International Journal of Genetics and Molecular Biology Vol. 2(7), pp. 147-157, July 2010
Available online at http://www.academicjournals.org/IJGMB
ISSN2006-9863 ©2010 Academic Journals

Full Length Research Paper

The frequency of Y chromosome microdeletions in infertile men from Chennai, a South East Indian population and the effect of smoking, drinking alcohol and chemical exposure on their frequencies

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Accepted 13 July, 2010

The aim of the study were to estimate the frequency of Y chromosome microdeletion in infertile men from a new geographical ethnic region, Chennai, South East India, to explore the effect of smoking, alcohol drinking, chemical exposure and cellular chromosomal aberration on the frequency of infertility in 34 azoospermia and 55 oligospermia patients. The frequency of Y chromosome microdeletion was estimated using 12 STS markers and the chromosomal aberrations were estimated in leukocyte cultures. In azoospermia the frequency of microdeletions in AZFa, AZFb, AZFc and AZFd were 27, 4, 56 and 13% respectively. In oligospermia they were 33, 7, 48 and 12% in the same order. These frequencies of Y chromosome microdeletion are significantly higher than that of European population. The chromosome aberrations per cell in azoospermia and oligospermia were higher than that of the control at the level of p > 0.001. The percentage of microdeletion observed in unexposed azoospermia had 15%, azoospermia smokers 22%, azoospermia smokers and alcoholics 25%; whereas the unexposed oligospermia had 7%, oligospermia smokers 12%, oligospermia smokers and alcoholics 37%. It seems that the etiology of male infertility may differ between ethnic populations and smoking, alcohol drinking and chemical exposure may have deleterious effect on human fertility.

Key words: Y chromosome microdeletion, sequence-tagged site (STS), chemical exposure, chromosomal aberrations, ethnic region.

INTRODUCTION

Infertility, defined as the inability to conceive after 12 months of unprotected intercourse, affects 10 – 15% of all couples (Mosher and Pratt, 1991). In roughly half of the cases, a male factor is identified, while an occult male factor may be involved in 15–24% percent of cases in which no etiology is uncovered (“unexplained” infertility) (Skakkebaek et al., 1994; Templeton and Penney, 1982). A variety of occupational exposures have been linked to impaired male fertility (Sheiner et al., 2003). However, studies have been limited by inadequate sample sizes, inappropriate study designs, and/or selection bias (Lahdetie, 1995; Bonde et al., 1996; Cohn et al., 2002). Hence well defined further study with a good sample size of 34 azoospermia and 55 oligospermia patients is planned.

The AZF (azoospermia factor) region Yq11 contains genes vital for spermatogenesis. Vogt et al. (1996), Affara et al. (1999) subdivided this region into AZFa, AZFb and AZFc. Deletions within these sub-regions cause various spermatogenic and infertility phenotypes (Affara and Mitchell, 2000). Molecular studies have shown that microdeletions at Yq11 may represent the etiological factor in as many as 10 - 15% of cases with idiopathic azoospermia or severe oligozoospermia (Reijo et al., 1995; Vogt et al., 1996). The microdeletion events appear in three critical regions of the long arm of the Y chromosome, initially considered non overlapping, called

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Azoospermia factor (AZFa, AZFb and AZFc) (Vogt et al., 1996). Around 71% of men with Y chromosomal microdeletions and severe oligozoospermia or idiopathic azoo-spermia were found to have AZFc deletions compared with 13% with AZFa and 31% with AZFb deletions (Ferlin et al., 1999; Reynolds and Cooke, 2005). It is planned to estimate the frequency of various Y chromosome micro-deletion in 34 azoospermia and 55 oligospermia patients selected from a new geographical and ethnic population from Chennai and its surroundings in South India.

