86, DFFRY, (AZFa), SY113, SY118, SY127, RBM1Y, XKRY, SY134, SY143 (AZFb), SY153, SY148, SY157, SY255, SY254, SY158, SY160 (AZFc) in addition to SY14 (SRY). Genomic DNA was used for PCR assay and was used with necessary reagents for detection of microdeletions in AZFa, AZFb, AZFc, AZFa+b and AZFb+c sub regions and others [9,15,36,37] for ensuring optimal results [10,38-40].

Testing of Yq microdeletions has many applications for correct diagnosis for the cause of infertility [38,39]. For correlation of testicular phenotypic manifestations specific guidelines are strictly followed, while performing spermeogram analysis [41]. Accordingly, the cases were grouped into azoospermia (obstructive and non-obstructive) oligozoosospermia (5-15×10^6/mL), severe oligospermia, normozoospermia (normal values of sperms in the ejaculate),

astherozoospermia (low level of motility, <50%), teratozoospermia (<30% of normal sperm morphology) and aspermia. Other causes include cryptorchidism, varicocele, endocrinological, obstruction of seminal pathways, infection, alcohol and chemotherapy in addition to genetic defects like cytogenetic disorders, gene mutation, Yq microdeletion [1,36,42]. Among genetic defects authors revealed the current knowledge of Yq microdeletions in the genes leading to infertility and their correlation to the phenotypic manifestations, implications, their distribution and prevalence in India.

Data Collection

The information, latest was collected using Google on YCMs and male infertility in India and research paper collected. As cited earlier this information was categorised for the prevalence of Yq microdeletion, region wise, phenotype of semen, type of q deletions, STS markers by PCR-STS method. The data of Yq deletions were subjected to percent values if necessary, from chosen 30 study groups of seven Indian Zones; Western (Gujarat, Maharashtra); Central Zone (Lucknow, Varanasi); North (Delhi); North-East (Assam); East (West Bengal); South Central (Hyderabad) and South-East (Tamil Nadu, Kerala), respectively [Table/Fig-2]. No data was duplicated in this study.



[Table/Fig-2]: Geographic distribution of Yq microdeletions in Indian infertile man.

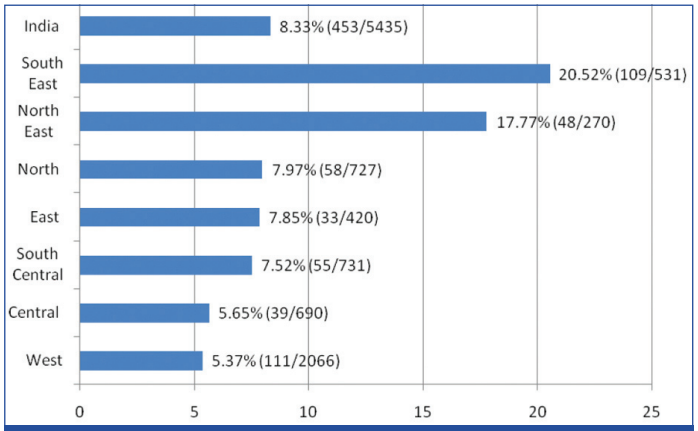
Statistics

The data of 30 group studies were added according to our need with simple statistics like percentage in this cohort. No duplicate of data was observed in present study, as it might lead to erroneous information.

RESULTS AND DISCUSSION

Zonal/Regional Deletion Analysis

In Gujarat Western part of India, screening of Yq microdeletion [43], reported 141 cases with oligospermia (41) and azoospermia (100) screen microdeletions. These authors in Anand (Gujarat) detected 24.11% (34/141) deletion with higher rate of AZFc deletions [43]. Analysis of oligospermia (3), azospermia (3), asthenospermia (2), cryptozoospermia (3), oligoasthenospermia (OAS) (4) and infertile normo-zoospermia (26) totally (41), revealed 7.31% (3/41) microdeletions [44]. In Mumbai and Nagpur, the cases were idiopathic type following the classification of World Health Organisation (WHO) [Table/Fig-3] [41] and their screening found 3.4% consisting of 56 from 1.636 infertile men, where AZFc subregion was higher in Mumbai, Maharashtra [36]. From Nagpur, Ambulkar PS and Pande SS obtained 10.6% deletion (17/160) from an analysis of oligozoospermia and azoospermia phenotypes with a dominant of AZFc followed by others [9]. A study conducted with 88 cases by Nagvenkar P et al., consisting of (42) oligospermia and (46) severe oligospermia detected only 1.1% microdeletions (1/88) with AZFc [44]. Thus, this Western region (Gujarat, Maharashtra) constituted only 5.37% (111/2066) YCM with AZFc dominance [Table/Fig-4].



