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Observational study of Y chromosome microdeletion, EAA markers and non EAA markers in Chhattishgarh

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ABSTRACT

Genetic factors contribute to 15% of all causes of male infertility. Y chromosome microdeletion is the second most common genetic cause of male infertility. Screening is important for Yq microdeletion as the defect can be transferred to offspring. Aim of our study is to detect the frequency of Y chromosome microdeletion in idiopathic infertile men using both EAA and non EAA markers in central region of India. Forty men from infertility clinic, seeking treatment of infertility were recruited in the study as cases. Thirty normal fertile men of same origin were recruited as controls. Semen analysis was done and cytogenetic normal infertile men were included in the study. Simplex and multiplex PCR methods were used to detect Yq microdeletions. Frequency of deletion was 11/40 (27.5%). Single deletion of AZF a,b,c were 12.5%, 7.5%, 2.5% respectively (Figure 1). Double deletions of AZF a+c and b+c were 2.5% each (Figure 2). Two subjects showed deletion for more than one loci. Overall frequency of deletion depends on sample size, no of markers used, inclusion criteria of subjects and geographic location. So, the screening is important for Yq microdeletion as the defect may be inherited to offspring.

1. Introduction

Outcome of pregnancy depends on the characteristics of male and female gametes. If one of them is not in existence or of not good quality, fertilization or quality of embryo formation would be affected. Male factor is responsible for approximately 50% of total infertility. Y chromosome microdeletion is an important factor in the occurrence of male infertility. There are many STS markers available to detect Y chromosome microdeletion. Usually six markers are tested at initial stage after semenogram that are recommended by European Academy of Andrology (EAA) society.[1] However other markers may be deleted even if EAA markers are not deleted. Therefore, testing of EAA markers may not serve the purpose in Indian population. Important non EAA markers should also tested along with EAA markers.

The development of male gamete takes place in various stages. Primordial germ cells which form in ectoderm of the bilaminer germ disc of human embryo and move to the wall of yolk sac give rise to spermatogonia. Spermatogonia converts in to primary spermatocytes by repeated mitotic division. Primary spermatocyte by meiotic division(I) develops secondary spermatocytes. Each secondary spermatocytes by meiotic division(II) forms two spermatids. The spermatids becomes spermatozoa by the process of spermiogenesis. This is the final stage in maturation. Overall process of spermatogenesis is regulated

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by gene present on Y chromosome. Quantity and quality of sperm depends on partial and complete deletion of genes.

Aim of our study is to determine the frequency of the Y chromosome microdeletion in idiopathic infertile men using both EAA and non EAA markers in central region of India. This microdeletion test is important for counseling of couples who opt for assisted reproductive technology (ART) as this defect in gene may get transferred to the offspring.

2. Materials and Methods

A total of forty men of Chhattisgarh origin presenting to infertility clinic seeking treatment of infertility were recruited in the study. Thirty normal fertile men of the same origin were enrolled as control. All infertile men and fertile men were of same age group ranging from 22-45 years. Infertile men in principle met the definition of infertility ie one year of unprotected intercourse and not leading to conception (regardless of fertility of partners). These were subjected to comprehensive questionnaire regarding their life style habits like smoking, alcohol and drug abuse, medical history, surgical history, marital age, sexual and family history. Every individual made available a minimum of two semen sample with abstinence period of three days. These specimens were evaluated on the basis of criteria of WHO 2010.[2] Informed consent was obtained from every couple. On the basis of mean sperm count, infertile men were grouped in azoospermia, severe oligozoospermia, oligzoospermia and normozoospermia. In addition, blood samples were obtained for DNA extraction.

