Y chromosome microdeletion and phenotype correlates with male infertility in Gujarati population

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ABSTRACT

Background: Y chromosome microdeletions are one of the genetic causes of male infertility. Evaluation of this is important clinically to assist couple with such genetic defects using polymerase chain reaction-based sequence-targeted-site (STS). **Objective:** The objective of the study was to evaluate Yq microdeletion in suspected infertile male patients referred to our genetic center. **Materials and Methods:** European Association of Andrology STS markers for azoospermia factor (AZF) region were used to analyze these anomalies. In addition to semen analysis, cytogenetic, and hormonal studies have also been carried out using respective techniques. **Results:** Three of 41 cases (7.3%) included in our study had Yq microdeletion, of which one case was of azoospermia and two cases of severe oligozoospermia in AZFa and AZFc subregions were detected. Hormonal levels and karyotypes were unchanged. **Conclusion:** Yq microdeletion in our study exhibited is similar to Maharashtra, but it varies region-wise and ethnicity, including across Indian populations. Such couples require counseling before opting assisted reproductive technologies.

Key words: *Azoospermia factor region, Gujarat, Male infertility, Semen quality, Sequence-targeted-site, Yq microdeletion*

The cause, diagnosis, and treatment of infertility are important fields of research. The frequency of infertility in couples varies between 10% and 15%, of which around 50% is contributory to male partners. The cause of infertility is due to several factors such as cryptorchidism, varicocele, endocrinological, infection, alcohol consumption, or chemotherapy, including genetic defects. Genetic defects include chromosomal copy number variations, mutation, polymorphism, and Yq microdeletions. Among these, karyotypic anomalies and microdeletions are leading genetic defects of male infertility. Comparatively, scanty data are available in regard to the latter. This could be due to variations occurring in ethnical, regional, food habits, and other environmental factors. Several researchers have reported different incidents in Northern, Eastern, Western, and Southern parts of India, ranging from 5.0% to 13.5% in Yq microdeletions [1]. In Asia, the incidence is 6.0–9.9% being the highest in East Asia. Globally, its variation is higher in North America (9.1%), and East Asia (9.9%) though its average percentage is reported to be 7.5% worldwide [2,3]. Studies conducted in West India, that is, Maharashtra had 7.0% deletions comprising Mumbai 3.4% and Nagpur 10.6% [1,4]. However, no reports are available about percent microdeletion in Gujarat state. Hence, this study was undertaken to evaluate Yq microdeletion in suspected infertile male patients referred to our genetic center in Gujarat state.

MATERIALS AND METHODS

The present study included infertile male volunteers from local clinics who were referred to our Genetic Diagnostic Center of Gujarat University, Ahmedabad. After taking written consent, semen samples were collected [5] to analyze sperm count, sperm motility, and sperm morphology of the patients. Five milliliters of peripheral blood was collected for hormonal assay, peripheral blood lymphocyte culture for karyotyping [6]. Genomic DNA extraction for microdeletion analysis was done using European Association of Andrology sequence-targeted-site-polymerase chain reaction (EAA STS-PCR) markers based gel electrophoresis, respectively [7]. Karyotyping was done following the International System for Human Cytogenetic Nomenclature manual [6]. Serum level of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were estimated using hormonal kits. The data were analyzed using descriptive statistical methods.

RESULTS

No statistical differences were noticed in serum hormonal level of testosterone, LH, and FSH level. There was insignificantly higher level of FSH in a few cases (2/41). The karyotype showed no change in chromosomal profiles. However, semen types were 26 cases of

infertile normozoospermia, 4 cases of oligoasthenozoospermia, 3 each of cryptozoospermia, severe oligozoospermia, azoospermia, and 2 cases of asthenozoospermia (Table 1). Of 41 cases, only 3 (7.3%) cases of Y chromosome analysis exhibited deletions. Of three, two were severe oligozoospermia and one with azoospermia. The deletions were of c and b, interstitial regions of azoospermia factor (AZF) (Table 2). Fig. 1 summarizes Yq microdeletion reported in South, North, East, and West regions of Indian subcontinent.