[Table/Fig-3]: Regional zonal distribution of Yq % microdeletions in Indian population.

S. No.	Medical/Clinical term	Condition/Definition
1	Aspermia	Absence semen upon ejaculate
2	Zoospermia	Presence of spermatozoa in the semen
3	Azoospermia	A complete absence of sperm in the semen (both OA and NOA)
4	Normozoospermia	All normal semen values in ejaculate
5	Oligozoospermia	Presence of an abnormally low number of sperm in a semen (5-20×10^6/mL)
6	Severe Oligozoospermia	Sperm counts fall between 0 and 5 million sperm/mL
7	Asthenozoospermia	Reduced sperm motility
8	Oligoasthenozoospermia	Combination of reduced sperm motility and low sperm count
9	Teratozoospermia	Presence of higher abnormal morphology in the semen
10	Oligoasthenoteratozoospermia	Condition that includes low number, poor sperm movement and high abnormal forms
11	Polyzoospermia	Higher than normal values/mL
12	Hematospermia	Semen with Red Blood Cell (RBC)
13	Pyospermia	Semen with White Blood Cell (WBC)

[Table/Fig-4]: Semen variables according to WHO [41].
OA: Obstructive azoospermia; NOA: Non-obstructive azoospermia

In South Eastern (SE) region (Tamil Nadu, Kerala) six groups studied microdeletions. Abhilash VG et al., from Chennai had screened 89 cases with 34 azoospermia and 55 oligozoospermia cases for microdeletions [45]. They reported an average percent of 24.7% (22/89) consisting high frequency of AZFc. A study from Tamil Nadu, reported 72 semen samples of oligozoospermia (5), asthermo (7) and Oligoasthenozoospermia (OAS) (7), exhibited 12.9% deletions (19/72) having AZFc deletions at higher rate [46] (Sakthivel PJ and Swaminathan M, 2008). Viswambharan N, et al., documented 13.3% microdeletions (4/30) from their study cohort [47], Suganthi R et al., [48] from South Eastern Part of India described 36% deletions (18/50) in oligo and azoospermia infertile cases with higher frequency of AZFc

sub-locus. Previous studies by this group [49,50] in 215 and 75 cases documented 11.2% (24/215) and 29.3% (22/75) Yq microdeletions, respectively. Thus, this zone possessed 20.52% YCMs (109/531) [Table/Fig-3].

In South Central Zone of India (Hyderabad), the results depicted in a different pattern. The screening of 20 cases of 10 each of azoospermia and oligospermic men, the observed deletions in 3 cases was 15% (3/20), with AZFc dominance [51]. Another study [52] identified in 251 infertile men only 3.98% Yq microdeletions (10/251) with high percent of AZFc deletions. There were cases of 194 idiopathic infertily and 57 were of varicocele having zoospermia followed by oligozoospermia, Oligoasthenoteratozoospermia and teratozoospermia [50]. Swarna M et al., from Hyderabad mapped 4 cases from 50 infertile cases (4/50; 8%) and 9 cases with Yq deletions (9/70, 12.8%) containing 70 idiopathic infertile cases of various testicular phenotypic manifestations with high AZFc deletions and similarly [53,54], Thangaraj K et al., from South India (Hyderabad) also reported an analysis of 340 azoospermia, where 29 had microdeletion (8.5%) of AZFc >AZFb >AZFa [55]. Thus, South Central region had the frequency of 7.52% (55/731) deletions comparatively lower than South East region [Table/Fig-3].

In Eastern region (West Bengal), recent report indicated 5% microdeletions in a study cohort documented by Ray A et al., in 80 infertile men with 19 azoospermia and 61 oligozoospermia (4/80) where AZFc was dominated [56]. Sen S et al., reported in their article, the deletions marked were 8.5% (29/340) [36]. The total average of Yq in the region remained 7.85% (33/420) [Table/Fig-3]. In North East (NE), the results varied where Mahanta R et al., [57] screened only 100 infertile cases with 5% YCM (5/100) where AZFc was dominant. In a study of Barbhuiya PN et al., published 25.30% Yq deletions in Assam (43/170) [58]. These 170 infertile men had various types of semen. They reported high frequency of AZFa and c in inferstitial deletion of AZF locus. The total deletions were of 17.17% (48/270) [Table/Fig-3].