To what extent there is a genetic contribution is unclear. It has been reported that in a certain ethnic group, men with a particular haplotype (II) have a lower sperm concentration compared with men with haplotypes (III) and (IV) and, the frequency of haplotype (II) is more common in azoospermic men compared with normal men (Kuroki et al., 1999). Based on this, it appears that the genetic contribution towards male fertility on account of a de-creased sperm concentration might be significant in some ethnic groups (Seshagiri, 2001). Hence it is planned to study the frequency and pattern of infertility in a new ethnic population at Chennai in South India.

In recent years, there has been increasing concern about the possible deleterious effects of sperm quality. Amongst other factors, cigarette smoke is one factor which can theoretically influence male fertility in several ways. Unfortunately, the results of several studies in this area have been contradictory. In some studies, no relation could be demonstrated between tobacco consumption and sperm quality whilst in other reports, an association has been described between smoking and low sperm count, a relatively higher proportion of abnormal spermatozoa and reduced sperm motility (Oldereid et al., 1989). Hence it requires further study to get a clear picture.

There appears to be a worldwide concern over decreasing human sperm concentration but this has been highly controversial (Seshagiri, 2001). Decreasing sperm counts are attributed to the deleterious effects of environmental contamination by heavy metals and chemical exposure during working in chemical industry (Mehta and Anandkumar, 1997; Benoff et al., 2000; Sharpe, 2000). Hence it is planned to study the effect of chemical exposure to understand the degree of deleterious effect on sperm count.

Microdeletion of the Y chromosomes that remove associated fertility genes have received attention of late (Chandley, 1998). Structural abnormalities do lead to phenoltypic male reproductive disorder or may predispose to severe congenital abnormality when gametes are formed (Diemer and Desjardins, 1999). A study is planned to estimate and detect the frequency of chromosomal aberrations in all the patients studied.

It may be concluded that though it was known that azoos-permia and severe oligospermia cause infertility in human yet it is further decided to detect in this study the impact of certain life style like smoking, drinking alcohol, exposure to some toxic chemicals and environmental induced chromosomal aberrations on the severity of infertility infertility.

MATERIALS AND METHODS

34 azoospermic and 55 oligospermic men, selected from the Andrology Department, Stanley Medical College and Hospitals, Chennai, India, were included in the present study. The age groups of azoospermic men ranged from 24 - 38 years and oligospermic men age ranged from 16 - 37 years. With the help of an experienced urologist at Stanley Hospital, a detailed case history and clinical examination of every patient were carried out. The life style habit and chemical exposure of the probands were recorded, including smoking habit, alcohol drinking and exposure to toxic chemicals.

Semen analysis is routinely performed on the male partner of couple coming for infertility treatment. Hundred random fertile Indian men were included in this study as control. Blood samples from each azoospermic, oligospermic and control men were collected by the Physicians with the written consent. For this study ethical clearance was obtained from the Madras Medical College, Chennai, India.

Cytogenetic analysis

2 ml of intravenous blood was collected from every patient and control by using sodium heparin coated vaccutainer. The cytogenetic studies were carried out to find out the karyotype, frequency and type of chromosomal aberrations. Chromosome preparations were obtained from PHA-stimulated peripheral blood lymphocytes by using modified method of Hungerford, (1965). At least fifty well spread metaphase plates were scored by direct microscopic analysis. Scoring of chromosomal aberrations including chromatid and chromosomal breaks and deletions were carried out and recorded from well spread and stained cells under oil immersion objective lens (100×) of the light microscope. Well spread metaphases were photographed under oil immersion objective lens (100X) of Leica DM2000 microscope with Metasystems camera and the photomicrographs of banded spreads were karyotyped using automatic IKaros software (Metasystems). The karyotype was described according to the International System for Human Cytogenetic Nomenclature (Shaffer and Tommerup, 2005).

Molecular analysis

The molecular study was carried out in all the patients and control sample to make a thorough analysis to detect the Y chromosome microdeletion in AZF region.

DNA extraction and quantification

9 ml of intravenous blood was collected from all the patients and control sample by using EDTA coated vaccutainer. The genomic DNA was extracted from peripheral blood by using modified method of Lotery et al. (2000). Qualitative analysis of DNA was carried out by 0.8% Agarose gel electrophoresis and quantification of DNA by using Biophotometer (Eppendorf). Dilutions of DNA were made up to 10 ng/µl concentration by using TE buffer, pH 8.0. The 10 ng/µl of concentrated DNA solution was checked on 0.8% agarose gel.