DISCUSSION

Molecular studies of Y chromosome have exposed a region on the short p-arm specifies for sex determination region Y which controls the sex differentiation in humans. The long q-arm of it has candidate genes required for physiological process of spermatogenesis. Hence, male infertility is associated with Yq of it [2,4]. Recognition of AZF region on the Yq is the second most accountable genetic cause of infertility. Molecular screening of this region has revealed tripartite (AZFa, AZFb, and AZFc) organization regulating spermatogenic process and has harbored 12 genes/gene families responsible for it. Microdeletions in these subregions harbor spermatogenesis to various semen phenotypes such as azoospermia and oligospermia [1,3,8,9]. These microdeletions vary from region to other, in sample size, ethnic groups, STS marker used, and other lifestyles [2]. In Indian population, the percentage differs from 3.0% to 28.6% depending on several factors [1]. Dada *et al*. [10] stated that the overall frequency of microdeletions varies from 1% to 55%. However, globally its prevalence is attributed to 7.5% [2]. Hence, this study cohort has been investigated where very scanty data are available in Gujarat.

We have analyzed 41 suspected infertile male cases for investigation using EAA PCR-based sequence-based sites (STS) sY84, sY86 (AZFa), sY127, sY134 (AZFb), and sY254, sY255 (AZFc), which are 97% feasible clinically [1]. We classified semen categories according to the WHO [5], infertile normozoospermia, oligoasthenozoospermia/oligozoospermia, cryptozoospermia, asthenozoospermia, severe oligozoospermia, and azoospermia. The g-DNA was processed for PCR-based STS analysis and revealed that oligospermia and azoospermia cases showed Yq microdeletion (7.3%).

The subregions of AZF were a (2.4%) and c (4.9%) making to 7.3% in which c subregion was higher than AZFa and AZFb subregions of AZF in this study. This is in accordance with the data of Sen *et al*. [1] and other researchers [2,4]. The AZFc region is