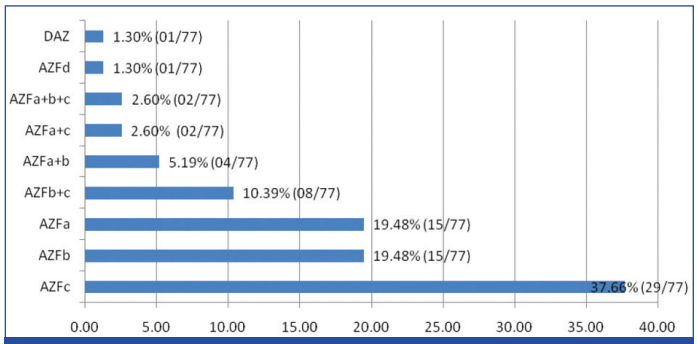
In Lucknow and Varanasi (Central Indian Zone), various reports are available contributing 5.65% YCMs (39/690) from various groups [Table/Fig-3]. Ambasudhan R et al., detected 5% microdeletions from 177 infertile men with various phenotype manifestations in Varanasi (9/177) with high frequency of c deletions [59]. Khan FH et al., in their cohort, 100 infertile cases consisting of azoo, oligo and asthenozoospermia had 10% Yq deletions (10/100) [60]. Mainly, they found AZFa and AZFc including b+c types. The study reported by Pandey LK et al., had 64 infertile men with 3.3% deletions (2/64), where AZFc was dominant in their semen types [61]. Similarly, Singh K and Raman R in their study obtained 4.8% (13/270) in azoo- and oligospermic men with infertility possessing of AZFc and AZFd deletions [62]. In Lucknow Mittal RD et al., in 79 infertile men, with oligo (25), azoospermia (54), screened for Y Chromosome Microdeletion (YCM), who reported 6.5%(5/79) with AZFc and AZFb deletions followed by b+c types [63].

In Northern Part of India (Delhi), Dada R et al., documented 6% deletions in a study having oligo and azoospermia (8/133) [64]. Earlier study by these researchers in 70 infertile cases with varicocele, 11.4% microdeletions was reported (8/70) [65]. Mitra A et al., in their study group containing 14 azoospermia with Klinefelter Syndrome (KFS) had 4 cases of AZFa and AZFc deletion (4/14; 28.6%) [66]. In another study with 119 azoo and 51 oligospermia cases had 5.29% microdeletions (9/170), spanning AZFc followed by b+c a and b deletions [67]. Recently, Dada R et al., analysed 140 cases of oligo-and azoo with varicocele and found 5.7% deletions (8/140) [68]. They observed AZFc and a+b are higher than b and b+c deletions. Sachdeva K et al., claimed that out of 200 infertile cases with spermatogenic failure at testicular level had deletions 10.5% (21/200) [69]. The protocol for identification of Y deletions in their cases was of Non Obstructive Azoospermic (NOA) and oligospermic men. Hence, in north zone, the average YCMs are 7.97% (58/727) having dominance of AZFc subregion [Table/Fig-3].

This mapping of Yq microdeletions in Indian studies of these 30 groups/citations indicated a range from 1.1 to 28.6% with an average frequency of 8.33% from total analysis of 5435 chromosomes (453/5435). It includes different groups, region and diverse classes of infertile men. Sub continent analysis of large databases has earlier revealed the percent of microdeletions ranged from 8-10% [70], in support of the observations. But reports presented by Sen S et al., had significantly lower frequencies (3.4%, 56/1636; 5.8% 215/3647) than the data (8.33%) [Table/Fig-3] [36].

