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A STUDY OF RBMY1 DELETION IN Y CHROMOSOME OF INFERTILE MALES OF NORTH EAST REGION, OF INDIA

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ABSTRACT

Even though curtailing population growth is a major concern, a substantial number of infertile couples in the Indian population have an equally great concern, that of having a child. This is an equally important problem concerning reproductive health.An attempt was made to study deletion mutation in 50 individuals for RBMY class of genes which has been established as a reason for males being infertile. This study is based on one of the class of RBMY i.e RBMY1 using PCR technique. Out of the 50 individuals studied deletion was observed in 11 samples accounting to 22% out of which 7(14%) were Azoospermic and 4 (8%) were Oligospermic . RAPD was done with two different primers.RAPD Analysis gives clear distinction between Azoospermic and Oligospermic samples the both of which again were different from fertile male.

INTRODUCTION

Infertility is a major problem among a substantial segment of Indian population. Research has been carried out in different regions of the world on the various aspects of infertility. Globally, the incidence of infertility is estimated to be about – 13-18% (Hull *et al.,* 1985, Thonneau *et al.,* 1991) in the human population, regardless of race, ethnic group etc.

Infertility may be due to problems in either male or female partner. Male problems may be contributory to 30% to 40% of infertile couples, and in another 20% of cases both men and women are affected. The male factor is therefore responsible in about 50% of infertile couples. The infertile male partner has qualitative or quantitative abnormalities of sperm production (Dada, Gupta and Kucheria 2002). In more than 60% of cases the origin of reduced testicular function is unknown (Krausz *et al*., 2000). Approximately 15% of the men seeking help at infertility clinics suffer from idiopathic azoospermia, the absence of mature sperm cells (spermatozoa) in seminal fluid. A significant proportion of infertile male with azoospermia and severe oligospermia (sperm counts of less than 5 to 10 million per ejaculation) have a genetic etiology for reproductive failure.

In men, the main causes of infertility are oligospermia, asthenospermia, teratozoospermia and azoospermia, which account for 20–25% of cases (Egozcue *et al.,* 2000; Hargreave 2000). A number of risk factors such as STD involving *N. gonorrhoeae* and *C. trachomatis* are also involved which cause changes in semen quality and chronic infection and thus may lead to a block of the *vas deferens* or seminal vesicles (Megory *et al.,* 1987) According to Simoni *et al.*, (2004) Microdeletions of the Y chromosome are the second most frequent genetic cause of spermatogenetic failure in infertile men after the Klinefelter syndrome. The molecular diagnosis of Y-chromosomal microdeletions is routinely performed in the workup of male infertility in men with azoospermia or severe Oligozoospermia

Morton (1991) reported that the Y chromosome is the smallest chromosome with 60 million base pairs. It is divided into long arm (Yq) and a short arm (Yp).The proximal portion of the AZFa region contains pseudogene sequences homologous to genes at Xp22. Other genes in the region include *DFFRY* (human homologue of the *Drosophila* development fat facets gene), dead box on the Y (*DBY*), ubiquitously transcribed tetretricopeptide repeat gene on the Y (*UTY*), and *AZFaT1* (Sargent *et al*.,1999) Chai *et al*, (1997) has divided the RNA binding motif protein on Y chromosome (RBMY) located in the AZFb region into six subclasses(RBMY1-6) consisting of 30 genes and pseudogenes. *RBM* may function in RNA metabolism. Deletion of the murine homologue of *RBM* results in male sterility, suggesting a similar role for the gene when it is absent in a small percentage of infertile men (Delbridge *et al*., 1997).

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Various researchers have carried works on the issue of Male infertility. However, little scientific study has been undertaken on the cases of North Eastern part of India till date. The North Eastern part of India consists of Mongoloid tribes, Adivasis and Arians as such it consist of diverse ethnic groups with different cultural and socio economic lifestyles.

The present work was restricted to the ethnic groups of North Eastern states. This work is based on the PCR analysis of a particular locus of Y chromosome, RBMY1 and RAPD Analysis of the samples.

