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The frequency of Y chromosome microdeletions and importance of genetic counselling in infertile male: A metropolis experience

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Abstract
Aim: To study the incidence & type of microdeletions in patient with infertility by Y Microdeletion by Polymerase chain reaction (PCR) studies.
Materials and Methods: In 763 patients aged from 21-47 years with suspicion of Y microdeletion PCR based Y microdeletion studies carried. 6-8 ml of peripheral blood was collected in EDTA Vacutainers and the genomic DNA were extracted and analysed by PCR.
Results: Microdeletion was observed in 30 patients with a total deletion rate of 3.9%. Of these deletions, AZFc deletions was seen in 2.6%, AZFb deletions in 0.8%, microdeletion of AZFb and AZFc in 3 patients with a deletion rate of 0.4%, 1 patient showed deletion in AZFa, AZFb and AZFc with a deletion rate of 0.1%.
Conclusion: Knowing the genetic status of Y chromosome in male infertile individuals currently in the era of advanced assisted reproductive technology (ART) can be very helpful to the couple.

Keywords: multiplex PCR, AZF, infertility

1. Introduction
Infertility is a one of the major health problem affecting almost 10-17% of the couple [1]. Most of the individual in there adulthood wants to become a parent and have a family but when this ends in experiencing infertility leads to various psychosocial issues including depression, anger, stress and anxiety. If a couple doesn't achieve a pregnancy after having an unprotected intercourse for duration of more than 12 months is an infertile couple [2]. There are different causes for infertility and one of the most common causes is male infertility [3]. Of the most common causes of male infertility genetic cause as an etiology is commonly seen. The genetic causes could be because of chromosomal abnormality which is studied by cytogenetic testing and usually in males it is numerical abnormality in the form of extra copy or copies of sex chromosome X or structural abnormalities of sex chromosomes in the form of deletion or translocations [4]. When the cytogenetic studies shows normal pattern in a male patient with history of infertility and clinical suspicion of Y Microdeletion the possibility of Y Microdeletion by PCR based study should be considered [5]. A genetic factor is located on the Y chromosome at the band position q11 is reported to be an important region for the proper male germ cell development the cluster of genes on this region is named as azoospermia factor(AZF) [6-8]**.** There are 3 associated loci identified and they are named as AZFa, AZFb, AZFc. There are 300 sequence tagged sites (STS) mapped for these three regions and they can be amplified by PCR based technique. The contribution of Y Microdeletion as a cause of male infertility is well established and clinical presentation is usually different in deletion of different AZF locus. The percentage varies according to case selection and if all other causes of oligospermia/azoospermia are ruled out the proportion due to AZF deletions is reported to be 10-20% [9]. Thus Y Microdeletion can lead to reproductive failures in couple and may lead to infertility. Identification of specific Microdeletion helps the genetic counsellor to counsel the couple and the specialist to select the mode, method and type of ART [4, 10]. The purpose of this retrospective study was to analyse the frequency and types of Y microdeletion abnormalities in individuals with history of infertility and suspicion of Y Microdeletion. It is to be noted that since the study was performed in a tertiary care laboratory, the percentage of findings may vary.

2. Materials and methods
DNA from 763 individuals, ranging between 21-47 years of age, was extracted using Qiamp DNA Mini kit (Qiagen, Hilden, Germany) and quantified using Biophotometer (Eppendorf, Hamburg, Germany). Individuals with clinical history of infertility, azoospermia, oligospermia, oligoasthenoteratozoospermia, etc that were referred to Metropolis Healthcare Pvt. Ltd. from Jan 2014 to June 2018 were included in this study. Five-six ml of peripheral blood

was requested in EDTA Vacutainers and multiplex PCR was carried out where 16 STS markers of the AZF (Azoospermia Factor) locus along with ZFY and SRY markers were amplified. The primers used for amplification were as described in table 1. Reactions with a total volume of 25µl were prepared using 8.5µl PCR grade water, 12.5µl of 2X Kappa mix (Kapa Biosystems, Wilmington, MA, USA), 2µl of the primer mix and 2µl of extracted DNA. The PCR program used for amplification of these STS markers is as follows:

Initial denaturation at 94℃ for 5 minutes, 35 cycles at 94˚C for 30 seconds, 57℃ for 45 seconds, 72℃ for 1 min 30 sec and a final extension at 72℃ for 7 minutes.