Polymerase chain reaction analysis

The polymerase chain reaction (PCR) based studies for

microdeletion on azoospermic, oligospermic and control men were carried out using STS markers on the long arm of Y chromosome. Screening for AZF region was done using 12 STS markers. The AZFa region was analyzed with sY82 and sY84. The AZFb region was analyzed with sY164. The AZFc region was analyzed with sY158, sY160, sY240, sY254, sY255, sY277 and CDY. The AZFd region was analyzed with sY145 and sY152. The lyophilized primers were ordered and received from the company (Ist Base Pvt. Ltd, Singapore). Polymerase chain reaction consisted of 10µl PCR reaction mixture and included 1.0 µl PCR buffer (10×), 1.0 µl MgCl2 (25 mM), 0.8 µl deoxynucleotide tri-phosphates (10 mM), 0.5 pM of each primer, 1unit of Taq Polymerase (1 unit/ul) and 20 ng of genomic DNA. All the reagents for PCR were purchased from Vivantis Technologies (Malaysia). Each marker was amplified separately in a 0.2 mL thin wall tube using an Eppendorf thermal cycler with a female negative control sample. PCR conditions used for STS markers were as follows: initial denaturation (95°C for 5 min), subsequent denaturations (94°C for 1min) and extension (72°C for 1 min) were the same for all the samples. Different annealing temperatures that were used for different STS markers were as follows: 60°C for 15 s for sY82, sY254 and sY277; 60°C for 1 min for sY158, sY160, sY240, and sY255; 57°C for 30 s for sY84, Chromodomain Y(CDY) and sY145; 58°C for 30 s for sY164 and sY152. The PCR products were separated by eletrophoresis on 2% agarose gel. A 100 bp DNA ladder was loaded with PCR products to estimate band size. The gel was stained with ethidium bromide and visualized under UV transilluminator and photographed.

RESULTS AND DISCUSSION

Cytogenetic analysis

Spermatogenesis is a complex process and it is subject to the influence of many genes. Genetic factors involved in male infertility are manifested as chromosomal disorders, monogenic disorders, multi-factorial disorders and endocrine disorders of genetic origin (Diemer and Desjardins, 1999; Egozcue et al., 2000). Chromosomal abnormalities are common in infertile men, for example, Klinefelter syndrome (Egozcue et al., 2000). Besides numerical abnormalities, structural abnormalities also lead to phenotypic male reproductive disorder or may predispose to severe congenital abnormality when gametes are formed (Diemer and Desjardins, 1999).

In the present study no chromosomal abnormality was observed. G banded metaphase analyses of all 34 cases of azoospermia revealed 46, XY normal karyotype. The frequency of naturally occurring chromosome aberrations per cell in the patient was 0.18 and in the control it was 0.014. In the patients the frequency of chromosomal aberrations was significantly higher than that of the control at the level of $p > 0.001$.

In the same manner in all 55 cases of oligospermia revealed 46, XY normal karyotype. The frequency of chromosome aberrations per cell in the patient was 0.16 and in the control it was 0.014. In the patients the frequency of chromosomal aberrations was significantly higher than that of the control at the level of $p > 0.001$. These increased cellular chromosome aberrations may lead to phenotypic male reproductive disorder and may predispose to congenital abnormalities resulting in increased infertility. The sperm count was less than 4 - 20 million per ml in the oligospermic patients and for controls more than 20 million/ml were observed.

The Y chromosome microdeletion frequency analysis

In Tiepolo and Zuffardi (1976) observed for the first time the involvement of Yq deletions in male infertility when they were analyzing cells from idiopathic infertile males. Since then, many structural abnormalities in the Y chromosome have been observed, including microdeletions detectable only by molecular methods. In a foreign population in England Pryor et al. (1997) showed Y chromosome microdeletions in azoospermia are 16%. Mole-cular studies have shown that microdeletions at Yq11 may represent the etiological factor in as many as 10 - 15% of cases with idiopathic azoospermia or severe oligozoospermia (Reijo et al., 1995; Vogt et al., 1996).