AZF Locus Microdeletions

AZF microdeletions and semen phenotypic correlations are well appreciated in relation to infertility by several authors [1,17,37,71]. Overall 25-55% males with testicular pathologies like hypo spermatogenesis, sperm maturation arrest, Sertoli Cell Only Syndrome (SCOS) and about 5-25% males are affected by SOS or azoospermia harbouring YCMs as indicated earlier [32]. In this cohort more azoospermia cases (referred/selected 66%; affected 61%) in 30 and 15 study groups, respectively, than oligo-and others are attributed to AZFc locus [Table/Fig-2,3]. The sub-region microdeletions delivered in present study were AZFc (37.66%; 29/77), AZFa and AZFb (19.48%; each15/77), AZFb+c (10.39%; 8/77), AZFa+b (5.19%; 5/77), AZF a+c and AZFa+b+c (2.6%;each 2/77) and 1.3% each of AZFd and DAZ (1/7) respectively in a total of 77 deletions in present study [Table/Fig-5].



[Table/Fig-5]: Summary of percent (%) AZF sub region micro deletions (77) in our study.

AZFa Microdeletions and Phenotype Correlation

Microdeletions in this region contributed to 5%, as it contains only 4 single copies of genes [16,17]. Mostly these infertile cases have spermotogenic failure and restricted to SCOS [33,72,73]. Few workers believe it is related to oligospermia [33]. In present study, few study groups (7/30) mapped high rate of AZFa deletion and related to oligo/severe oligospermia. Thus, this region deletion depends on amount of genetic material deleted ranging from azoospermia to normozoospermia [74,75]. Present study review data thus had indicated low frequency of microdeletion contributing to testicular pathology [Table/Fig-6] as seven group had AZFa deletions at higher rate.

AZFb Deletion and Phenotypes

This region is also comparatively smaller than c region containing 3.2 Mb spanning few genes [76] that are single and multiple copies of DNA repeats and 14 amplicons and contributes about 16% deletions. This region is important for early stages of spermatogenesis and frequently responsible for hyperspermatogenesis [77]. In present

Sr. No.	Author	Phenotypes selected			AZF locus detected	Deleted/Total cases	Percent deletion (%)	Locations	Indian zones
		Azoo	Oligo/SOS	Others					
1	Nailwal M and Chauhan JB [43]	41	100	-	AZFc, AZFb, AZFa	34/141	24	Anand	Western
2	Sen S et al., [36]	992	600	44	AZFc, b+c, a, b, a+b, a+c, a+b+c	56/1636	3.4	Mumbai	
3	Shah PS et al., [42]	3	3	35	AZFc, AZFa	3/41	7.3	Ahmedabad	
4	Ambulkar PS and Pande SS [9]	90	70	-	AZFc, b+c, b, a, a+b	17/160	10.6	Nagpur	
5	Nagvenkar P et al., [44]	40	48	-	AZFc	1/88	1.1	Mumbai	
6	Babu SR et al., [51]	10	10	-	AZFc	3/20	15	Hyderabad	South central
7	Rao L et al., [52]	104	90	57	AZFc, a=b+	10/251	3.89	Hyderabad	
8	Swarna M et al., [54]	15	55	-	AZFc	9/70	12.8	Hyderabad	
9	Swarna M et al., [55]+++	10	40	-	AZFc	4/50	8	Hyderabad	
10	Thangaraj K et al., [55]	340	-	-	AZFc, b, a	29/340	8.5	Hyderabad	
11	Suganthi R et al., [48]	30	20	-	AZFc, b+c, a=b+	18/50	36	Tamil Nadu	South east
12	Suganthi R et al., [49]	215 infertile cases			AZFc, b, b+c, a+b+c, a+c, a	24/215	11.2	Tamil Nadu	
13	Suganthi R et al., [50]	75 infertile cases			-	22/75	29.3	Tamil Nadu	
14	Vishwambaran N et al., [47]	17	13	-	AZFc, a=b+	4/30	13.3	Tamil Nadu	
15	Sakthivel PJ and Swaminathan M [46]	72 infertile cases	AZFc, a	19/72			12.9	Coimbatore	
16	Abhilash VG et al. [45]	34	54	-	AZFc	22/89	24.7	Chennai	
17	Sen S et al., [36]	340 infertile cases			-	29/340	8.5	West Bengal	Eastern
18	Ray A et al., [56]	19	6	-	AZFc	4/80	5	West Bengal	
19	Mahanta R et al., [57]	100 infertile cases			AZFc	5/100	5	Assam	North east
20	Barbhuiya PN et al., [58]	50	82	38	AZFc, d	43/170	25.3	Assam	
21	Khan FH et al., [60]	100 infertile cases			AZFa, c, b+c	10/100	10.0	Varanasi	Central
22	Pandey LK et al., [61]	64 infertile cases			AZFc	2/64	3.48	Varanasi	
23	Singh K and Raman R [62]	270 infertile cases			AZFc, DAZ, AZFb	13/270	4.80	Varanasi	
24	Mittal RD et al., [63]	54	24	-	AZFc, b	5/79	6.3	Lucknow	
25	Ambasudhan R et al., [59]	142	33	2	AZFc	9/177	5.1	Varanasi	
26	Mitra A et al., [66]	14 infertile cases			AZFa, c	4/14	28.6	New Delhi	North zone
27	Mitra A et al., [67]	119	51	-	AZFc, a=b+, b+c	9/170	5.3	New Delhi	
28	Dada R et al., [65]	40	30	-	AZFc, b, a+b	8/70	11.4	New Delhi	
29	Dada R et al., [64]	133 infertile cases			AZFc, b, a, a+b	8/133	6	New Delhi	
30	Dada R et al., [68]	114	16	-	AZFc, a+b, b, b+c	8/140	5.7	New Delhi	
31	Sachdeva K et al., [69]	200 infertile cases			AZFc, a,b, b+c	21/200	10.5	New Delhi	
	Total (30)@				Deletion types	453/5435	8.33%		All zones (7)

