Screening of Y-Chromosomal Microdeletions Using 8 STS Primers in Idiopathic Infertile Men from Coimbatore District, Tamil Nadu

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Abstract—India is one of the most populous country in the world with 2.5 as an average fertility rate. Nevertheless, the trend of fertility rate has been tumbling down in Indian states. Earlier, women were considered to be lone reason for infertility. It took several decades to understand that infertility in males which may either be due to genetic or non-genetic factors. Y-Chromosomal microdeletion is one of the major genetic factor which cause infertility in males. Analysis of Sequence Tagged Sites (STS) markers in Azoospermic Factor regions (AZFa, AZFb, AZFc) at Yq part helps us to know about the deletion frequency in infertile males. Infertile males who adopt Assisted Reproductive Technique (ART) are likely to transmit the infertility to their sons. Molecular diagnosis of Y chromosomal microdeletion is one of the paramount and prognostic techniques to acquire information about the infertility passing from one generation to another. Only little research has been carried out on Y chromosomal microdeletions in Tamilnadu. The aim of our research is to scrutinize the frequency of Y chromosomal microdeletions in infertile males of Coimbatore district, Tamil Nadu. We utilized eight STS primers (sY84, sY86 for AZFa region; sY 99, sY100, sY 134 for AZFb region; sY 156, sY 254, sY 255 for AZFc region) to detect the microdeletions. Blood samples of thirty infertile men were scrutinized for Y chromosomal microdeletion. Our results showed that the frequency of deletion in AZFb region accounted for 53%; AZFb – 50%; AZFc region- 60% respectively. AZFc region was highly deleted when compared to AZFa and AZFb.

Keywords— Y-Chromosomal Microdeletions, Azoospermic Factor Regions, Sequence Tagged Sites Primer.

I. INTRODUCTION

World Health Organization (WHO) reports that infertility affects 8-12% of the couples in the world [1]. The infertility rates differ among the Indian states. In olden days, women are the only reason to be blamed for infertility. A research in India indicated that male reproductive disorders contribute 50% to infertility [2,3,4]. The predominant factors to know about the infertility in male are sperm count, motility, structure and shape. Later, Scientists has intrigued about the genetic factors behind male infertility. Y chromosomal microdeletion is one of the breakthrough technologies to acquire knowledge about genetic factors behind infertility [5].

The Y Chromosome is divided into short arm (Yp) and long arm (Yq). The X chromosome will recombine with the ends of Y chromosome which is known as pseudo autosomal regions (PARs). The Y chromosome region which does not involve in recombination is termed as nonrecombining region(NRY). The euchromatin part of NRY consists of (Azoospermia factor region) AZFa, AZFb and AZFc gene clusters play a predominant role in spermatogenesis [6].

The deletion of AZF locus which can be detected by using STS PCR rather than cytogenetic test. The first identified candidate gene RBMY (RNA binding Motif on the Y chromosome) from AZFb expressed in the testis and its actual function in the male germ cell development is ambiguous [7]. After RBMY, DAZ (deleted in azoospermia) candidate gene from AZFc has been noticed and it is expressed in testis. Though there are other candidate gene such as CDY1 (Chromo domain Y) in AZFc, the high ubiquity of DAZ deletion in infertile men make them as an important candidate gene [7,8]. The candidate gene of AZFa is USP9 (Ubiquitin-specific protease 9, Y chromosome) and it has been expressed ubiquitously. DBY (Dead box on the Y chromosome) is another candidate gene from AZFa [9, 10,11].

The basic set of STS Primers has been used for detecting the deletions in AZF regions [12,13]. In this study, we tried to analyze the microdeletions in AZF regions from thirty infertile male samples in Coimbatore, India using eight STS primers.

II. MATERIALS AND METHODS

A. Collection of Samples
Ethical clearance for human research (Proposal number: 10/357) was issued by Institutional Human Ethics Committee of PSG Institute of Medical Sciences and Research in Coimbatore, India. Blood samples were collected from thirty infertile males with unspecified factors which caused infertility or also termed as idiopathic cases. Five samples
which were obtained from fertile male and female were used as positive and negative control respectively.

B. DNA Isolation from Blood Samples

Salting out method was used to isolate the DNA from blood samples [14]. The integrity of isolated DNA from samples were checked using agarose gel electrophoresis. The purity of DNA was determined by the $A_{260}/A_{280}$ ratios [15]. The quantification of DNA was done by using Nano-spectrophotometer.

C. Selection of STS Markers

The eight STS primers for this study were selected according to the guidelines of European Academy of Andrology and European Molecular Genetics Quality Network which helped to test the Y-Chromosomal Microdeletions [12,13]. The details of STS Markers are presented in the Table 1.