Genomic DNA was obtained from Blood lymphocytes using Qiagen kit. Primers were designed for EAA markers sY86, sY 127, sY254, sY84, sY 134, sY255 and Non-EAA markers sY746, sY182, sY121, sY128, sY130, sY143, sY145, sY160 (Table 1). All PCR reactions were performed in a total reaction volume 13μl. PCR were in simplex or multiplex fashion. Additional STS marker for internal control was SRY gene. Fertile male and female DNAs were used as external controls. Five microliter of genomic DNA, 1X amplification buffer, mM dNTPs, 10-25 pmol of each primer and 1.25 IU of taq polymerase were used in reaction mixture. After initial denaturation 5min, annealing reaction was carried out at a temperature specific for that primer. PCR products were separated in 1.2- 2% agarose by gel electrophoresis.

3. Result

All cytogenetically normal infertile and fertile patients were subjected for deletion testing. Y chromosome microdeletion were confirmed for eleven STS markers. Eleven subjects showed deletion out of forty cases whereas no deletion was observed in controls. Out of eleven cases, two (18%) showed deletion for more than one loci (Tables 2 and 3). Single deletion of AZF a,b,c were 5/11, 3/11, 1/11 respectively (Figure 1). Double deletions of AZF a+c and b+c were observed in 1/11, 1/11 respectively (Figure 2). Total oligozoospic cases, severe oligozoospermic cases and azoospermic cases were 17/40, 11/40 and 12/40 respectively. Over all frequency of deletion in AZFa, AZFb and AZFc regions were found 7(12.5%), 4(7.5%) and 2(2.5%) cases respectively.

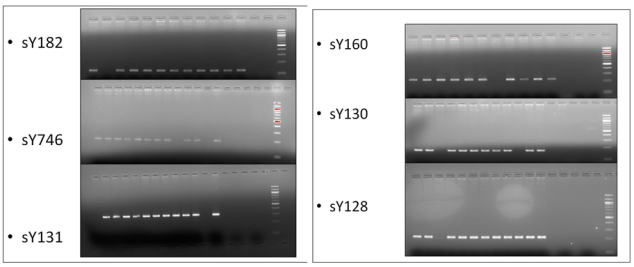

Fig. 1: Agarose gel analysis showing microdeletions of STS markers

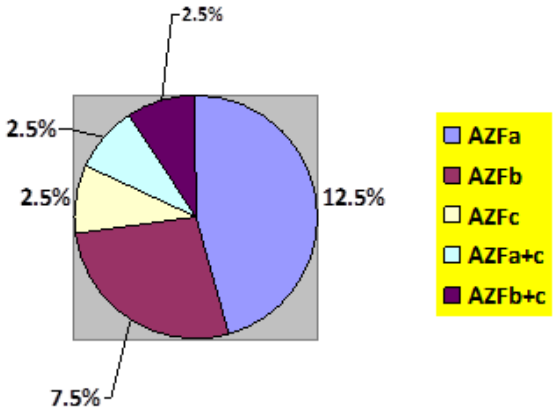

Fig. 2: Percentage deletion of AZF region in eleven subjects

4. Discussion

Many causes of infertility are treatable whereas many are not. Most of the genetic causes are not treatable and irreversible. Many males presenting without sperm in ejaculate have deletion of Ychromosome region. These deleted regions include azoospermia factor (AZF) gene. This AZF gene is located in long arm of Y chromosome. Tiepolo and Zuffardi[3] first localized factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm.

In earlier studies, there is distinguished variation in microdeletion frequency. These variations are depending on the selection criteria of patient, number of markers used, semenogram, and cytogenetic variability in studies. Arrest in different stages of spermetogenesis is reflection Yq microdeletion. The microdeletion in AZFa region

