

ORIGINAL ARTICLE

Prevalence of Y chromosome microdeletion in azoospermia factor subregions among infertile men from West Bengal, India

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Abstract

Background: Etiology of male infertility is intriguing and Y chromosome microdeletion within azoospermia factor (AZF) sub-regions is considered major cause. We conducted a screening for Y chromosome microdeletion in an infertile male cohort from West Bengal, India to characterize Y chromosome microdeletion among infertile men.

Methods: We recruited case subjects that were categorized on the basis of sperm count as azoospermia (N = 63), severe oligozoospermia (N = 38), and oligozoospermia (N = 17) and compared them with age, demography, and ethnicity matched healthy proven fertile control males (N = 84). Sequence Tagged Site makers and polymerase chain reaction based profiling of Y chromosome was done for AZF region and SRY for cases and controls.

Results: We scored 16.1% of cases (19 out of 118) that bear one or more microdeletions in the studied loci and none among the controls. The aberrations were more frequent among azoospermic males (17 of 19) than in severe oligozoospermic subjects (2 of 19).

Conclusion: Our study provides the results of screening of the largest Bengali infertile men sample genotyped with the maximum number of STS markers spanning the entire length of Y chromosome long arm. Y chromosome microdeletion is a significant genetic etiology of infertility among Bengali men.

KEYWORDS

azoospermia, azoospermia factor, male infertility, severe oligozoospermia, Y-chromosome microdeletion

1 | INTRODUCTION

Infertility is a major challenge to reproductive health and the etiology involves both genetic and environmental risk factors. Approximately 15% of couples are infertile across the globe,

and the origin of problems is shared almost equally by both the sexes (Poongothai et al., 2009). The problem of male infertility remains unaddressed in Indian society owing to male domination and illogical male chauvinism. Low sperm count is a clinically established cause of infertility among Indian

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men and more than 90% of reported cases of male infertility have either been characterized by reduced concentration of sperm or inferior quality of spermatozoa present in the semen (Nailwal & Chauhan, 2017). The cause behind this pathological condition is heterogeneous that includes, but is not limited to, infection in genital regions, varicocele, immunological factors, genetic anomalies, and other idiopathic causes. Surprisingly, the cause of male factor infertility in more than half of the cases remains unknown. It could be congenital or acquired and can be categorized grossly on the basis of the poor quality of spermatozoa in the semen and unexplained infertility with normal semen parameters (Atia et al., 2015).

Spermatogenesis, which is a multistep process of sperm production in testes, is regulated by many Y chromosome-specific genes. Spermatogenic interruption results in the qualitative and quantitative reduction of sperm. Male with reported impairment of spermatogenesis are frequently diagnosed as the cases of either azoospermia or oligozoospermia (Suganthi et al., 2013). Majority of these genes are located on the long arm of the human Y chromosome in a specific region termed as the azoospermia factor region (AZF; Suganthi et al., 2014). Molecular characterization and genetic dissection of Yq11.23 have identified three distinct, non-overlapping subregions termed as AZFa, AZFb, and AZFc, each carrying candidate genes implicated in spermatogenesis and/or sperm maturation (Vogt et al., 1996). A new pattern of AZF deletion has been identified in a subsequent study where the distal region of AZFb overlaps with the proximal section of AZFc, termed as AZFd subregion (Kent-First et al., 1999).

Initially, Tiepolo and Zuffardi (1976) reported a strong correlation between microdeletions on the long arm of the human Y chromosome with abnormal spermatogenesis and quantitative reduction of sperm in semen sample (Tiepolo, & Zuffardi, 1976). The estimated prevalence of microdeletions is 12% in azoospermic, 3.4% in oligozoospermic, and 1%–7% among severely oligozoospermic men (Pieri et al., 2002; Shamsi et al., 2011). According to the position of microdeletion, the phenotypic manifestation of spermatogenic failure may vary among the patients. Infertile men carrying proximal deletions (AZFa and AZFb regions) exhibit drastic defects in spermatogenesis, with higher incidence of Sertoli cell-only syndrome (SCOS), whereas deletions of distal AZFb and AZFc regions result in residual spermatogenesis (Foresta et al., 2001; Hopps et al., 2003; Raicu et al., 2003). Although deletions of these subregions display variation in the severity of disruption in spermatogenesis, the functions of these three “loci” and their associated genotype–phenotype correlation are still unclear (Pieri et al., 2002).