MATERIAL AND METHODS

As many as 50 samples were collected from clinically diagnosed patients.DNA was extracted using 200ul of Blood using Geneipure^{TM} Blood Genomic DNA Isolation Kit (Genei,Merck Bioscience) following the manufactures protocol. Following the DNA extraction, Polymerase Chain Reaction was carried out with primers from earlier Literature (Viswambharan *et al.,* 2007). PCR was performed using TC-512 Gradient thermal Cycler (Techne make,Mktd By Genei,Merck Bioscience) for 35 cycles with initial denaturation of 94°C for 5 minutes, denaturation at 94°C for 30 sec, Annealing at 55°C for 30 secs ,extension at 72°C, final extension at 72°C for 5 mins and final hold at 10°C.(Reaction mixture in Table I).A known fertile male was taken as Positive control and female blood was taken as negative control. In one of the wells water was added to avoid any chances of contamination. Following PCR the product was loaded in 2% Agarose Gel stained with ethidium bromide. RAPD was performed to compare the Azoospermic, Oligospermic and Normal fertile males using two primers *RHu 1 Acc* No: AM765824 and *RHu 2* Acc No: AM773780 (Genei,Merck Bioscience) with 94°C initial denaturation, denaturation at 94°C for 45 secs,annealing at 35°C for 1min ,extension at 72°C for 10 cycles and another 40 cycles was done with denaturation at 94°C for 45 sec,annealing at 37°C for 45Sec and extension at 72°C for 1min and finally extension at 72°C for 10 min. Following PCR the amplicons were loaded in 2% Agarose gel and visualised in a gel documentation system.(The reaction mastermixes were as per Manufactures protocol;Genei Merck Bioscience)

RESULTS

Of the 50 samples tested 30 were Azoospermic and 20 were Oligospermic. Out of the 50 samples deletion was observed in 11 samples accounting to 22% out of which 7(14%) were Azoospermic and 4 (8%) were Oligospermic (Fig. 3). The fertile (normal) male showed the Band while no bands were visible in the female (Fig. 1). Distinct polymorphism was observed in the RAPD result too. 7 bands were visible with Primer *RHu1* in case of Normal male in comparison to 6 bands in Oligospermic and 4 bands in case of Azoospermic. Whereas with the use of primer *RHu2* 8 bands was visible in case of normal males while there were 2 in case of Azoospermic and 7 in case of Oligospermic (Fig. 2).This shows some deletion in case of Azoospermic and Oligospermic samples in comparision to Normal

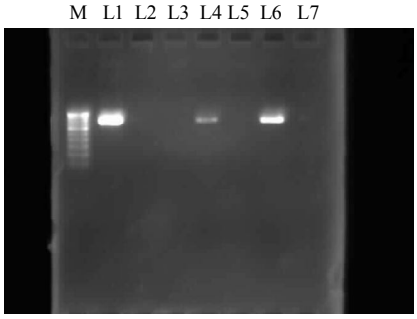

Fig.1: Gel Photograph of RBMY1 Gene in 2% Agarose Gel

Legend:
M: Marker(100bp DNA Ladder)
L1: Normal Male(Fertile)
L2: Negative Control(female)
L3: Water
L4: Sample (Azoospermic)
L5: Sample (Deletion)Azoospermic
L6:Sample(Oligo)
L7: Sample(Oligo) Deletion

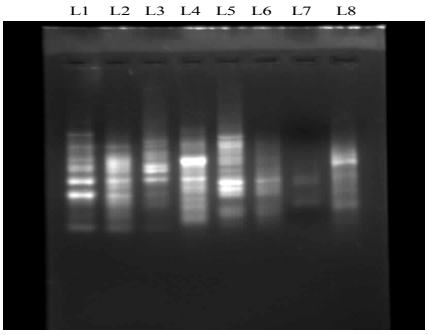

Fig 2: RAPD Profile

Legend:

PRIMER.RHu I Acc No: AM765824

Lane 1: Normal
Lane 2: Oligospermic
Lane 3: Azoospermic
Lane 4: Oligospermic

PRIMER RHu 2: Acc No: AM773780

Lane 5: Normal
Lane 6: Oligospermic
Lane 7: Azoospermic
Lane 8: Oligospermic

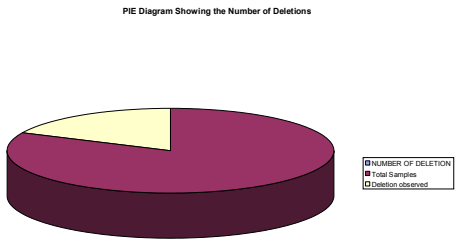

Fig 3 Pie Diagram Showing the Number of Deletion.

