

Gene Scanning for Microdeletions in the Azoospermia Factor Region of Y-Chromosome in Infertile Men of Gujarat, India

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ABSTRACT

Introduction: Azoospermia Factor (AZF) microdeletions in Yq chromosome is one of the most frequent genetic cause associated with failure of spermatogenesis in males with infertility.

Aim: To figure out the Yq chromosome microdeletions frequency in infertile men from Gujarat region of India.

Materials and Methods: In this study, 141 infertile men with azoospermia (n=41) and oligozoospermia (n=100) were examined along with 159 normozoospermic men. Eleven different markers spanning the azoospermia factor region of human Yq chromosome, amplified by sequence-tagged site Polymerase

Chain Reaction (PCR) to detect the microdeletions. Sperm morphological analysis was done using papanicolau staining method.

Results: Thirty four infertile men out of 141 presented Yq chromosome microdeletions. The frequency of AZF microdeletions was 31.71% in azoospermia and 21% in oligozoospermia patients. Only two oligozoospermia patients showed morphological defects.

Conclusion: Due to the presence of high frequency of Yq chromosome microdeletions in Gujarati infertile men, it is imperative to implement the AZF microdeletion screening in such patients as it results in male spermatogenesis dysfunctioning.

Keywords: Male infertility, Sequence-tagged site polymerase chain reaction, Yq chromosome

INTRODUCTION

Infertility in couples is about 15%-20% and can be due to female, male or combined factors. Male factor infertility contributes approximately half of the cases and around 90% or above are characterized by low sperm concentration or by poor quality of spermatozoa in semen [1,2]. Sperms in human are produced by synergy of genes and proteins, through a complicated process called spermatogenesis [3]. Human Y-chromosome is a sex determining chromosome and required for maintenance and development of male germ cells [4,5].

Earlier in 1976, Tiepolo L and Zuffardi O established the strong association between Yq chromosome microdeletions with abnormal spermatogenesis which has given a new insight in the study of Y chromosome long arm for identification and characterization of genetic grounds in spermatogenesis process [6]. After this, Bardoni and colleagues identified the human spermatogenesis locus at Yq 11.23 interval [7]. In 1996, Vogt PH et al., had given evidence that three sub regions (AZFa, AZFb and AZFc) in the AZF associated to specific spermatogenic disruptions [8]. Yq chromosome microdeletions are the second most important genetic cause for spermatogenic arrest in male with infertility after Klinefelter syndrome [9]. AZF region in Yq chromosome contain genes which are involved in spermatogenesis and microdeletions in AZF region leads to spermatogenic defects [10].

Published data showed that there is paucity of AZF microdeletions data in the Gujarati cohort, it is important to have such data as AZF sub regions microdeletions are correlated with spermatogenic failure. Therefore, present study aimed to screen and identifies the Yq chromosome microdeletions in AZF region using PCR as microdeletions cannot be detected by conventional cytogenetic techniques.

MATERIALS AND METHODS

Study Population

The molecular genetic study was conducted using total 300 patients from Gujarat with oligozoospermia, azoospermia and

normozoospermia classified according to the WHO manual 2010 criteria [11], along with two fertile female attending the infertility clinics were incorporated in this study. The study was conducted at Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, Gujarat, India, during the period of 2014 -2016. Among the 300 patients, 41 were azoospermic, 100 were oligozoospermic and 159 were normozoospermic. The study was approved by the Institutional Ethical Committee of the S.G. Patel Ayurveda Hospital and Maternity Home (Approval No. IEC-3/GJPIASR/2015-16/E/4), Gujarat, India. Written informed consent was signed and collected from all the patients. The study protocols described were in consent as per ICMR human ethical guidelines with respect to human.

The inclusion criteria for our study were oligozoospermia and azoospermia patients aged between 22 to 45 years, couple with infertility having inability to conceive after an unprotected regular intercourse at least for one year, patients with primary infertility attending IVF clinics and male with abnormal seminogram, whereas males with less than 22 years and above 45 years of age, patients with proven immunological, hormonal, chromosomal or other causes of male infertility were excluded from this study.

Morphological Analysis of Spermatozoa

The sperm morphological analysis was carried out by using rapid papanicolau staining kit (Biolab diagnostics, Mumbai). The slides were air dried and observed in light microscope with camera.

DNA Isolation

Semen samples from oligozoospermic and normozoospermic men whereas blood samples from azoospermic patients were collected for AZF microdeletion screening. All the patients instructed through laboratory technicians to follow guidelines for semen sample collection [11] whereas azoospermic blood samples were collected in the EDTA vacutainer tube. Isolation of genomic DNA from the peripheral blood cells and semen samples were carried out using phenol: chloroform method. Qualitative analysis of isolated DNA

was performed in 0.8% agarose gel electrophoresis. Quantitative analysis of DNA was carried out by Nanodrop.