As far published literature are concerned, the frequency of Yq microdeletions may vary among different ethnical populations across the globe. Surprisingly, the study on

Y chromosome microdeletion in the Bengali population is limited. The only study by Ray et al in 2014 (Ray et al., 2014) suggested the existence of microdeletion among the Bengali infertile men though the study suffered from smaller sample size as well as genotyping was performed using relatively few STS markers of the Yq. Another study (Thangaraj et al., 2003) on infertile male samples that included some Bengali men with idiopathic infertility reported the presence of Y-chromosome microdeletion, though the sample was heterogeneous and had individuals from different ethnic varieties from different parts of India. In the present study, we have analyzed azoospermic, oligozoospermic, and severely oligozoospermic infertile men from our local Bengali-speaking population of West Bengal (WB), and have screened the Y chromosome to estimate the frequency of microdeletion as an etiologic factor of male infertility. This data might be important to the clinicians of IVF clinics for designing management and treatment strategies for infertile men of West Bengal with a personalized approach.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

The study design was approved by the Institutional Ethics Committee constituted by the University of Calcutta, West Bengal, Kolkata, India. (Approval No. CU/BIOETHICS/HUMAN/2306/3044; Dated 04/12/2017). We have followed the guidelines as described in the “declaration of Helsinki” and the Indian Council of Medical Research (ICMR) for working with human subjects.

2.2 | Study subjects

We included a cohort of total of 118 infertile male patients (age ranged between 20 and 50 years with an average age of 35.9 ± 4.97 years) who were referred randomly to the Institute of reproductive medicine (IRM), Kolkata IVF clinic from March 2018 to December 2019. Simultaneously, the age-matched control group of 84 males (age ranging between 20 and 50 years with an average age of 34.9 ± 5.48 years), who are normozoospermic and fathered at least one child, was also included in this study. All the participants gave consent for donating tissue. Interview was taken for each subject in person to records epidemiological detail related to their personal, conjugal, and lifestyle in a pre-printed questionnaire. All the records were kept anonymously at the laboratory and highest confidentiality was maintained.

2.3 | Inclusion and exclusion criteria

The inclusion criteria were idiopathic infertile patients of age ranging between 20 to 50 years and having a history of inability to conceive after unprotected regular intercourse for one year at least, patients with primary infertility attending IVF clinics and male with abnormal seminogram. Male patients of age with less than 20 years and above 50 years, having proven chromosomal, hormonal, immunological, or other reasons of male infertility were excluded from this study.

2.4 | Morphological analysis of spermatozoa and categorization

The morphological analysis of the sperm was performed by “Easy Morpho Kit” (Cryo Lab International). Air-dried slides were observed under the light microscope fitted with a camera. The subjects stratified according to their seminal parameters into azoospermic (no spermatozoa in ejaculate), oligozoospermic ($<15 \times 10^6$ million spermatozoa/ml of ejaculate), severe oligozoospermic ($\leq 5 \times 10^6$ million spermatozoa/ml of ejaculate), and normozoospermic (normal values of sperms in the ejaculate) classified following the criteria laid in WHO manual 2010 criteria (World Health Organization, 2010). The spermogram revealed 63 were azoospermic, 38 were oligozoospermic, and 17 were severe oligozoospermic among the participating subjects. This stratification was performed blindly without knowing the outcome of the molecular analyses.

2.5 | Tissue sample collection

Blood samples from study patients as well as from normozoospermic fertile individuals were collected after donors consented. The laboratory technicians drawn blood through the venepuncture method into the 3ml of EDTA vacutainer tube (BD, USA).

2.6 | DNA extraction

Genomic DNA was extracted from blood samples using the Genomic DNA purification kit (Qiagen) according to the manufacturer's instructions. The quality of isolated DNA was analyzed through 0.8% agarose gel electrophoresis. The quantity of the DNA was estimated by NanoDrop (Implen).