Total Individuals 50
Deletion was found in 11
Azoospermic 7
Oligospermic 4

PIE Diagram Showing the Percentage of Deletion wrt Azoospermic and Oligospermic

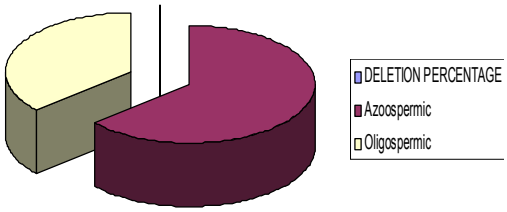

Table 1 Master mix used for PCR (50ul)

Reaction Components	Company	Amount Used
Sterile water	Genei (Merck Bioscience)	39ul
Forward Primer: 5' – CTT TGA AAA CAA TTC CTT TTC C – 3'	Genei (Merck Bioscience)	1ul
Reverse Primer: 5' – TGC ACT TCA GAG ATA CGG – 3'	Genei (Merck Bioscience)	1ul
dNTP Mix, 10 mM (2.5 mM each)	Genei (Merck Bioscience)	1ul
Genomic DNA		2ul
10X assay buffer	Genei (Merck Bioscience)	5ul
Taq DNA polymerase 3U/ul	Genei (Merck Bioscience)	1ul(3U)
	Total	50 ul

DISCUSSION

The PCR based study of Y chromosome microdeletions has emerged as diagnostic tool to detect the chromosomal aberrations which is otherwise not possible to detect with the help of Karyotyping or other conventional cytogenetic studies. To determine the Y chromosomal microdeletion will help to diagnose patients properly so that the genetic transfer of the deletions can be controlled. The deletion factors may be carried to the offspring. The frequencies of deletions of Yq, reported in different studies, range between 3% and 18% of males with non-obstructive azoospermia or severe oligospermia. (Reijo *et al.,* 1995). Viswambharan et *al.,* (2007) in their screening of 30 infertile males, reported that four patients carrying microdeletions corresponding to a frequency of 13.3% (four out of 30 patients had microdeletions).

CONCLUSION

From the study it can be concluded that the microdeletions are prevalent in the north eastern region of ndia. Pryor et *al*., 1997 reported that the micrdeletions in Y chromosome are between 13%-23% as such our study using a particular locus of the Y chromosome lies in between the earlier reported data.

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References

Chai NN,Salido EC,Yen PH.1997 Multiple Functional copies of the RBM family,a spermatogensis candidate on the human Y chromosome.Genomics .45.355-361

Dada R, Gupta NP and kucheria K 2002 AZFc microdeletions associated with Idiopathic and non idiopathic case with cryptorchidism and varicocele, Asian J. Androl 4 259-263.

Delbridge ML, Harry JL, Toder R, O'Neill RJ, Ma K, Chandley AC, Graves JA. 1997
A human candidate spermatogenesis gene, RBM1, is conserved and amplified on the marsupial Y chromosome. *Nat Genet.*1997; 15:131 –136.

de Vries JW, Hoffer MJ, Repping S, Hoovers JM,Leschott NJ,van der Veen F.,2002.
Reduction copy number of DAZ genes in subfertile and infertile men. Fertil Steril ;77:68-75.

Egozcue S, Blanco J, Vendrell JM, Garcia F, Veiga A, Aran B, Barri PN, Vidal F, Egozcue J 2000. Human maleinfertility: Chromosome anomalies meiotic
disorders,abnormal spermatozoa and recurrent abortion. Hum Reprod, 6: 93-105.

Foote S, Vollrath D, Hilton A, Page DC.1992. The human Y chromosome: overlapping DNA clones spanning the euchromatic region. Science
258:60-6.