The assay was standardized such that 5 sets of reactions, covering STS markers in AZFa, AZFb and AZFc regions, need to be performed for each sample. The following markers were included in the multiplex PCR:

AZFa: sY81, sY84, sY86, sY182
AZFb: sY121, sY124, sY127, sY128, sY130, sY134
AZFc: sY153, sY157, sY254, sY255, sY145, sY152

These markers were split into 5 sets of reactions depending on the PCR product sizes expected for each marker. The amplified products were then loaded on a 2% agarose gel and the results were analysed based on the separation of the PCR products. Absence of a particular band was interpreted as deletion of the corresponding STS marker. The results were reported as per standard International Guidelines and CAP and NABL guidelines.

Table 1: Primers sets used for multiplex PCR for Y chromosome micro deletion

Marker Name	Primer Sequence
ZFY-F	ACC RCT GTA CTG ACT GTG ATT ACA
ZFY-R	GCA CYT CTT TGG TAT CYG AGA AAG T
SRY-F	GAA TAT TCC CGC TCT CCG GA
SRY-R	GCT GGT GCT CCA TTC TTG AG
sY86-F	GTG ACA CAC AGA CTA TGC TTC
sY86-R	ACA CAC AGA GGG ACA ACC CT
sY134-F	GTC TGC CTC ACC ATA AAA CG
sY134-R	ACC ACT GCC AAA ACT TTC AA
sY127-F	GGC TCA CAA ACG AAA AGA AA
sY127-R	CTG CAG GCA GTA ATA AGG GA
sY254-F	GGG TGT TAC CAG AAG GCA AA
sY254-R	GAA CCG TAT CTA CCA AAG CAG C
sY255-F	GTT ACA GGA TTC GGC GTG AT
sY255-R	CTC GTC ATG TGC AGC CAC
sY152-F	AAG ACA GTC TGC CAT GTT TCA
sY152-R	ACA GGA GGG TAC TTA GCA GT
sY145-F	CAA CAC AAA AAC ACT CAT ATA CTC G
sY145-R	TTG AGA ATA ATT GTA TGT TAC GGG
sY128-F	GGA TGA GAC ATT TTT GTG GG
sY128-R	AGC CCA ATG TAA ACT GGA CA
sY81-F	AGG CAC TGG TCA GAA TGA AG
sY81-R	AAT GGA AAA TAC AGC TCC CC
sY153-F	GCA TCC TCA TTT TAT GTC CA
sY153-R	CAA CCC AAA AGC ACT GAG TA
sY157-F	CTT AGG AAA AAG TGA AGC CG
sY157-R	CCT GCT GTC AGC AAG ATA CA
sY121-F	AGT TCA CAG AAT GGA GCC TG
sY121-R	CCT GTG ACT CCA GTT TGG TC
sY130-F	AGA GAG TTT TCT AAC AGG GCG
sY130-R	TGG GAA TCA CTT TTG CAA CT
sY208-F	GGA CAT AGT CCT GCT TAA GAA AAG TGG
sY208-R	ACG TGG TTC AGG AGG TCT ACT ATT CTA
sY84-F	AGAAGGGTCTGAAAGCAGGT
sY84-R	GCCTACTACCTGGAGGCTTC
sY124-F	CAG GCA GGA CAG CTT AAA AG
sY124-R	ACT GTG GCA AAG TTG CTT TC
sY182-F	TCA GAA GTG AAA CCC TGT ATG
sY182-R	GCA TGT GAC TCA AAG TAT AAG C

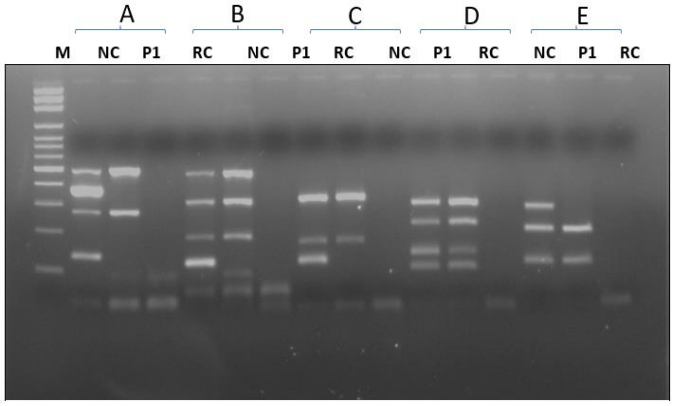
2.1 Ethical statement

Our study has been done on live subjects and written and informed consent was not necessary in our diagnostic setting. The informed consent was obtained by clinician when this material was collected for diagnosis. This is an amalgamation of our data of testing of these results. All these tests referred were part of the routine diagnostic procedure and clinical details were part of the Test Requisition Forms which were

analysed anonymously and the need for ethics committee approval was ruled out by The Institutional review board.