Microdeletions Analysis by PCR

Eleven sets of Yq chromosome specific Sequence Tagged Sited (STS) markers were utilized to screen all the three sub-regions of AZF locus using PCR techniques. These STS-primer sets were considered as per European Academy of Andrology, EAA [12]. The following STS markers were used:

AZFa: sY84 and sY86

AZFb: sY121, sY127 and sY134

AZFc: sY153, sY254, sY255, sY1191, sY1197 and sY1291

Internal control: sY14 (SRY)

Independent PCR reactions were designed for all the STS markers for the analysis of microdeletions in AZF region of Yq chromosome. Normal female DNA and reaction mixture with deionized nuclease free water instead of DNA added as negative controls, whereas normal fertile males were used as positive controls. The reaction mixture of PCR was of 25 µL total, which consist of 3 µL genomic DNA templates with concentration of 100 ng and PCR master mix (XcelGen- Premix Taq Version 2.0), nuclease free sterile distilled

water, Dimethyl Sulfoxide (DMSO) and STS markers specific primers (Sigma-Aldrich). Amplification of reaction mixture was performed in Eppendorf thermal cycler with initial denaturation at 94°C for four minutes followed by 34 cycles of denaturation at 94°C for 30 seconds, annealing varying according to STS markers [Table/Fig-1] and extension at 72°C for 40 seconds. The final extension was carried out at 72°C for three minutes followed by cooling at 4°C. PCR products were separated on 2% agarose gel prepared using Tris-acetate-EDTA (TAE) buffer and electrophorized at 100 mA for one hour. Ethidium Bromide (EtBr) stained gel was subjected to UV transilluminator and photographed.

RESULTS

Morphological Analysis of Spermatozoa

In the present study, 300 semen (azoospermia, oligozoospermia and normozoospermia) samples analyzed for morphological defect. Different types of sperm defects were observed which includes head, mid-piece, tail and mixed defects. In this study, only two oligozoospermia samples (Sample O89 and O91) showed complete (100%) morphological defect.

AZF sub regions	STS markers	Chromosomal Location	Gene Bank Accession no.	Primer sequence (5'-3')	Annealing temperature (°C)	PCR product size
SRY (Internal Control)	sY14	Yp11.3	G38356	F- GAATATTCCTCCGCTCCGGA R- GCTGGTGCTCCATTCTTGAG	56	472 bp
AZFa	sY84	Yq11.221	G12019	F-AGAAGGGTCTGAAAGCAGGT R-GCCTACTACCTGGAGGCTTC	57	327 bp
	sY86	Yq11.221	G49207	F-GTGACACACAGACTATGCTTC R-ACACACAGAGGGACAACCCT	57	320 bp
AZFb	sY121	Yq11.222	G38341	F-AGTTCACAGAATGGAGCCTG R-CCTGTGACTCCAGTTTGGTC	61	190 bp
	sY127	Yq11.223	G11998	F-GGCTCACAACGAAAAGAAA R-CTGCAGGCAGTAATAAGGGA	56	274 bp
	sY134	Yq11.223	G12001	F-GTCTGCCTCACCATAAAACG R-ACCCACTGCCAAAACCTTCAA	56	301 bp
AZFc	sY153	Yq11.223	G12004	F- GCATCTCATTTTATGTCCA R- CAACCCAAAAGCACTGAGTA	60	139 bp
	sY254	Yq11.223	G38349	F- GGGTGTACCAGAAGGCAAA R- GAACCGTATCTACCAAAGCAGC	60	380 bp
	sY255	Yq11.223	G65827.1	F- GTTACAGGATTCGGCGTGAT R- CTCGTCATGTGCAGCCAC	56	124 bp
	sY1191	Yq11.223	G73809	F- CCAGACGTTCTACCCCTTCG R- GAGCCGAGATCCAGTTACCA	58	385 bp
	sY1197	Yq11.223	G67168	F- TCATTGTGTCTCTCTTGGGA R- CTAAGCCAGGAACCTGCCAC	58	453 bp
	sY1291	Yq11.223	G72340	F- TAAAAGGCAGAAGTCTGCAACG R- GGGAGAAAAGTCTGCAACG	61	527 bp

[Table/Fig-1]: Sequence-tagged sites primers details.

Patients	AZFa		AZFb			AZFc					
	sY84	sY86	sY121	sY127	sY134	sY153	sY254	sY255	sY1191	sY1197	sY1291
A2	MDel	MDel	-	-	-	-	-	-	-	-	-
A3	-	-	-	MDel	-	-	MDel	-	-	-	-
A6	-	MDel	-	MDel	-	MDel	MDel	-	-	-	-
A7	MDel	MDel	-	-	-	MDel	MDel	-	-	-	-
A9	MDel	-	-	-	-	MDel	-	-	-	-	-
A11	-	-	-	MDel	-	-	-	-	-	-	-
A16	-	-	-	-	-	-	MDel	-	-	-	-
A23	-	-	-	-	-	MDel	-	-	-	-	-
A26	-	-	-	-	-	MDel	-	-	-	-	-
A29	-	-	MDel	-	-	-	-	-	-	-	-
A30	-	-	MDel	-	-	-	-	-	-	-	-
A35	-	-	-	-	-	-	-	MDel	-	-	-
A39	-	-	-	-	-	-	-	-	-	-	MDel

[Table/Fig-2]: Results of Y chromosome microdeletions in azoospermia Gujarati males. A: Azoospermia patients and MDel: Microdeletion