2.7 | Y chromosome microdeletion analysis by PCR

The Y chromosome-specific Sequence Tagged Sites (STSs) were used to design AZF region-specific primers. A set of 23 markers (Table 1) was used to cover the entire genomic segment of interest to characterize the microdeletion. All case and control subjects were genotyped with all the markers arranged contiguously from 5' to 3'. We confirmed the size of the PCR products of particular STS markers through the genome browser of the University of California, Santa Cruz (UCSC) and male-specific region of the Human Y chromosome (MSY) breakpoint mapper. The STS markers in the present study were selected according to the European Academy of Andrology (EAA) and the European Molecular Genetics Quality Network (EMQN) guidelines (Krausz et al., 2014) for diagnostic testing and most recent works. Apart from classical YCMD (involving typical AZFa, b, and c) markers two other markers, namely, sY152 and sY153 were used to screen another region termed as AZFd (overlapping region of AZFb and AZFc) were used (Kent-First et al., 1999). The segment-specific markers are given as follows:

1. Diagnosis of AZFa deletions: The most commonly used gene specific STS markers to detect and study the extension of AZFa deletions are *DFFRY*, *DBY*, sY83, sY86, sY121, and sY182.
2. Diagnosis of AZFb deletions: The STS markers, sY127 and sY134 are routinely employed for the primary detection of AZFb deletion and sY34, sY143, sY142, sY124, and sY130 are used for the extension analysis.
3. Diagnosis of AZFc deletions: The *DAZ* gene (OMIM: 400003; <https://www.ncbi.nlm.nih.gov/gene/1617>) specific STS markers sY254 and sY255, are frequently used for the preliminary screening of AZFc deletions. STS markers sY239, sY242, sY1197, sY1191, and sY1291 helped in identifying the extension of the deletion.
4. Diagnosis of AZFd deletions: Two important STS primers which are commonly used for the detection of this particular region is sY152 and sY153.
5. Internal control: sY14 representing the sex-determining region on Y-chromosome (*SRY*).
6. We used DNA samples of euploid female as a negative control and that of proven fertile male as a positive control. DNA contamination was checked by nuclease-free water (Promega) as a blank in the place of genomic DNA. Total 10 μ l of PCR reaction mixture consisted of PCR master mix (GoTaq Green Master Mix, Promega), STS marker-specific forward and reverse primers (Integrated DNA Technologies), genomic DNA template (with a concentration of 25 ng) and nuclease-free water (Promega).