Table 1: Showing Non EAA markers and their primer sequence

Primers	Forward sequence	Reverse sequence
sY746	5'-TTGACTGCTTATTCTACACAA-3'	5'-CAGGGGAAATTGGGTTTT-3'
sY182	5'-TCAGAAGTGAAACCCTGTATG-3'	5'-GCATGTGACTCAAAGTATAAGC-3'
sY121	5'-AGTTCACAGAATGGAGCCTG-3'	5'-CCTGTGACTCCAGTTTGGTC-3'
sY128	5'-GGATGAGACATTTTTGTGGG-3'	5'-AGCCCAATGTAAACTGGACA-3'
sY130	5'-AGAGAGTTTTCTAACAGGGCG-3'	5'-TGGGAATCACTTTTGCAACT-3'
sY143	5'-GCAGGATGAGAAGCAGGTAG -3'	5'-CCGTGTGCTGGAGACTAATC-3'
sY145	5'-CAACACAAAAACACTCATATACTCG-3'	5'-TTGAGAATAATTGTATGTTACGGG-3'
sY160	5'-TACGGGTCTCGAATGGAATA-3'	5'-TCATTGCATTCCTTTCCCATT-3'

Table 2: Different Primer tested and cases showing deletions

S.No.	STS markers	Deletions in case	Bp	Locus	
1	sY86	No	320	AZFa	EAA STS markers
2	sY 127	C13	274	AZFb	
3	sY254	C13	380	AZFc	
4	sY84	No	326	AZFa	
5	sY 134	C13	301	AZFb	
6	sY255	C13	126	AZFc	
7	sY746	C28, C39	216	AZFa	
8	sY182	C11, C15, C22, C32	125	AZFa	
9	sY121	No	190	AZFb	Non EAA markers
10	sY128	C13	228	AZFb	
11	sY130	C13, C20	173	AZFb	
12	sY143	C4, C31,	311	AZFb	
13	sY145	C22,	143	AZFc	
14	sY160	C27,	236	AZFc	
16	sY14 SRY	No	476		

Table 3: Cases, deleted regions and semen condition

S.No.	Case no	Deleted AZF region	Semenogram
1	C4	AZFb	Oligozoospermia
2	C13	AZFb+c	Azoospermia
3	C11	AZFa	Sever Oligozoospermia
4	C15	AZFa	Sever Oligozoospermia
5	C20	AZFb	Oligozoospermia
6	C22	AZFa+c	Oligozoospermia
7	C27	AZFc	Azoospermia
8	C28	AZFa	Oligozoospermia
9	C31	AZFb	Oligozoospermia
10	C32	AZFa	Oligozoospermia
11	C39	AZFa	Sever Oligozoospermia

is associated with SOC syndrome and azoospermia. The microdeletion of AZFb region is associated with meiotic maturation arrest. The microdeletion of AZFc region is associated with arrest at spermatid stage or hypospermatogenesis.[4,5]

The microdeletion of AZFb region is associated with hypospermatogenesis. Most common deletion observed in our study was AZFa. These individuals were oligozoospermic and severe oligozoospermic. Deletion in AZFa region in oligozoospermic and azoospermic men were common.[6,7] Studies show highest frequency of deletion was AZFc type.[8,9] In our study it was AZFa type which may be due to small sample size.

Double deletion was observed in two subjects; AZFa+c and AZFb+c (Table 3). Deletion type AZFa+c is rare. AZFa+c was observed in oligozoospermic case. Only complete deletion of AZFa is associated with absence of sperm.[10]

The AZFb microdeletion is associated with developmental arrest of germ cells at spermatid stage and resulted to meiotic maturation arrest. AZFc deletion is associated with arrest of germ cell development at

spermatid stage and hypospermatogenesis with sperm count.

Sen et al. stated that frequency of deletion was significantly high in the group using both EAA and non EAA markers.[11] Based on this analysis approximately 3.1% cases would be missed if only EAA markers are used. In our study, only one case exhibited deletion of EAA markers out of forty cases and it was double deletion.

5. Conclusion

These findings add to the growing literature of Yq microdeletion. Both EAA markers and non EAA markers should be tested for Yq microdeletion. Frequency of EAA markers deletion was found in one case out of forty cases and it was double deletion. Very less frequency of Yq microdeletion was observed with EAA markers. Chances of missing of infertility cases can happen. This test is important to screen the patients who are opting assisted reproductive techniques as it may be transmitted in offspring.

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7. Conflict of Interest

None.

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