Patients	AZFa		AZFb			AZFc					
	sY84	sY86	sY121	sY127	sY134	sY153	sY254	sY255	sY1191	sY1197	sY1291
O18	-	-	-	-	-	-	MDel	-	-	-	-
O24	-	-	-	-	MDel	-	-	-	-	-	-
O34	-	-	-	-	-	-	-	-	-	-	MDel
O39	-	-	-	-	-	-	-	-	-	-	MDel
O41	-	-	-	-	MDel	-	-	-	-	-	-
O42	-	-	-	-	-	-	-	-	MDel	-	-
O51	-	-	-	-	-	-	-	-	MDel	-	-
O53	-	-	-	MDel	-	-	-	-	-	-	MDel
O56	-	-	MDel	-	-	-	-	-	-	-	-
O57	MDel	-	-	MDel	-	-	-	MDel	-	-	MDel
O62	-	-	-	-	-	-	-	-	-	MDel	-
O67	MDel	MDel	-	-	-	-	MDel	MDel	-	MDel	-
O68	MDel	MDel	-	-	-	MDel	MDel	MDel	-	-	-
O69	-	-	-	-	-	-	-	-	-	-	MDel
O76	-	-	-	-	-	-	-	MDel	-	-	-
O79	-	-	-	-	-	-	-	-	MDel	MDel	-
O83	-	MDel	-	-	-	-	-	-	-	-	-
O89	-	-	-	-	-	-	-	-	-	-	MDel
O90	-	MDel	MDel	-	-	-	-	-	MDel	MDel	-
O91	-	-	MDel	-	-	-	-	-	-	-	-
O92	-	-	MDel	-	-	-	-	-	-	-	-

[Table/Fig-3]: Results of Y chromosome microdeletions in oligozoospermia Gujarati males.
O: Oligozoospermia patients and MDel: Microdeletion

DNA Isolation

DNA was isolated from all the 259 semen (oligospermic and normozoospermic) and 41 azoospermic blood samples by phenol: chloroform method and subjected to electrophoretic analysis on 0.8% agarose gel. The electrophoretic pattern of DNA isolated, indicates good quality of DNA. DNA quantification of all the semen and blood samples were performed using Nanodrop spectrophotometer.

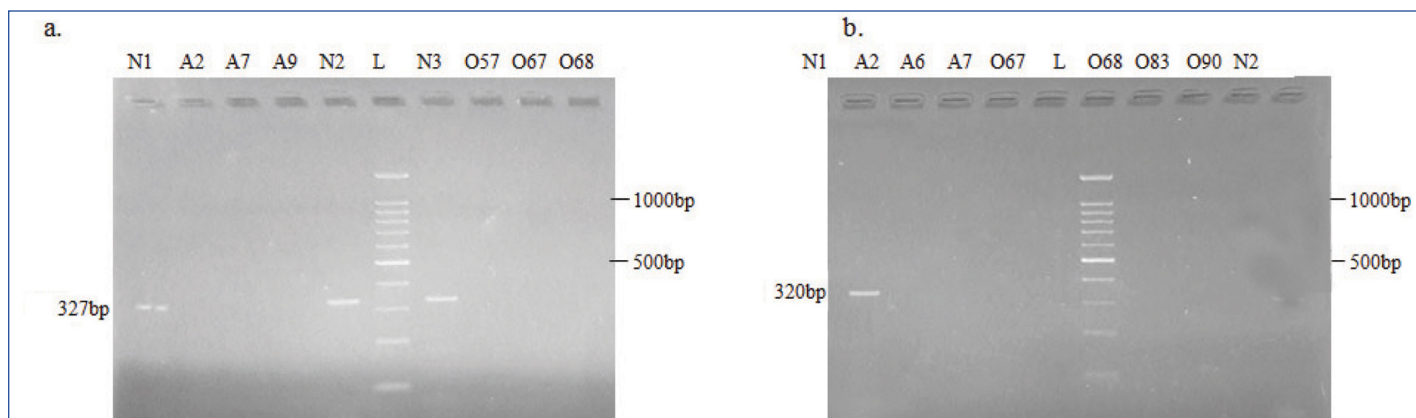
Azoospermia Patients and Microdeletion

Microdeletion analysis: In the present study, screening of 141 infertile men with the AZF region specific STS markers showed microdeletions in 34 individuals, which accounts for 24.11% of total infertile men analyzed for Yq chromosome microdeletions. Microdeletion pattern of azoospermic individuals and oligozoospermic individuals are represented in [Table/Fig-2,3] respectively. Among the azoospermia patients, 13 (31.41%) out of 41 while 21 (21%) out of 100 oligozoospermia patients showing AZF microdeletions [Table/Fig-4-8]. [Table/Fig-9] showing frequencies of microdeletions in each sub-region of AZF. None of the normozoospermic men showed microdeletions in any of the 11