TABLE 1 Sequence-tagged sites (STSs) primers details used in the study

Region	STS	Primer sequence	Annealing temperature (°C)	product size
SRY	sY14 (internal control)	F-GAATATTCCTCCGCTCTCCGGA R-GCTGGTGCTCCATTCTTGAG	57	470 bp
AZFa	DFFRY	F-GAGCCCATCTTTGTCAGTTTAC R-CTGCCAATTTCCACATCAACC	56	111 bp
	DBY	F-AGTTTATTCTAACCTAGG CAAACG R-TCCAACCAGGCCTGTAGTGAGGCC	62	165 bp
	sY83	F-CTTGAATCAAAGAAGGCCCT R-CAATTTGGT TTGGCTGACAT	54	277 bp
	sY86	F-GTGACACACAGACTATGCTTC R-ACACACAGAGGGACAACCCT	59	318 bp
	sY121	F-AGTTCACAGAAATGGAGCCTG R-CTGTGACTCCAGTTTGGTC	55	189 bp
	sY182	F-TCAGAAGTAAAACCCTGTATG R-GCATGTGACTCAAAGTATAAGC	53	125 bp
AZFb	sY34	F-GTCTGCCTCACCATAAAAACG R-ACCACTGCCAAAACCTTCAA	55	303 bp
	sY124	F-CAGGCAGGACAGCTTAAAAG R-ACTGTGGCAAAGTTGCTTTC	55	109 bp
	sY127	F-GGCTCACAACGAAAAGAAAA R-CTGCAGGCAGTAATAAGGGA	55	274 bp
	sY130	F-ATAAGATTCTGGGTCTCTTCC R-AGCCCACTTGTGGACTTTATC	54	286 bp
	sY134	F-GTCTGCCTCACCATAAAAACG R-ACCACTGCCAAAACCTTCAA	56	303 bp
	sY142	F-AGCTTCTATTGAGGGCTTC R-CTCTGCAATCCCTGACA	55	196 bp
	sY143	F-GCAGGATGAGAAGCAGGTAG R-CCGTGTGCTGGAGACTAATC	53	311 bp
	AZFc	sY254	F-GGGTGTACCAGAAGGCAAA R-GAACCGTATCTACCAAAGCAGC	55
sY255		F-GTTACAGGATTCGGCGTGAT R-CTCGTCATGTGCAGCCAC	56	124 bp
sY239		F-CATTCATCTTCCCTTTTGAAGG R-ATGCAAGTCGCAGGAAATCT	54	200 bp
sY242		F-ACACAGTAGCAGCGGGAGTT R-TCTGCCACTAACTGTAAGCTCC	59	234 bp
sY1197		F-TCATTTGTGTCCTTCTCTTGGA R-CTAAGCCAGGAACTTGCCAC	55	453 bp
sY1191		F-CCAGACGTTCTACCTTTTCG R-GAGCCGAGATCCAGTTACCA	59	385 bp
sY1291		F-TAAAAGGCAGAAGTCCAGG R-GGGAGAAAAGTTCTGCAACG	59	527 bp
AZFd	sY152	F-AAGACAGTCTGCCATGTTTCA R-ACAGGAGGGTACTTAGCAGT	56	125 bp
	sY153	F-GCATCCTCATTTTATGTCCA R-CAACCCAAAAGCACTGAGTA	54	139 bp

Abbreviation: bp, base pair.

Amplification was performed in the thermal cycler (Applied Biosystems by Thermo-Fischer Scientific) using reaction condition for each cycle as 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s (annealing temperature given in Table 1) and extension at 72°C for 30 s. The final extension was carried out at 72°C for 5 min followed by cooling at 4°C. Amplified PCR products were separated on 2% agarose gel (Sisco Research Laboratories Pvt. Ltd.) at 90V and 400mA current for 1 hr. The results were visualized and documented by Gel Doc EZ imager (Bio-Rad). Samples with deletions were repeated at least thrice to confirm the deletion of any given marker.

2.8 | Data analysis

The histopathological outcome of the semen samples and the results of molecular analyses were put together to obtain the final outcome of the study. As the control subjects did not exhibit any deletion, we did not perform any statistical test to measure the difference between case and control. Only the frequency of different types of deletion was estimated among the cases.

3 | RESULTS

In the present study, we have genotyped a cohort of 118 idiopathic infertile male patients, which were further stratified into azoospermic, (63/118; 53.39%), oligozoospermic (38/118; 32.2%), and severe oligozoospermic (17/118; 14.41%), according to the histopathological quality of their semen samples. Further, we screened all the subjects for Yq microdeletion and compared them with 84 normal fertile men which were tested as a control. Total 19 patients of 118 tested (17 azoospermic and 2 severe oligozoospermic) showed microdeletions of one or more specific STS markers corresponding to AZF locus which accounts for 16.1% (19/118; Table 2) of total infertile men of the sample cohort. The frequencies of AZF microdeletions in the entire study group are displayed in Table 2. Two patients were found with AZFc+d microdeletion and only one patient was with AZFb+c microdeletion comprising 1.69% (2/118) and 0.85%

(1/118) frequencies, respectively (Table 4). In normal fertile women which were taken as a negative control, STS failed to amplify. The highest percentage of deletion was among the AZFc region (11/118; 9.32%), followed by the AZFa (5/118; 4.24%) while only 2.54% (3/118) of deletions were found in both AZFb and AZFd (Table 4).

