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Anatomy

Y-CHROMOSOME MICRODELETION AND ITS DIAGNOSTIC IMPORTANCE IN MALE INFERTILITY.

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ABSTRACT To identify chromosomal aberrations of male Infertility cases and to evaluate the patterns of Y-chromosome micro deletion in Male Infertility cases (N=147) attending the infertility clinic. PCR based Y chromosome microdeletion analysis revealed nearly 14.28% (21/147) deletions in the Infertile men with idiopathic infertility, of which 5.4% deletion among Azoospermic men, 2.72% among severe Oligospermic men, 4.08% among Oligospermic men and 2.04% among Asthenozoopermic, Oligoastheno-teratoazoospermic, Astheno-teratozoospermic men. In summary the incidence of AZFc deletion was more frequent in Azoospermic which could be the cause for the severe spermatogenic failure in Subfertile men opting for Assisted reproductive procreation technique. Hence PCR based Y chromosomal microdeletion analysis is essential to prevent the transmission of defective genes to the offspring before the ART

KEYWORDS : Male Infertility, Azoospermic factor, Y-chromosome microdeletion, STS-Sequence tagged sites.

Introduction:

Natural desire of human beings is to propagate their lineage and is a part and parcel of human evolution. Infertility is a major problem prevailing world-wide which accounts for about 15% of all couples and 50% of this problem is the contribution by male factor [1]. A recognizable decline in the semen quality been observed in the south Indian population [2]. Infertility affects 1 in 5 couples of reproductive age group and in most of the cases the infertility is idiopathic [3]. The most commonly observed chromosomal abnormality was Klinefelter syndrome, Robersonian translocation, Y- chromosome microdeletion, polymorphism of chromosome 9 and increase in the length of heterochromatic segments of Y chromosome [4].The cytogenetic analysis of men undergoing assisted reproductive treatment depict microdeletion in the long arm of Y chromosome in about 18.2% of the cases [5] which will be inherited by the male offspring and causes deleterious effects in the progeny [6] .

The AZFc (3.5Mb length) is located in the distal part of Yq in the interaval 6 and contains DAZ gene – Deleted in Azoospermia and Chromodomain Y (CDY) (Fig:1).The DAZ gene is present in multiple copies and was found that nearly four DAZ genes were present within the AZFc region together they are called as DAZ gene family are the more commonly deleted in the Y Chromosome of men with Azoospermia and severe Oligospermia.

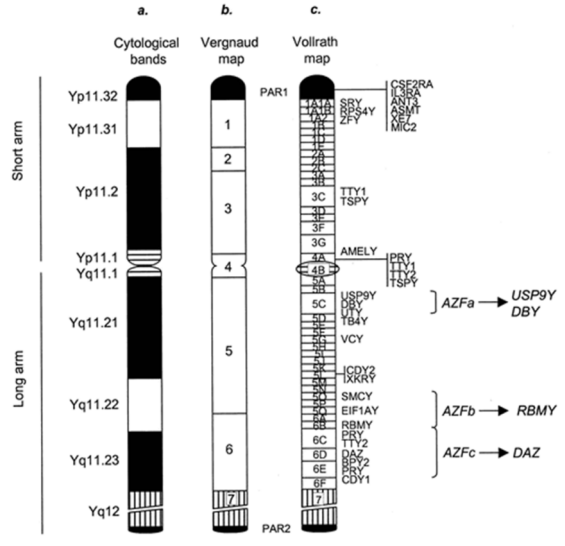

Fig:1. Y chromosome Map showing the Azoospermic regions and the associated genes. [7].

The Y chromosome microdeletion deletion may be quite difficult to analyse through manual cytogenetic method, hence molecular analysis of Y chromosome using the STS (sequence tagged sites) is essential in men with severe spermatogenic failure, which helps to counsel the couple undergoing infertility treatment.