[Table/Fig-6]: Summary of referred/screened phenotypes cases vs AZF locus with percent deletion in Indian population. @ Sr. No. 2 and 17: Same but Indian Zones are compared from this citation.
Deletion frequencies in study groups (30):AZFc*(27)>AZFb**(9)>AZFa***(7). Numbers in parenthesis indicate study groups in the [Table/Fig-3] (1-30); *:1-16, 18, 19, 20, 22-25, and 27-31 (27 study groups) **:1, 4, 10, 12, 23, 24, 28-30 (9 Study groups); ***: 2, 3, 15, 21, 26, 31 (6 Study groups); +=equal.: Total deletion=77; Referred/Selected cases (21 Groups); Azoospermia=66%(2264/3445);OS/SOS=30% (1045/3445); Others=4%(136/3445); ΨSOS: Severeoligozoospermia

review report, AZFb microdeletions exhibited high frequency of these deletions found relatively in 9 study groups (9/30) [Table/Fig-6]. Phenotypes range from spermatogenic anomalies and oligo- spermia/hypo-oligospermia [42,76]. The deletions hence in this AZFb, however had more than AZFa types in infertility cases with oligo to SOS [Table/Fig-6] [11,36].

AZFc Deletions and Phenotype Correlation

The AZFc region comprises of repeated sequences and palindromes making it suitable for deletions with partial types. Hence, these deletions correlated with testicular phenotypes ranging from severe oligospermia to azoospermia [27,78,79]. Patients having these deletions show progressive decline in sperm counts from oligo to severe and absence of sperm [80-83]. In our cited study groups (30) [Table/Fig-6], more (66%) infertile men were referred for screening and affected (61%) were azoospermia followed by oligospermia correlating with high frequency of AZFc deletions comparatively. Colaco S and Modi D; Sen S et al., and Shah PS et al., and several researchers also noticed high prevalence of AZFc microdeletions due to its complex structure [1,36,42]. Twenty seven study groups (27/30) delivered high rate of AZFc microdeletions amongst all 30 groups [Table/Fig-6].

Other Microdeletions and Phenotypes

Few study groups in our cohort reported AZFb+c, AZFa+b, AZFa+c, AZFa+b+c, AZFd and DAZ having other double and triple deletions that are related to hypospermetogenesis and other testicular pathologies. These deletions were low in frequency as compared to AZFc, a and b types and are depicted in [Table/Fig-6]. Ambulkar PS and Pande SS and Suganthi R et al., detected high rate of AZFc microdeletions comparing to abnormal semen types in comparison to a and b deletions [9,37]. Dada R et al., in their studies revealed high rate of AZF a+b over AZFb micro deletions [Table/Fig-6] [65,67].