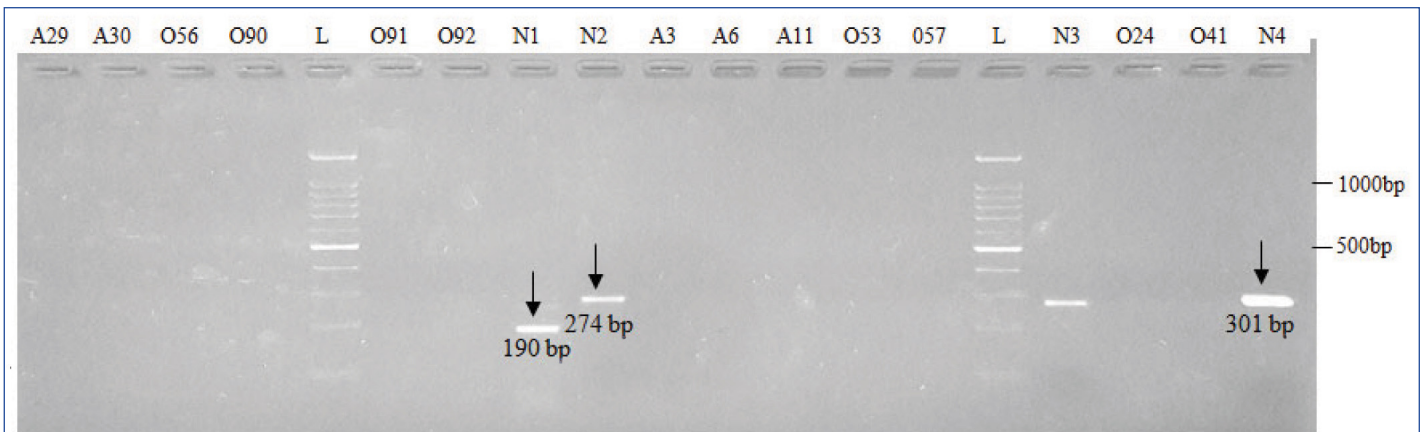
selected STS markers, which were used for the screening for the AZF microdeletions (AZFa, AZFb and AZFc sub-regions).

DISCUSSION

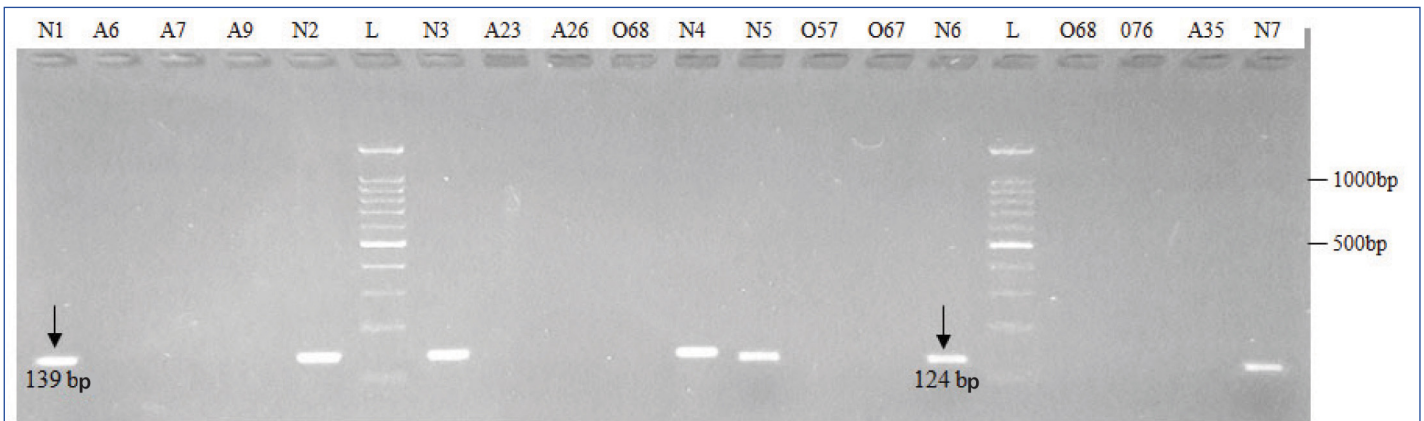
After Klinefelter syndrome, the AZF microdeletion in Yq chromosome is considered as second cause of spermatogenic arrest [12]. There was variable but acceptable percentage of sperm morphological defects (head, mid-piece, tail and mixed defects) as per the WHO, 2010 guidelines (upto 96% acceptable). The morphological defects observed in all the sperms in sample number O89 and O91. Both the samples are of oligozoospermia and there is a deletion of only single STS sY1291 in O89 and sY121 in O91. Interestingly, sample O67 and O68 had microdeletion in five STS out of 11 but they had acceptable sperm morphological defects. This proves that there were no association between sperm morphological defect and Yq microdeletion in the present study. In our study, we determined the prevalence of AZF microdeletions in Yq chromosome for infertile men, with the aim to find out the correlation between AZF microdeletions with male infertility in the population of Gujarat. A bunch of genes on the Yq chromosome regulates the spermatogenesis. In recent years, Yq chromosome microdeletions have appeared as a major cause of