SUBJECTS AND METHODS:

The study was conducted in 147 Subfertile men with severe Spermatogenic failure compared with control group of 150 healthy men with normal seminal profile and who have fathered a child. Incidental sampling method was used. The study population was evaluated by eliciting complete medical history. Based on the semen profile the study group was classified as Azoospermia (N = 44), Severe Oligospermia (N=43), Oligospermia (N= 34), Oligoasthenoteratozoospermia (N=13), Asthenozoospermia (N=8), Asthenoteratozoospermia (N=5). The PCR based Y Chromosomal microdeletion was carried out using the STS (Sequence tagged sites) markers on the long arm of the Y-chromosome (Table: 1). The AZF a, b and c region deletion was analysed using the following primers (Table:1) [9].

Table 1: Primer sequence for the PCR based Y Chromosome microdeletion analysis

S. No	Primer Name	Primer Sequence	Product Size
1.	AZFa : DYS273	Forward: 5`-AGA AGG GTC TGA AAG CAG GT-3` Reverse: 5`-GCC TAC TAC CTG GAG GCT TC-3`	(326bp)
2.	AZFa: DYS148	Forward: 5`-GTG ACA CAC AGA CTA TGC TTC-3` Reverse: 5`-ACA CAC AGA GGG ACA ACC CT-3`	(320bp)
3.	(AZFb): DYS218	Forward: 5`-GGC TCA CAA ACG AAA AGA AA-3` Reverse: 5`-CTG CAG GCA GTA ATA AGG GA-3`	(274bp)
4.	(AZFb): DYS224	Forward: 5`-GTC TGC CTC ACC ATA AAA CG-3` Reverse: 5`-ACC ACT GCC AAA ACT TTC AA-3`	(301bp)
5.	(AZFc): DAZ254	Forward: 5`-GGG TGT TAC CAG AAG GCA AA-3` Reverse: 5`-GAA CCG TAT CTA CCA AAG CAG C-3`	(400bp)
6.	(AZFc): DAZ255	Forward: 5`-GTT ACA GGA TTC GGC GTG AT-3` Reverse: 5`-CTC GTC ATG TCA TGT GCA GCC AC-3`	(274bp)

1.8% agarose was prepared by melting the agarose in a microwave or hot water bath until the solution becomes clear. Solution was cooled to about 50-55°C, swirl the flask occasionally to cool evenly. Then ethidium bromide was added. Ends of the casting tray were sealed with two layers of tape. Combs were placed in the gel casting tray and melted agarose solution was poured into the casting tray and allowed to cool until it got solidified.The tape was then removed from sides and a dry run to remove any ions and to check integrity of wells was done. Gel was placed in the electrophoresis chamber and enough TAE buffer was added so that there is about 2-3 mm of buffer over the gel.

5 µl samples was mixed with 6X loading dye and carefully pipetted into separate wells in the gel. 2 ml DNA ladder was added in a separate well. Power supply was kept at about 100 volts. Maximum allowed voltage varied depending on the size of the electrophoresis chamber. The gel was removed and bands were visualized under UV light in gel document system (UVitec, Lark innovative Inc.)

Results:

PCR based Y chromosome micro deletion analysis revealed nearly 14.28% (21/147) deletions in the subfertile men with idiopathic infertility (Table 2), of which 5.4% (8/147) deletion among Azoospermic men, 2.72% (4/147) among Severe Oligospermic men, 4.08% (6/147) among Oligospermic men and 2.04% (3/147) among Asthenozoopermic, Oligoastheno-teratoazoospermic, Astheno-teratozoospermic men (Table 2).

Table 2: Y Chromosomal microdeletion observed in the Infertile Men

Phenotype	Total number of cases	Number of. Deletions					
			AZFa D148	AZFb Dy218	AZFb Dy224	AZFc Dy254	AZFc Dy255	AZFa Dy273
Azoospermic	44	8	--	3	--	--	5	--
Severe oligospermic	43	4	--		1	2	1	--
Oligospermic	34	6	--	--	--	3	3	--
Severe Oligo astheno teratozoospermic	26	3	---	-	--	2	1	--
Total number of cases	147	21						

The AZFc region was found to be frequently deleted in the Infertile men in comparison to the AZFb and AZFa region. A total number of 17 AZFc deletions were observed in the present study in which five deletions were present in Azoospermic cases, three deletions in Severely Oligospermic cases, six deletions in Oligospermic cases and three deletions in Asthenozoopermic, Oligoasthen oteratoazoospermic, Astheno- teratozoospermic men (Table 2).The AZFc was the most frequently deleted AZF loci in this study population with idiopathic male infertility (Fig 3 & 4)