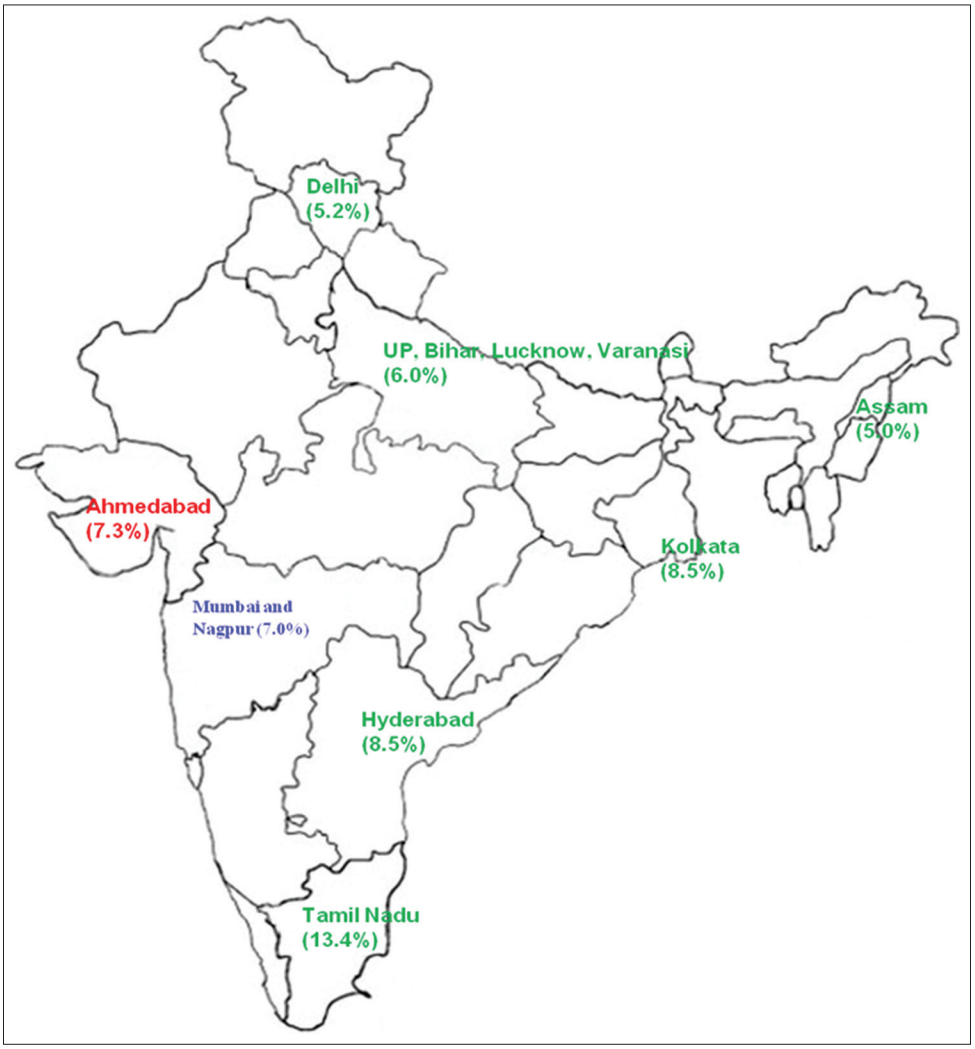

Figure 1: Geographical distribution of Yq microdeletions in India (Modified from Sen *et al*. [11])

Table 1: Frequency of Yq microdeletions in present data with semen phenotypes

Semen type	Sample	Deletion	Percentage
Azoospermia	3	1	2.4
Severe oligozoospermia	3	2	4.9
Asthenozoospermia	2	0	0
Cryptozoospermia	3	0	0
Oligoasthenozoospermia	4	0	0
Infertile normozoospermia	26	0	0
Total infertile	41	3	7.3

Table 2: Subregion wise distribution of Yq microdeletions in AZF

Deletion type	Numbers of deleted n=41	Percentage
AZFa	1	2.4
AZFb	0	0
AZFc	2	4.9
AZFbc	0	0

AZF: Azoospermia factor

known to be complex with more candidate genes and the deletions in it are related to oligozoospermia and azoospermia, although other loci cause different spermatogenic alterations [1,3,8,10]. Similarly, the global prevalence of these deletions also indicated same results contributing to 60–70% of deletions in this region (AZFc), followed by AZFa (0.5–4%), AZFb (1–5%), and AZFb+c (1–3%) deletions to support our observation [2,11,12].

The frequency of Yq microdeletion also varies from North, South, East, and West regions of India [1]. In Maharashtra, the percent reported by Sen *et al*. [1] was 3.4% in Mumbai and Ambulkar and Pande reported 10.6% in Nagpur making an average of 7% which is close to Gujarat region, but differing to other reports where Southern (8.7%), Eastern (7.7%), and Western (5.4%) India. Similarly, North America has a higher percent of Yq microdeletions (9.1%), with the highest of East Asia (9.9%) followed by lowest in Europe (2.6%) [2] contributing an average frequency of 7.5% deletions. Thus, worldwide, several studies in recent years have investigated the association of infertile male phenotypes with selected gene sequence polymorphism. Such couples are subjected to genetic counseling those who opt for assisted reproductive technologies (ARTs). The heterogeneity of phenotype due to selection criteria, population structure, ethnic background, environmental influence, food styles, and epigenetic factors is the most important limitation [4,13-16]. Thus, our study confirms that Y chromosome microdeletion frequency changes according to these factors. However, the limited sample size of our study limits the generalization of our study results. We further recommend studies with more sample size with both EAA and non-EAA STS markers for accurate Yq microdeletion analysis. We also believe that Yq microdeletions can be considered as one of the indices in the assessment of donor sperm used in ARTS.

CONCLUSION

Our data in Gujarat demonstrate that Yq microdeletions (7.3%) are similar to Western region and differ globally, including India. Several factors affect the incidence of microdeletions such as selection criteria, region, ethnicity, population structure, food habits, and other environmental factors in addition to chromosomal aberrations. Hence, clinical evaluation of microdeletions is an important aspect of male infertility so that such couples can seek genetic counseling before the adoption of ARTs.

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