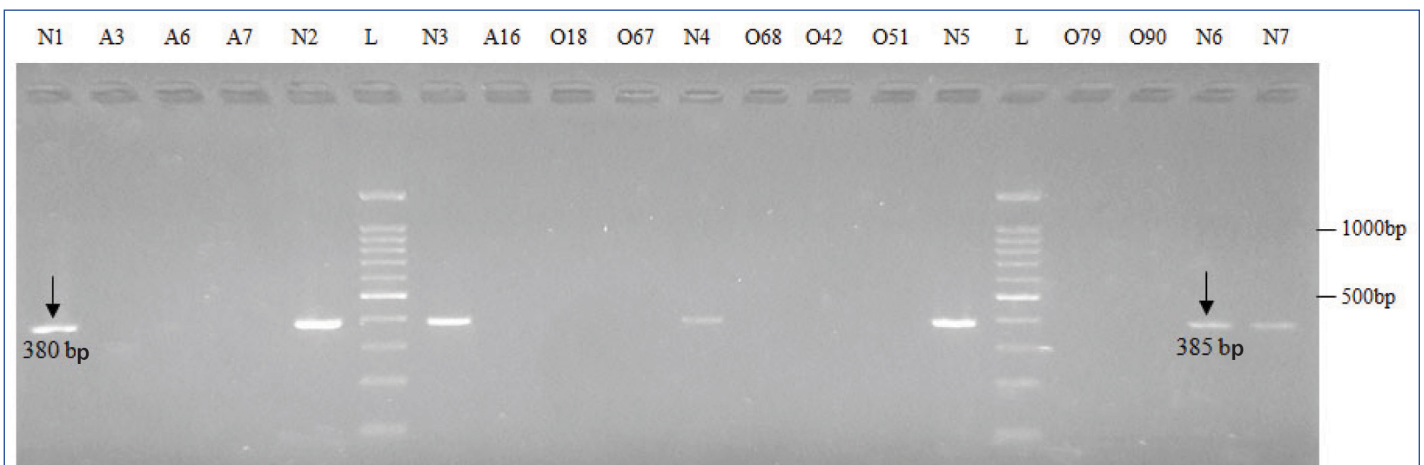
[Table/Fig-4]: Electrophoretic pattern of microdeletions generated by amplification of DNA using AZFa STS specific primer: a) sY84; b) sY86.
N: Normospermic semen samples, O: Oligospermic semen samples, A: Azoospermic blood samples and L: DNA Ladder (100 bp)



[Table/Fig-5]: Electrophoretic pattern of microdeletions generated by amplification of DNA using sY121 (Lane 1 to 8), sY127 (Lane 9 to 16) and sY134 (Lane 17 to 19) AZFb STS specific primers.
N: Normospermic semen samples, O: Oligospermic semen samples, A: Azoospermic blood samples and L: DNA Ladder (100 bp)



[Table/Fig-6]: Electrophoretic pattern of microdeletions generated by amplification of DNA using sY153 (Lane 1 to 11) and sY255 (Lane 12 to 20) AZFc STS specific primers.
N: Normospermic semen samples, O: Oligospermic semen samples, A: Azoospermic blood samples and L: DNA Ladder (100 bp)



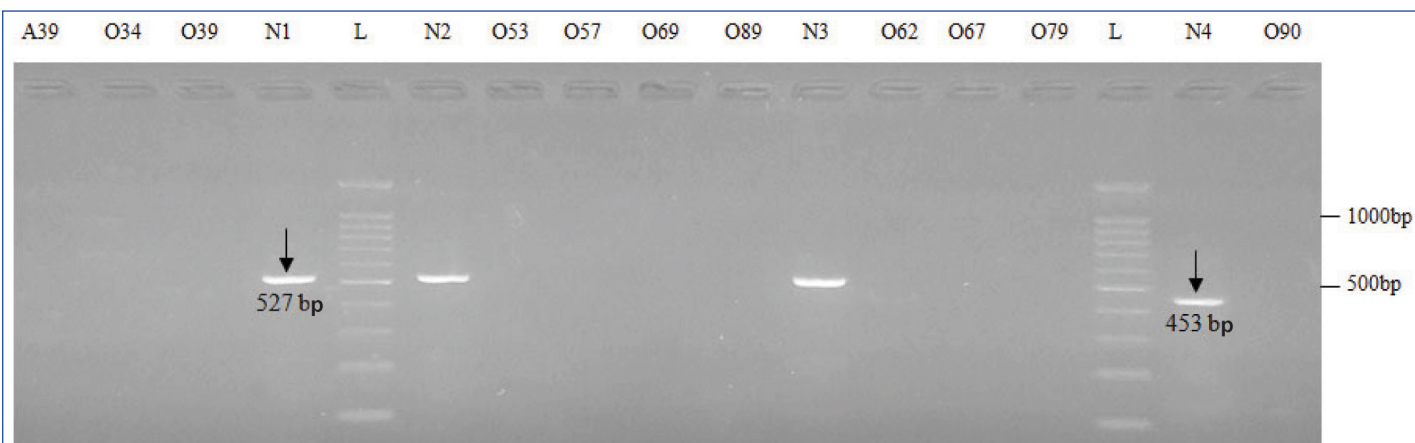
[Table/Fig-7]: Electrophoretic pattern of microdeletions generated by amplification of DNA using sY254. (Lane 1 to 12) and sY1191 (Lane 13 to 20) AZFc STS specific primers.
N: Normospermic semen samples, O: Oligospermic semen samples, A: Azoospermic blood samples and L: DNA Ladder (100 bp)

male factor infertility. Yq chromosome microdeletion is reported as second most frequent genetic cause in male factor infertility after Klinefelter syndrome [13].