The microdeletion events appear in three critical regions of the long arm of the Y chromosome, initially considered non overlapping, called Azoospermia factor (AZFa, AZFb and AZFc) (Vogt et al., 1996). Around 71% of men with Y chromosomal microdeletions and severe oligozoospermia or idiopathic azoospermia were found to have AZFc deletions compared with 13% with AZFa and 31% with AZFb deletions (Ferlin et al., 1999; Reynolds and Cooke, 2005). Challenges for current research include the elucidation of the genomic mechanisms that generate such recurrent deletions and also the identification of the genes that cause infertility when deleted or damaged. Recombination between repetitive regions is believed to be the cause of the high incidence of de novo microdeletions in the Y chromosome long arm. For instance, Kuroda-Kawaguchi et al. (2001) demonstrated that 47 out of 48 men with AZFc deletions had the same proximal and distal breakpoints in 229 kb direct repeats flanking AZFc. Furthermore, Repping et al. (2002) demonstrated that recombination between repetitive regions in Yq can explain the majority of AZFb and AZFb + AZFc deletions.

Recently, it was shown that homologous recombination events are highly recurrent in the MSY region, especially at the AZFc locus (Repping et al., 2003; Skaletsky et al., 2003; Machev et al., 2004). The spermatogenic impairment caused by AZFb and AZFc deletions can be actually caused by genes mapped at these regions as proposed by several studies (Brown et al., 1998; Mahadevaiah et al., 1998). Since the first deletion mapping studies until the most recent and detailed physi-cal map of the human Y chromosome several genes rela-ted to spermatogenesis were discovered (Skaletsky et al., 2003).

The patients selected for this study from a new geographical ethnic population showed in 34 cases of azoospermia the microdeletion events in four critical regions of the long arm of the Y chromosome, namely azoospermia factor (AZFa, AZFb, AZFc and AZFd). 56% of azoospermia