The spatial pattern and distribution of microdeletions among the case subjects are represented in Figure 1. We did not find any microdeletions among oligozoospermic patients in our studied cohort. All the deletions were detected in the AZF region of the Y chromosome, while no deletions were seen in the SRY region. The distribution of the identified microdeletions and their frequencies in AZF subregions among the azoospermic (n = 63) and severe oligozoospermic (n = 17) subjects are shown in Table 3. Moreover, we did not find any deletions among the 84 control males.

4 | DISCUSSION

There are several fragile sites in the AZF subregions that are prone to small sub-microscopic deletions in the proximal Yq possibly due to errors in self-recombination. They are known as Y chromosome microdeletions (YCMD) and involves entire or partial removal of the AZF subregions (Colaco & Modi, 2019). It has been well established in earlier studies that Y chromosome microdeletion and defective spermatogenesis are closely associated (Krausz et al., 2014; Krausz & Riera-Escamilla, 2018; O'Flynn O'Brien et al., 2010). The Y-chromosome microdeletions are frequently diagnosed in a variable frequency among infertile men of different ethnicities worldwide. Data analysis has reported the presence of nearly 7.5% microdeletions from 40,000 human Y chromosomes (Colaco & Modi, 2019) among infertile males across the globe with some demographic, cultural, and ethnic variations. Thus, as a part of pre-treatment investigations, it is important to screen YCMD among men with idiopathic infertility (Raicu et al., 2003). Generally, patient's age and duration of infertility are among the decision-making factors to investigate, treat, and establish a reproductive prognosis (Ambulkar et al., 2014). Since the last two decades, incidences of YCMD in the Indian populations have been reported in several studies. It is stated that the variation in the frequency

TABLE 2 Frequencies of AZF microdeletions in the entire study population

Category of spermatogenic defect	Total patients screened	Sample studied	No. of STS markers used	Patients with microdeletions	Frequency of microdeletions (%)
Azoospermia	63	Blood	23	17	26.98
Oligozoospermia	38	Blood	23	0	—
Severe oligozoospermia	17	Blood	23	2	11.76
Normozoospermia	84	Blood	23	0	—
Total	202	—	23	19	16.1