It was also reported that microdeletions in AZF region can lead to a variable phenotype with a significant reduction in sperm count and increased loss of germ cells and progressive decline in semen quality. According to EAA guidelines for diagnostic testing [12,14] and most recent works [15,16], we have choose STS markers for our study.

PCR is a very sensitive technique to detect constitutional Yq microdeletions. A total of 259 semen samples and 41 blood samples of fertile and infertile men were screened for sub-microscopic Yq

chromosome deletions. Molecular analysis of AZF (AZFa, AZFb, AZFc) sub regions was carried out using eleven sY84, sY86, sY121, sY127, sY134, sY153, sY254, sY255, sY1191, sY1197 and sY1291 STS specific primers and additionally one internal marker sY14 (SRY). University of California, Santa Cruz (UCSC) genome browser and male specific region of the Human Y chromosome (MSY) breakpoint mapper is used to check the PCR product size of particular STS markers. In the present study, thirty four (24.11%) out of 141 infertile men showed microdeletions in Yq chromosome of AZFa, AZFb and AZFc sub-regions. No microdeletions were identified in any of the control males. The frequency of Yq microdeletion in India has shown vast variation ranging from 0 to 36%. For such vast



[Table/Fig-8]: Electrophoretic pattern of microdeletions generated by amplification of DNA using sY1291 (Lane 1 to 10) and sY1197 (Lane 11 to 17) AZFc STS specific primers.

N: Normospermic semen samples, O: Oligospermic semen samples, A: Azoospermic blood samples and L: DNA Ladder (100 bp)

Class	AZFa		AZFb			AZFc					
	sY84	sY86	sY121	sY127	sY134	sY153	sY254	sY255	sY1191	sY1197	sY1291
Azoospermia (n=41)	3 (7.3%)	3 (7.3%)	2 (4.9%)	3 (7.3%)	0 (0%)	5 (12.2%)	4 (9.8%)	1 (2.4%)	0 (0%)	0 (0%)	1 (2.4%)
Oligozoospermia (n=100)	3 (3%)	4 (4%)	4 (4%)	2 (2%)	2 (2%)	1 (1%)	3 (3%)	4 (4%)	4 (4%)	4 (4%)	6 (6%)
Normozoospermia (n=159)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

[Table/Fig-9]: Frequency distribution of Y chromosome microdeletion subtypes in infertile males.

variations in Yq microdeletion frequency is due to many reasons like geographical as well as racial variations [17], fluctuation in sample size [18] and number and types of STS markers used [19-21]. In Indian cohort, the very first report on Yq microdeletion was studied by Babu SR et al., which reported frequency of 5% [22]. In present study, the Yq microdeletion frequency is higher (24.11%) in Gujarati population which is very similar to 24.7% that was recorded by Abilash VG et al., 2010 in Southern East Indian population [23]. Yq microdeletion frequency recorded by Suganthi R et al., in Southern Indian population was 1.5 times more than this study [24]. Mitra A et al., found slightly higher frequency of Yq microdeletion (28.6%) in their study on Indian population [25].

Group	AZFa	AZFb	AZFc
Infertile group (n=141)	9/141 (6.38%)	13/141 (9.22%)	24/141 (17.02%)
Fertile group (n=159)	0 (0%)	0 (0%)	0 (0%)

[Table/Fig-10]: Number of infertile and fertile subjects within different sub-regions of AZF.

The frequency of microdeletion reported in our study matches with other published reports also. For example Hofherr SE et al., reported frequency of 24% and Omrani MD et al., reported a frequency of 24.2% in infertile male patients [26,27]. Yq chromosome microdeletions results in this study are higher than the studies reported by some other authors. For example, Behulova R et al., reported 14.07% of AZF microdeletion in Northeast China and Fu L et al., reported 10.80% of AZF microdeletion in Chinese infertile men [28,29]. However, the frequency of AZF microdeletions reported in our study is lower than the frequency reported by Arruda JT et al., Elhawary NA et al., and Elsaid H et al., which is 43.48%, 39.28% and 37.5% respectively [30-32]. The frequencies of all the three regions are shown in [Table/Fig-10]. Two azoospermia samples A2 and A7 showed deletions in both AZFa specific markers. Two oligozoospermia patients (O67 and O68) showed microdeletions in five STS markers out of eleven with respect to AZFa and AZFc. In the present study population, it has been observed that the AZFc region deletion frequency is comparatively higher followed by AZFb whereas lowest frequency is found in AZFa region. Among the 41 azoospermia

patients, 4 (9.75%) patients in AZFa, 5 (12.19%) patients in AZFb and 9 (21.95%) patients in AZFc carry microdeletions. Similarly, out of 100 oligozoospermia 5 (5%) patients in AZFa, 8 (8%) patients in AZFb and 15 (15%) patients in AZFc showed microdeletions. In azoospermic patients, two (A6 and A7) showed deletion of four STS-markers, three patients (A2, A3 and A9) showed deletion of two STS-markers and rest eight patients showed deletion only one STS-marker. In oligozoospermic patients, two (O67 and O68) showed deletion of five STS-markers, two patients (O57 and O90) showed deletion of four STS-markers, two (O53 and O79) showed deletion of two STS-markers and remaining 15 showed deletion of only one STS-markers [Table/Fig-2,3].