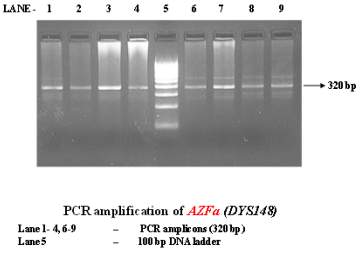

Figure 2: Agarose gel picture showing the PCR amplification of AZFa (DYS148)

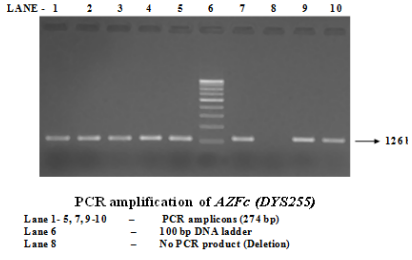

Figure 3: Agarose gel picture showing the amplification of AZFc (DYS255) and its deletion.

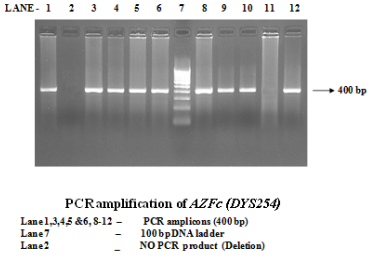

Figure 4: Agarose gel picture showing the amplification of AZFc (DYS254) and its deletion.

Discussion:

In the present study a total number of four deletions (2.72%) in AZFb region and the incidence of AZFc (11.56%) deletions were frequent and there was no AZFa deletion (fig;2). AZFa deletion was absent in the present study population, whose deletion causes sertoli cell only syndrome azoospermia and causes variable testicular phenotype [10]. Prevalemce of (5.29%) Y chromosome microdeletions in Azoospermic men (2AZFa, 1AZFb and 3 AZFb+c) [11] and absence of deletion in severe Oligospermic men were documented in the Indian population. Similar reports were documented in South indian population (Azoospermic men and Oligoasthenoteratozoospermic men) in which overall occurance of 12.6% of Y chromosome deletion was observed with increased frequency of AZFc (9.14%) deletion when compared to AZFb (2.28%) and AZFa (1.14%) [12]. AZFc is considered to be important region in Y Chromosome as it contains testis-specific chromodomain protein Y1 (CDY1) and (DAZ) cluster (Fig;1.). Multiple copies of the DAZ gene with more than 99% of sequence identity exist in the AZFc region. Since this gene also has an autosomal homologue in chromosome 3p24 (DAZLA) and due to the presence of various modifying genes and multiple copy number, there is a marked variation of phenotype in Men harboring AZFc deletions (Fig:1).

Normally men with AZFc deletion have severe spermatogenic failure, cannot father a child naturally and opt for assisted reproductive techniques but they are at risk of transmitting AZFc deletion to their offssprings. The Y chromosomal microdeletion is a denovo event, accumulated loss of Y-chromosome during the time of embryogenesis leads to vertical transmission of AZFc deletion to the offspring [13]. The frequency of the Y Chromosome Deletion increases in the germ cells compared to Blood DNA[14], hence proper screening of Y chromosome microdeletion is necessary to counsel the patients prior to the assisted reproduction procreation techniques. In summary the present study the incidence of AZFc deletion was more. There was no AZFa deletion and few AZFb deletions were observed. In Azoospermic men the AZFc deletions were frequent which could be the reason for severe Spermatogenic failure in Subfertile men.

The Infertile men with Y chromosome microdeletion cannot conceive a child normally, they opt for IVF treatment, hence prior screening of Y Chromosomal micro-deletion is essential for men opting for Assisted reproductive procreation technique inorder to prevent the transmission of abnormal Y chromosomal pattern to the male offspring. Proper Genetic counselling concerning the risk of genetic disorder transmitted to the offspring must be done for the Couple with Y-chromosome micro deletion before undergoing the IVF treatment.

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