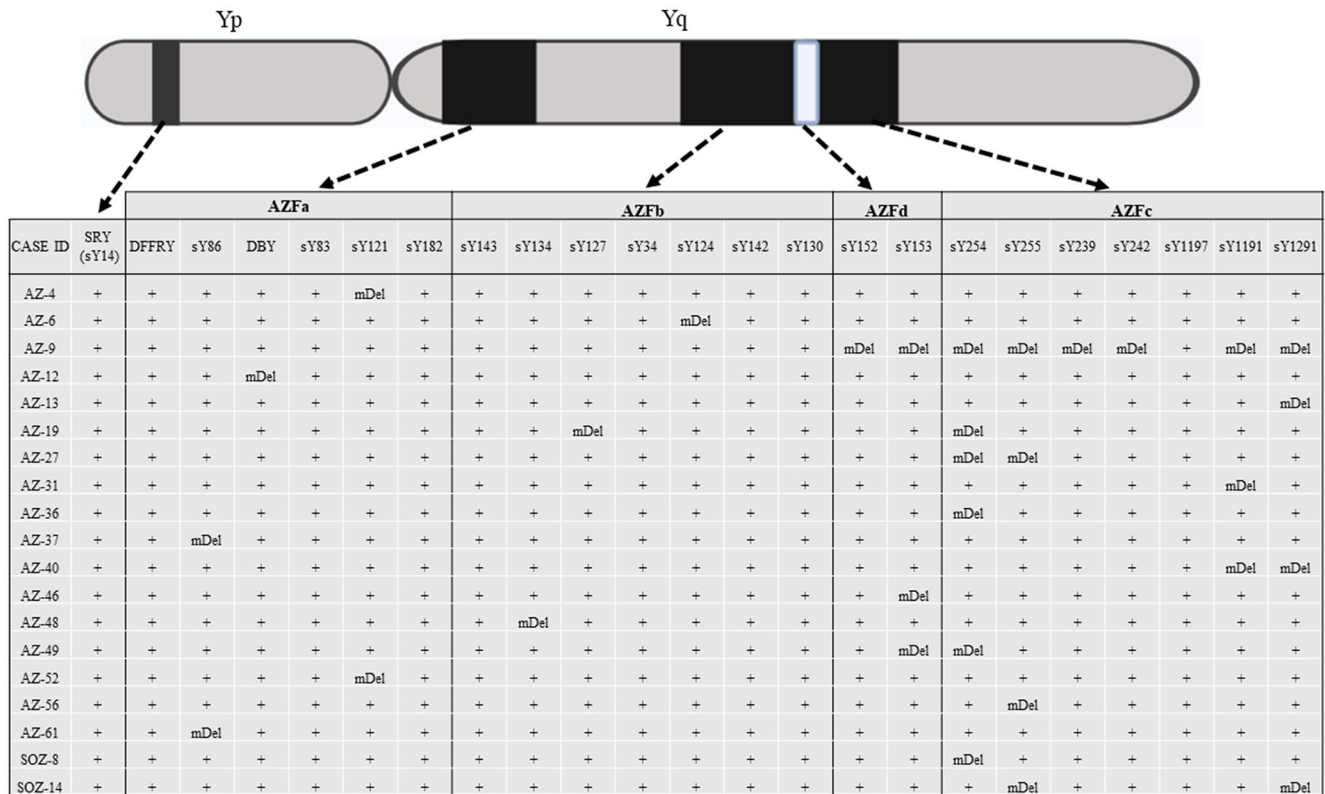


FIGURE 1 A schematic representation of the Y chromosome showing the AZF regions along with sequence-tagged sites (STS), and the Yq microdeletions pattern found in the study population. Positive PCR results for particular STSs are indicated by “+,” absence is shown in “mDel” which indicates microdeletion. The column on the left shows the number of patients in this study with the deletion pattern identified. AZ, azoospermic, SOZ, severe oligozoospermia

TABLE 3 Frequency distribution of AZF subregions of azoospermic and severe oligozoospermic males in the study population

Group	AZF subregions			
	AZFa	AZFb	AZFc	AZFd
Azoospermia (n = 63)	5 (7.94%)	3 (4.76%)	9 (14.29%)	3 (4.76%)
Severe oligozoospermia (n = 17)	0 (0%)	0 (0%)	2 (11.76%)	0 (0%)
Normozoospermia (n = 84)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

TABLE 4 Frequency of microdeletions of total infertile and fertile men within different AZF subregions.

Group	AZFa	AZFb	AZFc	AZFd	AZFb+c	AZFc+d
Infertile (n = 118)	5/118 (4.24%)	3/118 (2.54%)	11/118 (9.32%)	3/118 (2.54%)	1/118 (0.85%)	2/118 (1.69%)
Fertile (n = 84)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

of these micro-deletions is mostly because of differences in the study protocol and ethnic background (Suganthi et al., 2014). A meta-analysis study based on the YCMD frequency in the Indian population revealed the presence of AZF deletions in 10.02% of azoospermic and oligozoospermic men with the maximum frequency of AZFc deletions. Deletion frequency from 0.59% to 32.62% (average = 13.48%) has

been reported in a pooled analysis (Waseem et al., 2020). A large-scale study on the Indian population reported 3.4% among 1636 of infertile men had Yq microdeletions (Sen et al., 2013). Another very cryptic type of microdeletion called the “partial AZFc deletion” or AZFc sub-deletions has been reported in few studies (Repping et al., 2003). The partial AZFc deletions do not result in complete removal of the

AZFc locus but the reduction in the copy number of the genes within it (Colaco & Modi, 2019).

In the present study, we observed microdeletions in AZFa, AZFb, AZFc, and AZFd sub-regions of the Yq chromosome among the 19 karyotypically normal patients, and the overall frequency of the microdeletion in our study cohort is ~16.1%. Among the deletion positive cases, 17 (17/63; 26.98%) patients were azoospermic and 2 (2/17; 11.76%) patients were severe oligozoospermic (Table 2). We found no such microdeletions among oligozoospermic patients and control males. Region-specific microdeletion frequency among azoospermic and severe oligozoospermic patients is shown in Table 3 and Figure 1 and the frequency of microdeletions (n = 118) within different AZF subregions is represented in Table 4.