<table>
<thead>
<tr>
<th>S.No</th>
<th>STS Marker</th>
<th>AZF Region</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
<th>Size of PCR Products (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>sY84</td>
<td>AZFa</td>
<td>AGA AGG GTC TGA AAG CAG GT</td>
<td>GCC TAC TAC CTG GAG GCT TC</td>
<td>326</td>
</tr>
<tr>
<td>2.</td>
<td>sY86</td>
<td>AZFa</td>
<td>GTG ACA CAC AGA CTA TGC TTC</td>
<td>ACA CAC AGA GGG ACA ACC CT</td>
<td>320</td>
</tr>
<tr>
<td>3.</td>
<td>sY99</td>
<td>AZFb</td>
<td>GAC TCA GGG ATC CAG GTT G</td>
<td>GCA CTG CAA CTT TTA TGC CT</td>
<td>357</td>
</tr>
<tr>
<td>4.</td>
<td>sY100</td>
<td>AZFb</td>
<td>TAA AGG AAC TTC TGT GTG TAA ACA</td>
<td>TAA GCC AGA TAG GGG CTT CT</td>
<td>111</td>
</tr>
<tr>
<td>5.</td>
<td>sY134</td>
<td>AZFb</td>
<td>GTC TGC CTC ACC ATA AAA CG</td>
<td>ACC ACT GCC AAA ACT TCC AA</td>
<td>301</td>
</tr>
<tr>
<td>6.</td>
<td>sY156</td>
<td>AZFc</td>
<td>AGG AAC TGG CAG GAT TAG CC</td>
<td>ATG TCA GGG TTT CCT TTG CC</td>
<td>950</td>
</tr>
<tr>
<td>7.</td>
<td>sY254</td>
<td>AZFc</td>
<td>GGG TGT TAC CAG AAG GCA AA</td>
<td>GAA CCG TAT CTA CCA AAG CAG C</td>
<td>380</td>
</tr>
<tr>
<td>8.</td>
<td>sY255</td>
<td>AZFc</td>
<td>GTT ACA GGA TTC GGC GTG AT</td>
<td>CTC GTC ATG TGC AGC CAC</td>
<td>126</td>
</tr>
</tbody>
</table>

Table: 1 STS markers of AZF regions with sequences and their Product Size

D. PCR Analysis

The conditions of PCR for eight STS markers were standardized by testing the estimated annealing temperature, annealing time, extension time, primer concentration and DNA concentration. The standardized PCR conditions for eight STS markers were listed in the Table II. 1µl (200 ng) of DNA template from the individual samples were used for each reaction. Total volume of PCR reaction mix was 20 µl and it consisted of 10X PCR buffer,10mM dNTPs, forward and reverse primers (50 ng/µl), Taq polymerase (3U/µl), Double distilled water and DNA samples. PCR products were electrophoresed at 75V in 2% agarose gel. The PCR products were visualized under UV trans illuminator and then documented by alpha imager gel documentation system.
III. RESULTS AND DISCUSSION

Thirty infertile male samples were screened for Y chromosome microdeletions using eight STS primers (sY 84, sY 86 for AZFa region; sY 99, sY 100, sY 134 for AZFb region and sY 156, sY 254 and sY 255 for AZFc region). The PCR reactions were performed thrice to confirm the deletions in AZF regions. The number of samples with deletions and their percentage were listed in Table III and IV respectively.

The deletion of AZFa region results in Sertoli cell only syndrome (SCOS) [16]. When we analyzed with sY84 marker, thirteen samples showed deletion in AZFa region among the thirty samples and the percentage of deletion in AZFa region using sY84 marker was 43%. The other marker sY86 for AZFa resulted deletions in eight samples and accounted 26.6% frequency of deletions in AZFa region. In this study, the percentage of deletions in AZFa region using sY84 and sY 86 were 53%.

Azoospermia and maturation arrest of germ cells prevalently occur, when there is a deletion in AZFb region [17]. Three STS markers such as sY99, sY100 and sY134 resulted deletions of AZFb region in eleven, ten and nine samples out of thirty samples respectively. The Percentage of deletions in AZFb region using sY99, sY100 and sY134 were 36.6%, 33% and 30% respectively. The AZFb deletions were analyzed by using these three markers which accounted for 50%.

The deletions in AZFc region reported that the conditions such as mild or severe oligozoospermia and azoospermia would become dominant [17]. While we analyzed samples for the deletions in AZFc region utilizing sY156, sY 254 and sY 255, the deletions were observed in four, eight and twelve samples out of thirty samples respectively. Hence the percentage of deletions in AZFc region was 60%, which seemed to be higher compared to the deletions (53%) AZFa and (50%) AZFb region. Venn diagram depicted the distribution of AZF deletions in Fig. 7.