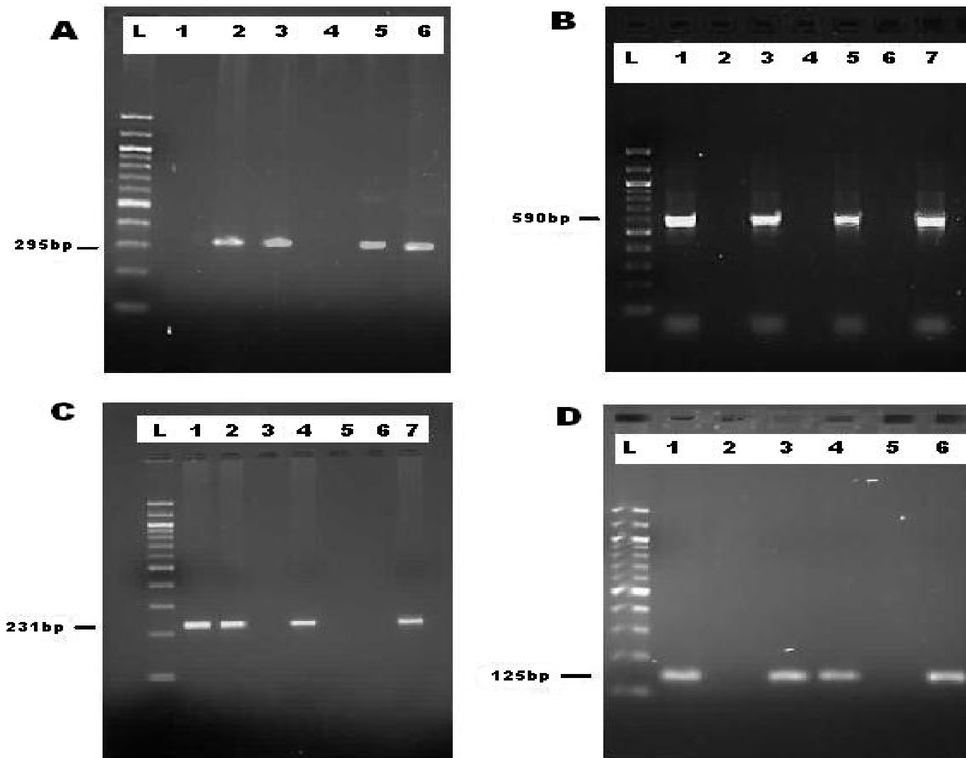

Figure 1. PCR analysis of Y chromosome microdeletions in azoospermic and oligospermic cases. (A) agarose gel electrophoresis analysis shows the microdeletion of AZFa region SY84 (295bp) STS marker of Y chromosome in azoospermia case (AZO17-1). L -100 bp DNA ladder, 1 - negative control, 2 - positive control, 3 - AZO16-1= azoospermia, 4 - AZO17-1= azoospermia (deletion), 5 - AZO19-1= azoospermia, 6 - AZO20-1= azoospermia. (B) agarose gel electrophoresis analysis shows the microdeletion of AZFb region SY164 (590bp) STS marker of Y chromosome in oligospermia cases (OLI33-1& 46-1). L - 100 bp DNA ladder, 1 - positive control, 2 - negative control, 3 - OLI32-1= oligospermia, 4 - OLI33-1= oligospermia (deletion), 5 - OLI34-1= oligospermia, 6 - OLI461= oligospermia (deletion), 7 - OLI47-1= oligospermia. (C) agarose gel electrophoresis analysis shows the microdeletion of AZFc region SY158 (231bp) STS marker of Y chromosome in oligospermia cases (OLI33-1& 35-1). L - 100 bp DNA ladder, 1 - positive control, 2 - OLI36-1= oligospermia, 3 - negative control, 4 - OLI32-1= oligospermia, 5 - OLI33-1= oligospermia (deletion), 6 - OLI35-1= oligospermia (deletion), 7 - OLI36-1= oligospermia. (D) agarose gel electrophoresis analysis shows the microdeletion of AZFd region SY145 (125bp) STS marker of Y chromosome in azoospermia case (AZO29-1). L -100 bp DNA ladder, 1 - positive control, 2 - negative control, 3 - AZO27-1= azoospermia, 4 - AZO28-1= azoospermia, 5 - AZO29-1= azoospermia (deletion), 6 - AZO31-1= azoospermia.

men with Y chromosomal microdeletions to have AZFc deletions as compared with 27% with AZFa, 4% with AZFb and 13% with AZFd deletions (Figures 1a, d and 2a and Table 1).

In oligospermia the microdeletion events in four critical regions (AZFa, AZFb, AZFc and AZFd) showed 48% of oligopermia men with AZFc deletions as compared with 33% with AZFa, 7% with AZFb and 12% with AZFd deletions (Figures 1b and c, 2b and Table 2). These results showed higher frequency when compared with European ethnic population.

Thus the overall frequency of Y chromosome micro-deletion detection in the infertile South Indian population in the present study was found to be (24.71%). The frequency of Y chromosome microdeletions was higher in azoospermic males (29.41%) compared with the cases of oligospermia (21.81%). In our report the frequencies of microdeletions were higher than that reported by Stuppia et al. (1996) and Yao et al. (2001) as 21 and 18.7% respectively.

AZFc region is one of the most important candidate genes involved in infertility, microdeletions in this region is known to cause sterility via meiotic arrest or absence of germ cells (Yao et al., 2001). In this study we observed 56% Y chromosome microdeletions in AZFc region in azoospermic cases and 48% in oligospermia cases. This

Table 1. The details of the karyotype, lifestyle and molecular analysis in azoospermia cases.