Partial Microdeletions and Phenotypes

The AZFc has partial deletions viz b2/b4, b1/b3, b2/b3 and gr/gr, where b1/b3 combination of AZFb and AZFc regions in addition to CNV. Among, gr/qr the most common type that are due to homologous recombination. These partial microdeletions are highly variable in multiple studies reported and are controvertial [1,28]. Similarly few reports are documented regarding to CNVs [1]. In present report, these are not well screened by any group and require further elucidation.

Prevalence and Distribution of Yq Microdeletions in India

Overall distribution of microdeletion screening notified that AZFc region has high frequency as it is a complexed structure. Most of the testicular phenotype cases with azoospermia followed by oligospermia possessed deletion of AZFc only. It is overall followed by AZF region of a, b, b+c and others [Table/Fig-6] to support the data of previous authors [36,37] in Indian infertile men.

In our review cohort, further South East (20.52%), North East (17.77%), North (7.97%), East (7.85%) and South Central (7.52%) contained comparatively high frequency of Yq deletions followed by Western (5.37%) and Central (5.65%) regions, respectively. Thus these YCM are minimum in Western and Central Indian Zones [Table/Fig-2,3]. This could be due to geographic regions, ethnicity, sample size, population, food styles, STS-kits used and other epigenetic factors. Such results with other authors are also documented in Indian population in related to prevalence and distribution of Yq microdeletions [36,42].

IMPLICATIONS

Yq Microdeletions Analysis and Methodologies in Future

It is suggested that analysis of Yq microdeletions involve use of multiple STS markers spanning various AZF loci [37]. Authors used EAA and non-EAA markers of 4 to 30, but it is better to use a good number of markers to detect Yq microdeletions. Further, EAA and EMQN strongly recommend two, STS which is specific to DAZ gene in the P2 and P1 palindromes. Partial deletions cited to b2/b4 pattern can also be analysed using Sy 160 STS markers. Conventional PCR is to be upgraded to multiplex PCR, which is less costlier less time and advantageous than former [23]. Commercial kits such as Diachem/Bird, Euroclone are available [84]. Bunyan DJ et al., reported a method for the detection of partial AZFc deletions and Multiplex Ligation dependant Probe Amplification (MLPA) probe mix (P360) known as MLPA assay [85]. Microarray developed by Osborne EC et al., for microdeletions are also recommended for these studies with better reproducible results to infertile men [86].

Microdeletions and ARTs

Mapping of microdeletions of Yq in infertile men correlates to phenotype of testis which is a good predictor of sperm retrieval during Testicular Sperm Ejaculates (TESE). Microdeletions in infertile cases carry them to offspring born after ICSI. Further, testing of sperm for microdeletions, which are useful for sperm banks related to ARTs. Sperm carrying higher microdeletions may lead poor quality embryos [87] after using ICSI. Further, patients with AZFc microdeletions presented high sperm recovery from testis than cases with AZFa and AZFb deletions who presented a poor prognosis [88]. Simoni M et al.,; Nailwal M and Chauhan JB also supported azoospermic cases with AZFc deletions are better for ICSIs [15,75]. Genetic counselling is adapted to couples undergoing such ARTs. Moreover, prediction of prognosis of male infertility with Yq microdeletion is also important for recovery of testicular phenotypes. Testing of AZF region for deletions/CNVs may also be essential for detecting testicular tumours. Additionally, microdeletions causing infertility in males undergoing ARTs through genetic counselling are also strongly associated with neuropsychiatric disorders [1].

Y-Deletion and DNA Damage

DNA fragmentation increases with Oxidative Stress (OS) in a sperm cell. This OS occurs in sperm of infertile cases by several factors such as heavy metals, free radicals, caspases during apoptosis. Such induced OS upsets oxido-redox ratios leading to DNA damage and mutations. Hence, it is proved that DNA Fragmentation Index (DFI) shoots up in sperm of infertile men due to reduced recombination repair, DNA package anomaly [17,89]. Thus, OS is implicated for DNA damage inturn relating to Yq microdeletions in infertile cases. Hence, OS DNA damage and deletion in Y chromosome are related in infertile males [71,90-93], but such couples with affected males need to be treated with antioxidants and evaluated and such cases are also to be counselled prior to adopt ICSI and other IVF techniques in future [34,37,42,94,95]. [Table/Fig-7] shows semen types deleted vs deletion correlation in 15 groups of Indian population. Azoospermia cases (163/266=61%) were affected than others types.