In our study, among azoospermic individuals, the AZFc region is showing 14.29% (9/63) of deletions involving nine patients followed by AZFa (5/63; 7.94%) with five patients then AZFb and AZFd subregion both with three patients (3/63; 4.76%). In contrast, two severe oligozoospermic patients [SOZ-8 and SOZ-14] showed deletion in AZFc subregion only (2/17; 11.76%; Table 3). Deletion of the AZFc region was detected altogether among 11 patients (9 azoospermic+2 severe oligozoospermic), which represents 9.32% (11/118) of total infertile males. Microdeletions in the AZFa region were detected among five (5/118; 4.24%) infertile males, AZFb region was detected in three (3/118; 2.54%) infertile males, and AZFd region was detected in three (3/118; 2.54%) when all the deletion positive men were considered. We observed highest frequency in AZFc type of deletions, which is concordant with the findings of other studies (Nailwal & Chauhan, 2017; Sen et al., 2013; Waseem et al., 2020). In comparison to AZFa and AZFb, the occurrence of several repetitive sequences in the AZFc region may be responsible for such observation (Suganthi et al., 2013). Deletion of AZFc+d and AZFb+c regions was detected in two [AZ-9 and AZ-49] (2/118; 1.69%) and one [AZ-19] (1/118; 0.85%) infertile males, respectively, who were azoospermic. The present study showed the frequency of microdeletion in AZFa, AZFb, and AZFd are lesser than that of the AZFc region.

Among all tested STS makers that we used for microdeletions screening, the STS sY254 & sY255 have been identified as most informative and these two cover the *DAZ* (Deleted in Azoospermia) gene. *DAZ*, a gene with multiple copy numbers containing several palindromic repetitive sequences in AZFc region, whose reduction of copy number enhances the risk of male infertility (Ambulkar & Pande, 2017). In the earlier study, it was evident that the deletions of the *DAZ* gene marked by sY254 and sY255 are co-incidental (Krausz et al., 2014). In contrast, some other later studies suggested that the deletions of segments covered by sY254 and sY255 are mutually exclusive (Batiha et al., 2018; Nailwal & Chauhan,

2017; Wang et al., 2010). In our analysis, we obtained deletion covering both the sY254 and sY255 among two azoospermic cases [AZ-9 and AZ-27]. Moreover, there are cases in our samples that exhibited alternate deletions too. Our results support the mutually exclusive occurrence of these two microdeletions. Azoospermic or severe oligozoospermic condition can be inflicted due to complete deletions of AZFa and AZFb of the Y chromosome (Vogt et al., 1996). Studies revealed that microdeletions limited to the AZFa region usually cause Sertoli cell-only syndrome (SCOS) and azoospermia in the patients whereas complete deletions of AZFb and AZFb+c are characterized by either SCOS or azoospermia owing to spermatogenetic arrest. Several reports claimed similarity between patients with complete AZFa region deletions with those having no spermatozoa upon attempts of testicular sperm extraction (TESE; Krausz et al., 2000, 2014; Vogt et al., 1996). In general, deletions of AZFc regions cause residual spermatogenesis which might also occur in men with severe oligozoospermia. Azoospermic men with AZFc deletion bear nearly 50% chance of spermatozoa retrieval through TESE and children can be conceived via intracytoplasmic sperm injection (ICSI; Kent-First et al., 1999; Krausz et al., 2014). Patients with AZFd microdeletions may either show mild oligozoospermia or normal sperm counts coupled with atypical sperm morphology (Kent-First et al., 1999). In our study, patients with AZFd deletion detected were all azoospermic [AZ-9, AZ-46, and AZ-49]. One azoospermic male patient AZ-9 was identified with AZFc+d large deletions with every STS marker (except sY1197) for the said region involved in our study.