S. NO	Lab code	AGE	SMO	ALO	CHE	Karyotype	AZFa		AZFb	AZFc							AZFd	
							SY82	SY84	SY164	SY158	SY160	SY240	SY254	SY255	SY277	CDY	SY145	SY152
1	AZO1–1	28	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
2	AZO2–1	29	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
3	AZO3–1	29	–	–	–	46,XY	P	P	P	P	P	P	D	P	P	P	P	P
4	AZO4–1	30	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
5	AZO5–1	33	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
6	AZO6–1	31	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
7	AZO7–1	32	–	–	–	46,XY	P	P	P	P	D	P	P	P	P	P	P	P
8	AZO8–1	31	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
9	AZO9–1	29	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
10	AZO10–1	28	–	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
11	AZO11–1	31	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
12	AZO12–1	34	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
13	AZO13-1	35	†	–	–	46,XY	P	D	P	P	P	P	P	D	P	D	P	P
14	AZO14-1	32	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
5	AZO15-1	36	†	†	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
16	AZO16-1	26	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
17	AZO17-1	28	†	–	–	46,XY	D	D	P	P	P	P	P	D	P	D	P	P
18	AZO18-1	31	†	–	–	46,XY	D	P	P	P	P	P	P	D	P	P	P	D
19	AZO19-1	36	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
20	AZO20-1	32	–	–	†	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
21	AZO21-1	37	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
22	AZO22-1	38	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
23	AZO23-1	32	–	–	†	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
24	AZO24-1	24	†	–	–	46,XY	P	D	P	D	D	P	D	P	P	P	P	D
25	AZO25-1	29	†	–	–	46,XY	D	D	P	P	P	D	P	P	P	D	P	P
26	AZO26-1	27	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
27	AZO27-1	31	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
28	AZO28-1	32	†	†	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
29	AZO29-1	35	–	–	†	46,XY	D	D	D	D	D	D	D	D	P	D	D	P
30	AZO30-1	36	†	†	–	46,XY	D	D	D	D	D	D	D	D	P	D	D	D
31	AZO31-1	34	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
32	AZO32-1	37	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P
33	AZO33-1	38	–	–	†	46,XY	P	D	P	P	P	P	P	D	P	P	P	D
34	AZO34-1	29	†	–	–	46,XY	P	P	P	P	P	P	P	P	P	P	P	P

P, Present; **†,** Exposed; **SMO,** Smoking; **CHE,** Chemical exposure; **D,** Deleted; **–,** unexposed; **ALO,** Alcoholics.

is in agreement with the earlier studies showing that the incidence of deletions in the AZFc region was higher when compared with the AZFa and AZFb regions (Martinez et al., 2003; Peterlin et al., 2002). The size of the deletion on the Y chromosome in our study did not show any significant correlation with the amount of sperm production in oligospermia.

The effect of smoking, alcohol drinking and chemical exposure on the frequency of fertility

In recent years, there has been increasing

Table 2. The details of the sperm count, lifestyle and molecular analysis in oligospermia cases.