When compared overall results with the seminogram, the present study results and the evidence found in various studies suggest that the infertile men sperm concentration less than $15 \times 10^6/\text{ml}$ [11], should be screened for AZF microdeletions, which help clinician to avoid expensive ART treatments. Comparing overall result together, it could be found that microdeletions in Yq chromosome AZFa, AZFb and AZFc sub-region in the infertile male is one of the main causative factor of infertility. The microdeletion in the azoospermia factor region (AZF) of Y-chromosome was scanned in the present study using PCR. It is very powerful technique where sensitivity and specificity depends on purity of DNA. Therefore, enough care should be taken during the collection and transport of the semen and blood samples as well as during isolation of DNA to avoid possibility of contamination. Additionally, microdeletion pattern reproduced atleast thrice by the laboratory along with Y-specific internal STS markers (sY14). In future, more number of STS markers in different sub-regions of AZF and large number of infertile patients are required for better understanding of genetic background and pathogenic significance in infertile man.

CONCLUSION

The frequency of microdeletion of the AZFc sub region was found to be higher followed by AZFb and AZFa sub region and the prevalence of AZF deletion in this Gujarati study population, was 24.11%. The Yq chromosome microdeletion analysis is highly recommended for oligozoospermia and azoospermia infertile males of Gujarat

to explore appropriate assisted reproductive techniques. It also lowers down the risk of transmission of AZF microdeletion to future generations. Frequency varies among different reports, this may be due to selection criteria of populations, different diagnostic methods and/or selection of STS markers by researchers.

ACKNOWLEDGEMENTS

The authors express appreciation to Charutar Vidya Mandal (CVM) and SICART, Vallabh Vidyanagar, Gujarat for providing research work platform. We acknowledge Director, Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar, for all the facilities and constant encouragements to carry out this work. This work was supported by INSPIRE division of Department of Science and Technology, New Delhi, India, in a form of INSPIRE fellowship to Ms. Mili Nailwal. Samples provided by the Akanksha IVF Clinic and Shanjivni Hospital along with preliminary patient information are thankfully acknowledged.