Foresta C, Ferlin A, Garolla A, Rossato M.Barbaux S,De Bortoli A.1997 Y- chromosome deletions in idiopathic severe testiculopathies. J Clin Endocrinol Metab ;82:1075-80

Hull MG,Glazener CM,Kelly NJ, Conway DI,Foster PA,Hinton RA,Coulson C,Lambert PA,Watt EM,Desai KM: Population Study of Causes,treatment,and outcome of Infertility. Br Med J(Clin Res Ed) Dec 14;291(6510):1693-7

Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L,McElreavey K and Skakkebaek E.2001 Double-blind chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletionsare specific for spermatogenic failure. J Clin Endocrinol Metab ; 86:2638-42.

Krausz C, Quintana-Murci,L and McElreavey K 2000: Prognostic value of Y deletion analysis: What is the clinical prognostic value of Y chromosome microdeletion analysis? Hum.Reprod.15 1431-1434

Maria Rosa Maduro, Kira C Lo, Weber W.Chuang and Dolores J Lamb:2003Genes and Male Infertility: What Can Go Wrong? Journal of Andrology, Vol. 24, No. 4, July/August 2003 Morton NE.Parameters of the human genome.Proc Natl Acad Sci USA 1991:88:7474-7476

Mosher WD.1985 Reproductive impairments in the United States 1965-1982.

Demography ; 22:415-30.

Pryor JL, Kent-First M, Muallem A, et al.Van Bergen Andrew H,Nolten Wolfram

E,Meisner Lorraine,Roberts Kenneth P 1997 Microdeletions in the Y chromosome of infertile men. N Engl J Med; 336:534-9.

Reijo R, Lee TY, Salo P, Alagappan R,Brown LG,Rosenberg M,Rozen S,Jaffe T,Straus D,Hovatta O 1995. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995; 10:383-93.

Selva J, Kanafani S, Prigent Y, Poncet V,Bergere M ,1997 Incidence of AZF (Azoospermia Factor) deletions and familial forms of infertility among patients requiring intracytoplasmic spermatozoa injection (ICSI). J AssistReprod Genet ; 14:593-5.

Simoni M, Bakker E, Eurlings MC, Matthijis G.Moro E,Muller CR,Vogt PH 1999. Laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions. Int J Androl ; 22:292-9.11.

Simoni M, Gromoll J, Dworniczak B, Rolf C Abshagen K,Kamischke A,Carani C,Meschede D,Behre HM,Horst J,Nieschlaq E.1997 Screening for of the Y chromosome involving the DAZ (Deleted in Azoospermia) gene in azoospermia and severe oligozoospermia. Fertil Steril ;67:542-7.

Simoni M, Kamischke A, Nieschlag E. 1998 Current status of the molecular diagnosis of Y-chromosomal microdeletions in the work-up of male infertility – Initiative for international quality control. Hum Reprod ; 13:1764-7.

Sargent CA, BoucherCA, Kirsch S,Brown G,Weiss B,Trundley A,Burgoyne P,Saut N,Durand C,Levy N,Terriou P,Hargeave T,Cooke H,Mitchell M,Rappold GA,Affara NA 1999.The critical region of overlap defining the AZFa male infertility interval of proximal Yq contains three transcribed sequences J med Genet :36:670-677

Tiepolo L, Zuffardi O. 1976 Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 34:119-24.

Thangaraj K, Gupta NJ, Pavani K, Pavani K, Reddy AG,Subhramainan S,Rani DS, Ghosh B,Chakravarty B,Singh L (2003) Y chromosome deletions in azoospermic men in India. J Androl ; 24:588-97.

Thonneau P. Marchard S, Talke A, Ferial ML, Ducot B, Lansac J, Lopes P,Tabaste JM and Spira A 1991, Incidence and main courses of infertility in a resident population (1,850,000) of three French region (1988-1989) Hum Reprod 6 811-816.

Viswambharan N,,Suganthi R, MSc, Simon AM,Manonayaki S, M. 2007:Male infertility: polymerase chain reaction-based deletion mapping of genes on the human chromosome.Singapore Med J;48(11):1140

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