When we compared the outcome of our present study with other published reports, we observed population and ethnicity-specific distribution of different Y chromosome microdeletions. While, the average frequency of microdeletions in South European population (Italy) is nearly 15%, the frequency of YCMD in North European population (France, Netherlands etc.) appeared to be comparatively less (1%–4%; Ambulkar et al., 2014; Foresta et al., 2001). In Spain, the estimated frequency was 3.3% as reported by Giacco et al (Lo Giacco et al., 2014). It was 9.4% among male infertile patients from the United States as reported by Stahl et al. (2010). The data recorded in Turkey and Jordan revealed frequencies as 3.3% (Sargin et al., 2004) and 4.93% (Batiha et al., 2018), whereas from two other Middle East countries Syria and Saudi Arab reported frequency of 28.4% (Al-Achkar et al., 2013) and 22% (Atia et al., 2015), respectively. Among the Asian populations, the study on the Chinese population by Liu et al. reported a frequency of 16.9% (Liu et al., 2016) in infertile male patients which is consistent with our study. Moreover, a study by Tse et al. (2000), Wang et al. (2010), Zhang et al. (2014) reported the frequency of 6.9%, 9.2%, and 8.70% respectively in infertile males among their study population in the provinces of China (Tse et al., 2000; Wang

et al., 2010; Zhang et al., 2014). The frequency of Y chromosome microdeletions among infertile males from Sri Lanka is only 1.5% (Wettasinghe et al., 2012) and in Pakistani infertile men, it is about 3.54% (Tabassum Siddiqui et al., 2013).

In the Indian cohort, the earliest report on Yq microdeletion estimates a frequency of 15% among 20 patients from the South Indian population (Babu et al., 2002). Since then, similar studies on Indian sub-population have reported frequency of 12.9% from a sample population of Tamil Nadu (Sakthivel & Swaminathan, 2008), 36% among 50 men from South India (Suganthi et al., 2013), 5% and 6.3% among North Indian male patients (Ambasudhan et al., 2003; Mittal et al., 2004) 24.11% among Gujarati males (Western India; Nailwal & Chauhan, 2017) and 3.9% among idiopathic infertile men from Maharashtra (Pande et al., 2018). Another study claims 8.5% Yq microdeletions among 340 azoospermic Indian men subjected to analysis (Thangaraj et al., 2003). Thorough and consecutive studies from Central India in the years 2014, 2015, and 2017 reported the frequency of Yq microdeletion as 8.33%, 12.8%, and 10.6%, respectively (Ambulkar et al., 2014; Ambulkar et al., 2015; Ambulkar & Pande, 2017). As stated by the previous studies, AZFc appears to be the region most susceptible to deletion in azoospermic or oligozoospermic men. To our best knowledge, there are no previous reports of the East Indian male population being subjected to such screening, thus this is perhaps the first report from a population of West Bengal that documents the prevalence of 16.1% AZF microdeletions which is in accordance with the previously reported results based on Indian population. As stated above, microdeletions were restricted in the azoospermic ($n = 63$) and severe oligozoospermic ($n = 17$) infertile male patients while we did not find any such deletion in oligozoospermic ($n = 38$) male patients, which is consistent with the findings reported by Sen et al. (2013). Variations in the Yq microdeletion frequency as stated in the reports from India may be due to fluctuation in sample size, selection bias of subjects, different diagnostic methods, choice of STS primers used in respective studies, different demographic, and environmental factors.

In conclusion, our findings of Y chromosome microdeletions (YCMD) restricted to AZF a, b, c, and d sub-regions of azoospermic and severe oligozoospermic infertile groups validates the essential involvement of genes located in these subregions for the spermatogenic regulation in one way or the other. This comprehensive, sensitive, and cost-effective PCR-based screening of YCMD must be recommended with utter importance for diagnostic as well as therapeutic mileages prior to the employment of suitable assisted reproductive techniques (ART). Our study reports the outcome of the screening of YCMD of Bengali men using dense STS markers on Yq and estimated the frequency of microdeletions in this population sample and have characterized the population-specific pattern of distribution of AZF aberrations which need confirmation by further study with larger sample size.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

SG conceived and designed the study. SD performed all the experiments, analyzed the data, and wrote the manuscript, PP and SP helped in experimentation, GB, RC, and BC cared for the patients, helped in sample collection, and acquired the clinical data, PG reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All datasets generated and analyzed for this study are included in this article.

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