Sample pictures of agarose gel analysis after PCR reactions exhibit microdeletions in the AZF region with respect to STS primers are displayed from Fig. 1. to Fig. 6.
Surprisingly, six infertile male samples (S5, S8, S22, S23, S24, S26) did not show any deletion using eight STS primers and their percentage of undeletion accounted for 20%. Though the STS markers were selected based on the European Academy of Andrology and European Molecular Genetics Quality Network, six infertile male samples showed no deletions in the AZF regions. The undeleted infertile samples should contain a deletion possibly in some other loci which could not be able to diagnose by these eight STS primers. The observation of undeleted samples in infertile samples were also reported in Greek and Nilgiri districts, South India [18, 19].

Table: 3 Microdeletions of AZF regions in infertile male samples

<table>
<thead>
<tr>
<th>S.NO</th>
<th>AZF Region</th>
<th>Sample Number with AZF deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AZFa</td>
<td>S1, S3, S6, S7, S9, S10, S11, S12, S14, S15, S17, S18, S19, S20, S21, S28</td>
</tr>
<tr>
<td>2.</td>
<td>AZFb</td>
<td>S1, S2, S6, S7, S10, S11, S13, S14, S15, S16, S18, S21, S25, S27, S28</td>
</tr>
<tr>
<td>3.</td>
<td>AZFc</td>
<td>S1, S2, S4, S6, S7, S10, S11, S12, S14, S15, S17, S18, S19, S20, S21, S25, S29, S30</td>
</tr>
</tbody>
</table>

Table: 4 Percentage of deletions in AZF regions for thirty infertile male samples

<table>
<thead>
<tr>
<th>S.NO</th>
<th>AZF Region</th>
<th>Number of samples with deletion</th>
<th>Percentage of deletions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AZFa</td>
<td>16</td>
<td>(16/30) 53%</td>
</tr>
<tr>
<td>2.</td>
<td>AZFb</td>
<td>15</td>
<td>(15/30) 50%</td>
</tr>
<tr>
<td>3.</td>
<td>AZFc</td>
<td>18</td>
<td>(18/30) 60%</td>
</tr>
<tr>
<td>4.</td>
<td>AZFa+b</td>
<td>1</td>
<td>(1/30) 3.3%</td>
</tr>
<tr>
<td>5.</td>
<td>AZFb+c</td>
<td>2</td>
<td>(2/30) 6.6%</td>
</tr>
<tr>
<td>6.</td>
<td>AZFa+b+c</td>
<td>9</td>
<td>(9/30) 30%</td>
</tr>
</tbody>
</table>

Fig. 1: Analysis of microdeletions in AZFc region using sY255

Fig. 2: Analysis of microdeletions in AZFa region using sY84
Fig. 3: Analysis of microdeletions in AZFb region using sY134

Fig. 4: Analysis of microdeletions in AZFb region using sY99

Fig. 5: Analysis of microdeletions in AZFc region using sY156

Fig. 6: Analysis of microdeletions in AZFc region using sY254.
AZFc region was highly deleted in infertile male patients compared to AZFa and AZFb region and this reports were congruous with previous research works [19,20,21,22]. AZF region contains palindromic sequences which are more susceptible to intrachromosomal recombination and therefore, this region has been deleted frequently in infertile male samples [23]. A research conducted in North India reported that the deletion of AZFc region was 58.8% [21] whereas our results showed that 60% of deletion in AZFc region. Deletion in AZFa+b region was 3.3% which found to be lower than AZFb+c region (6.6%). Deletions in AZFb+c region lead to either asthenozoospermia (reduced sperm motility) or severe oligozoospermia (low sperm motility). Azoospermia (absence of sperm) or failure of testicular sperm retrieval are associated with the deletions in AZFa+b region [19,24]. 30% of deletion was observed in all the three regions. Even though infertile male who has a deletion in AZFc Region can adopt Intracytoplasmic sperm injection (ICSI) treatment to father a child, the deletion pattern which causes infertility is likely to transmit to their offspring [25]. It is because most of the AZF deletions are occurred in the germ cell [26]. Hence, the Y chromosomal microdeletion analysis helps the clinicians to decide the deletion pattern of AZF regions for infertile male’s future generation. Clinicians could able to inform infertile couples about the risks in adopting either ICSI or assisted reproductive techniques.

IV. CONCLUSION

This study showed that the AZFc region has been highly deleted when compared to the AZFb and AZFa regions. Increase in sample size would have helped us to explore more about the frequency of AZF deletions. Examining Y Chromosomal microdeletions assist the clinicians to decide the suitable assisted reproductive techniques for the infertile patients. Analysis of Y chromosomal microdeletions help the clinicians to counsel the infertile patients prior to assisted reproductive techniques.

V. ACKNOWLEDGMENT

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REFERENCES