Sr. No	Author	Semen types deleted			Deleted/Total
		Azoo	oligo/SOS	Others	
1	Sen S et al., [36]	34	22	-	56/1636 (3.4%)
2	Shah PS et al., [42]	1	2	-	3/41 (7.3%)
3	Suganthi R et al., [48]	10	8	-	18/50 (36%)
4	Barbhuiya PN et al., [58]	13	18	12	43/170 (28.3%)
5	Ambasudhan R et al., [59]	8	-	1	9/177 (5%)
6	Nagrvenkar P et al., [44]	1	-	-	1/88 (1.1%)
7	Nailwal M and Chauhan JB [43]	20	14	-	34/141 (24.11%)
8	Rao L et al., [52]	5	4	1	10/25 (3.89%)
9	Ambulkar PS and Pande SS [9]	10	7	-	17/160 (10.6%)
10	Thangaraj K et al., [55]	34	-	-	34/25 (8.5%)
11	Swarna M et al., [53]	2	1	1	4/80 (8%)
12	Swarna M et al.. [54]	4	1	4	9/70 (12.8%)
13	Dada R et al., [64]	7	1	-	8/133 (6.0%)
14	Dada R et al., [68]	6	2	-	8/140 (5.7%)
15	Athalye AS et al., [94]	8	3	1	12/100 (12%)
	Total (15 Groups)	163	83	20	266/3036 (8.76%)

[Table/Fig-7]: Semen types deleted vs deletion correlation in 15 groups of Indian population. Azoospermia cases (163/266=61%) were affected than others.

YCMs in Indian Scenario

Published data earlier showed the frequency of microdeletions in Indian population ranged from 3 to 29.34% with an average frequency of 8.1% [37,42,47,61,65]. Thangaraj K et al., proposed 8.5% YCMs from the study of 340 azoospermia infertile cases [55]. In another study, it is reported that it increased to 9.63% [65]. Only Sen S et al., [36] documented to 3.4% frequency of deletions in Indian population of 1636 cases, which is significantly lower than others. Same group had also mentioned low frequency 5.8% deletions, but present study cohort exhibited 8.33% deletions from 5435 Y chromosomes analysed supporting the data of Thangaraj K et al., Pandey LK et al., and Dada R et al., [55,61,64]. Recently, Shah PS et al., and Rao MV, also reported similar data falling in range of 8-10% [16,42] and Waseem AS et al., in India these deletions ranged from 0.59-32.62% with an average of 13.48% [95], but his own data delivered only 10.02% deletion frequency. This variation could be due to ethnic background study protocol and other factors. The Indian population further is affected by AZFc deletion followed by

AZFb and AZFa in most of the testicular phenotypes as reported by others [37]. The azoospermia cases thus considered to be mostly affected than oligo/severe oligospermia and others. The AZFc deletions containing such azoospermic cases are better suitable than other pathologic phenotypes as suggested above [75,88,95] for associated reproductive technologies in present and future of Indian Scenario.

Limitation(s)

Male infertility caused by genetic factor needs to be assessed with proper genetic tests. Significance of microdeletions/ partial deletions are to be communicated to couples. Accurate methodology is to be developed for full success rate of deletions. Congenital malformations, genetic defects, loss of birth weight and its propagation to offspring are to be limited successfully.

CONCLUSION(S)

This study cohort clearly enumerates the Yq microdeletion (8.33%) leading to male infertility mainly falling in the range of 8-10% in India. Correlation between these types of deletions and associated testicular phenotypes provide identification of infertility type, detecting the prognosis of infertile males, treatment and other defects like cancer. Azoospermic men are affected with high frequency of AZFc deletions in present study having more percent in Eastern region of India. Herewith, it also provides opportunities for counseling the couple adopting ARTs, where male partner carries these specific deletions. Y chromosome deletion analysis is also necessary in sperm banks to curtile multiple abnormal embryo yield after ICSI and TESI, since the prevalence and distribution, of these complete and partial deletions vary region wise, population groups, ethnicity, sample size, STS marker used, selection criteria, haplotypes, environment and other epigenetic factors.

Further, hereby these studies definitely take forward new direction to evaluate infertile males possessing Yq deletions for correct treatment clinically. These advancements allow more widespread of this deletion screening and its implication in infertility clinics, IVF and andrology laboratories in our country and also around the globe in future.

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