3. Results

A total of 763 patients ranging from 21-47 years with history of infertility and clinical suspicion of Y microdeletion were referred. Of the 763 patients referred, 30 patients were found to have Y-chromosome microdeletion, with a deletion rate of 3.9% (30/763). Patients with Y-chromosome microdeletion ranged from 25-47 years. Of these 30 patients, 1 patient showed deletion in AZFa, AZFb and AZFc with a deletion rate of 0.1% (1/763) and all of them were azoospermic, 20 patients had AZFc deletions with a deletion rate of 2.6% (20/763), 6 had AZFb deletions with a deletion rate of 0.8% (6/763), 3 patients showed deletion in AZFb and AZFc with a deletion rate of 0.4% (3/763) [Table 2]. Since, Metropolis Healthcare Ltd is a Global Reference Laboratory; samples for Y Microdeletion are received from all over India. This could be the reason for variation in the percentage of abnormality detected in our study and also because of the selection bias since only the highly suspected samples were referred to our laboratory for chromosomal studies. Figure 1 shows example of AZFc microdeletion.



M – 100bp Ladder
NC – Normal Control – Not Deleted
P1 – Detected (AZFc: sY152, sY153, sY157, sY254, sY255, sY152 markers are deleted)
RC – Reaction Control
Fig1: Example of AZFc microdeletion

Fig 1

4. Discussion

The causes for male infertility could be genetic or non-genetic. For most of the non-genetic causes some treatment is available and some of the causes are reversible [11, 12]. Most of the genetic causes are irreversible and unfortunately concrete treatment modalities are not available. Hence genetic workup is of paramount importance in cases with male infertility as a cause. It is important to note that depending upon the region deleted consequence differs. The most common deletion involves the AZFc region in Yq11.23. It is reported that AZFa and AZFb are more severe in effect than AZFc and a mosaicism of sex chromosome can have an additional effect with AZFc deletion [13]. The highest frequency of Y microdeletion is seen in azoospermic individuals and it is reported to be 8-12%. Almost 3-7% of Oligozoospermia have Y microdeletion [14]. Y microdeletion is very rarely seen in individuals with sperm concentration more than 5 million of spermatozoa/ml. Out of the Y microdeletion cases AZFc deletion is most common and seen in 65-70% of the cases, deletions of AZFb, AZFb plus AZFc or AZFa plus AZFb plus AZFc is seen in almost 25-30% of the cases and deletion of AZFa is extremely rare which contributes for 5% of the cases [15]. Individuals with AZFc deletion, and at a younger age with an excellent fertility may not experience any obvious difficulty they can have slightly milder form of phenotype with residual spermatogenesis in about 45-50% of cases while individuals with AZFa though rare can have very sever phenotype and complete absence of spermatogenesis or azoospermia [16]. Individuals with AZFb deletions have almost zero possibility of having mature spermatozoa [17]. Possibility of obtaining sperm by ART is almost 50% in individuals with AZFc deletion but to best of our knowledge no sperm retrieval has been reported in patients with AZFb and AZFa deletion. A male child of an individual with constitutional Yq microdeletion would likely to have similar infertility that of father as it is transmitted to the son [7]. Genetic counseling is very important in couples with Y microdeletion as a cause of infertility as this helps the couple and the clinician for selection of appropriate ART which may help the patient also in avoiding extra expenses on infertility management.

5. Conclusion

Almost 5-10% of males with non-obstructive azoospermia or severe oligospermia have microdeletion of AZF regions on long arm of chromosome Y as they are hardly seen in normospermic men association of Y microdeletions and spermatogenic failure is proved. Infertility workup and management is costly. Genetic counseling and knowing the genetic status before utilization of ART is very important this can avoid the expenses of the patient as it will help the genetic counsellor to counsel the patient. Also if at all genetic abnormality in Y chromosome in the form of microdeletion is identified depending upon the type and nature of the abnormality options of reproduction can be discussed with couple to make them informed choice that's why this protocol should be usually followed in medical practice to rule out the cause as Y Microdeletion for male infertility.

Table 2: Frequency of deletion of AZFa, AZFb and AZFc regions in our cohort

Region	n	%
AZFc	20/763	2.6
AZFb	6/763	0.8
AZFa, AZFb and AZFc	1/763	0.1
AZFb & AZFc	3/763	0.4

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7. Conflict of Interest: No

8. References

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