REFERENCES

- [1] Quilter CR, Svennevik EC, Serhal P, Ralph D, Bahadur G, Stanhope R, et al. Cytogenetic and Y chromosome microdeletion screening of a random group of infertile males. *Fertil and Steril.* 2003;79:301-07.
- [2] Bourshi CL, Siffroi JP, McElreavey K, Dadoune JP. Y chromosome microdeletions and germinal mosaicism in infertile males. *Mol Hum Reprod.* 2000;6:688-93.
- [3] Pandey LK, Pandey S, Gupta J, Saxena AK. Loss of the AZFc region due to a human Y-chromosome microdeletion in infertile male patients. *Genet Mol Res.* 2010;9:1267-73.
- [4] McElreavey K, Krausz C, Bishop CE. The human Y chromosome and male infertility. *Results Probl Cell Differ.* 2000;28:211-32.
- [5] Li Z, Haines CJ, Han Y. Micro-deletions of the human Y chromosome and their relationship with male infertility. *J Genet Genomics.* 2008;35:193-99.
- [6] Tiepolo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent position of the human Y chromosome long arm. *Hum Genet.* 1976;34:119-24.
- [7] Bardoni B, Zuffardi O, Guioli S, Ballabio A, Simi P, Cavalli P, et al. A deletion map of the human Yq11 region: implications for the evolution of the Y chromosome and tentative mapping of a locus involved in spermatogenesis. *Genomics.* 1991;11:443-51.
- [8] Vogt PH, Edelmann A, Kirsch S, Henegar O, Hirschmann P, Kiesewetter F, et al. Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum Mol Genet.* 1996;5:933-43.
- [9] Kleiman SE, Yogev L, Gamzu R, Hauser R, Botchan A, Lessing JB, et al. Genetic evaluation of infertile men. *Hum Reprod.* 1999;14:33-38.
- [10] Krausz C, Meyers ER, Frydelund-Larsen L, Quintana-Murci L, McElreavey K, Skakkebaek NE. 2001. Double-blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure. *J Clin Endocrinol Metab.* 2001;86:2638-42.
- [11] World Health Organization, 2010 WHO laboratory manual for the Examination and processing of human semen, 5th edition, Cambridge: Cambridge University Press.
- [12] Krausz C, Hoefsloot L, Simoni M, Tüttelmann F. EAA/EMQN best practice guidelines for molecular diagnosis of Y-chromosomal microdeletions: state-of-the-art 2013. *Andrology.* 2014;2:5-19.
- [13] Naasse Y, Charoute H, Houate BE, Elbekkay C, Razoki L, Malki A, et al. Chromosomal abnormalities and Y chromosome microdeletions in infertile men from Morocco. *BMC Urol.* 2015;15:1-6.
- [14] Simoni M, Bakker E, Krausz C. EAA/EMQN best practice guidelines for molecular diagnosis of y-chromosomal microdeletions. State of the art 2004. *Int J Androl.* 2004;27:240-49.
- [15] Kdouss M, Zhioua F, Zhioua A, Gaided A, Merdassi G. Cytogenetic and Y chromosome microdeletions screening in Tunisian infertile men. *Donn J Genet Molecul Bio.* 2015;1:001-05.
- [16] Sathyanarayana SH, Malini SSN. Impact of Y chromosome AZFc subdeletion shows lower risk of fertility impairment in Siddi tribal men, Western Ghats, India. *Basic Clin Androl.* 2015;25:1-10.
- [17] Tse JY, Yeung WS, Ng EH, Cheng LN, Zhu HB, Teng XM, et al. A comparative study of Y chromosome microdeletions in infertile males from two Chinese populations. *J Assist Reprod Genet.* 2002;19:376-83.
- [18] Sen S, Pasi AR, Dada R, Shamsi MB, Modi D. Y chromosome microdeletions in infertile men: prevalence, phenotypes and screening markers for the Indian population. *J Assist Reprod Genet.* 2013;30:413-22.
- [19] Khan FH, Ganesan P, Kumar S. Y chromosome microdeletion and altered sperm quality in human males with high concentration of seminal heaxchlorocyclohexane (HCH). *Chemosphere.* 2010;80:972-77.
- [20] Mahanta R, Gogoi A, Roy S, Bhattacharyya IK, Sharma P. Prevalence of azoospermia factor (AZF) deletions in idiopathic infertile males in North-East India. *Int J Hum Genet.* 2011;11:99-104.
- [21] Viswambharan N, Suganthi R, Simon AM, Manonayaki S. Male infertility: polymerase chain reaction-based deletion mapping of genes on the human chromosome. *Singapore Med J.* 2007;48:1140-42.
- [22] Babu SR, Swarna M, Padmavathi P, Reddy PP. PCR analysis of Yq microdeletions in infertile males, a study from South India. *Asian J Androl.* 2002;4:265-68.
- [23] Abilash VG, Saraswathy R, Marimuthu KM. The frequency of Y chromosome microdeletions in infertile men from Chennai, a South East Indian population and the effect of smoking, drinking alcohol and chemical exposure on their frequencies. *Int J Genet Mol Biol.* 2010;2:147-57.
- [24] Suganthi R, Vijesh VV, Jayachandran S, Benazir JAF. Multiplex PCR based screening for microdeletions in azoospermia factor region of Y chromosome in azoospermic and severe oligozoospermic South Indian men. *Iran J Reprod Med.* 2013;11:219-26.
- [25] Mitra A, Dada R, Kumar R, Gupta NP, Kucheria K, Gupta SK. Y chromosome microdeletions in azoospermic patients with Klinefelter's syndrome. *Asian J Androl.* 2006;8:81-88.
- [26] Hofherr S E, Wiktor AE, Kipp BR, Dawson DB, Van Dyke DL. Clinical diagnostic testing for the cytogenetic and molecular causes of male infertility: the Mayo Clinic experience. *J Assist Reprod Genet.* 2011;28:1091-98.
- [27] Omrani MD, Samadzade S, Bagheri M, Attar K. Y chromosome microdeletions in idiopathic infertile men from west azarbaijan. *Urol J.* 2006;3:38-43.
- [28] Behulova R, Varga I, Strhakova L, Bozikova A, Gabrikova D, Boronova I, et al. Incidence of microdeletions in the AZF region of the Y chromosome in Slovak patients with azoospermia. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.* 2011;155:33-38.
- [29] Fu L, Xiong D, Ding X, Li C, Zhang L, Ding M, et al. Genetic screening for chromosomal abnormalities and Y chromosome microdeletions in Chinese infertile men. *J Assist Reprod Genet.* 2012;29:521-27.
- [30] Arruda JT, Bordin BM, Santos PR, Mesquita WE, Silva RC, Maia MC, et al. Y chromosome microdeletions in Brazilian fertility clinic patients. *Genet Mol Res.* 2007;6:461-69.
- [31] Elhawary NA, Seif-Eldin NS, Zaki M, Diab H, Teama S, Saleh SA. Common tag STSs in the AZF region associated with azoospermia and severe oligozoospermia in infertile Egyptian men. *Open Androl J.* 2010;2:11-18.
- [32] Elsaid H, Babiker E, Abu D, Alhassan EA, Abushama H. Detection of Y chromosome microdeletion in AZFb and AZFc loci using sequence tagged sites (STSs). *Scholars Acad J Biosci.* 2014;2:384-88.

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Date of Submission: **Jan 13, 2017**

Date of Peer Review: **Mar 04, 2017**

Date of Acceptance: **Jun 17, 2017**

Date of Publishing: **Aug 01, 2017**

FINANCIAL OR OTHER COMPETING INTERESTS: None.