S.NO	Lab code	Age	SMO	ALO	CHE	Sperm count per ml	AZFa		AZFb	AZFc							AZFd	
							SY82	SY84	SY164	SY158	SY160	SY240	SY254	SY255	SY277	CDY	SY145	SY152
1	OLI 1–1	32				20	P	P	P	P	P	P	P	P	P	P	P	P
2	OLI 2–1	29	–	–	–	18	P	P	P	P	P	P	P	P	P	P	P	P
3	OLI 3–1	36	–	–	–	> 19	P	P	P	P	P	D	P	P	P	P	P	P
4	OLI 4–1	30	–	–	–	15	P	P	P	P	P	P	P	P	P	P	P	P
5	OLI 5–1	28	–	–	–	16	P	P	P	P	P	P	P	P	P	P	P	P
6	OLI 6–1	27	–	–	–	> 14	P	P	P	P	P	P	P	P	P	P	P	P
7	OLI 7–1	28	–	–	–	19	P	P	P	P	P	P	P	P	P	D	P	P
8	OLI 8–1	31	–	–	–	>15	P	P	P	P	P	P	P	P	P	P	P	P
9	OLI 9–1	30	–	–	–	20	P	P	P	P	P	P	P	P	P	P	P	P
10	OLI 10–1	35	–	–	–	> 17	P	P	P	P	P	P	P	P	P	P	P	P
11	OLI 11–1	29	–	–	–	16	P	P	P	P	P	P	P	P	P	P	P	P
12	OLI 12–1	32	–	–	–	18	P	P	P	P	P	P	P	P	P	P	P	P
13	OLI 13-1	26	–	–	–	> 14	P	P	P	P	P	P	P	P	P	P	P	P
14	OLI 14-1	28	–	–	–	16	P	P	P	P	P	P	P	P	P	P	P	P
5	OLI 15-1	30	–	–	–	> 19	P	P	P	P	P	P	P	P	P	P	P	P
16	OLI 16-1	24	†	–	–	12	P	P	P	P	P	P	P	P	P	P	P	P
17	OLI 17-1	26	†	–	–	> 16	P	P	P	P	P	P	P	P	P	P	P	P
18	OLI 18-1	31	†	–	–	10	P	P	P	P	P	P	P	P	P	P	P	P
19	OLI 19-1	33	†	–	–	> 7	D	P	P	P	D	P	D	P	P	P	P	P
20	OLI 20-1	17	†	–	–	> 5	P	P	P	P	P	P	P	P	P	P	P	P
21	OLI 21-1	36	†	†		11	P	D	P	P	D	D	P	P	P	P	P	D
22	OLI 22-1	35	–	–	†	13	D	D	P	D	P	D	P	D	P	P	D	D
23	OLI 23-1	16	†	–	–	> 9	P	P	P	P	P	P	P	P	P	P	P	P
24	OLI 24-1	21	†	–	–	> 6	P	P	P	P	P	P	P	P	P	P	P	P
25	OLI 25-1	26	†	–	–	12	P	P	P	P	P	P	P	P	P	P	P	P
26	OLI 26-1	19	†	–	–	13	P	P	P	P	P	P	P	P	P	P	P	P
27	OLI 27-1	19	†	–	–	15	P	P	P	P	P	P	P	P	P	P	P	P
28	OLI 28-1	32	†	–	–	4	P	D	P	P	P	P	P	D	P	P	P	D
29	OLI 29-1	26	–	–	†	> 8	P	P	P	P	P	P	P	P	P	P	P	P
30	OLI 30-1	28	†	†	–	16	P	P	P	P	P	P	P	P	P	P	P	P
31	OLI 31-1	31	–	–	†	> 10	P	P	P	P	P	P	P	P	P	P	P	P
32	OLI 32-1	36	†	†		13	P	P	P	P	P	P	P	P	P	P	P	P
33	OLI 33-1	34	–	–	†	> 8	D	D	D	D	P	D	P	D	P	P	P	P
34	OLI 34-1	31	–	–	†	> 4	P	P	P	P	P	P	P	P	P	P	P	P
35	OLI 35-1	31	†	†	–	10	D	D	P	D	P	P	P	D	P	P	P	P

Table 2. Contd.

36	OLI 36-1	35	†	–	–	> 7	P	P	P	P	P	P	P	P	P	P	P	P
37	OLI 37-1	29	–	–	†	12	P	P	P	P	P	P	P	P	P	P	P	P
38	OLI 38-1	35	–	–	†	16	D	D	P	D	P	P	P	P	P	P	P	P
39	OLI 39-1	37	–	–	†	11	P	P	P	P	P	P	P	P	P	P	P	P
40	OLI 40-1	36	†	†	–	19	D	D	P	P	P	P	P	P	P	P	P	D
41	OLI 41-1	32	†	†	–	15	P	P	P	P	P	P	P	P	P	P	P	P
42	OLI 42-1	29	†	†	–	> 14	P	P	P	P	P	P	P	P	P	P	P	P
43	OLI 43-1	28	†	†	–	> 6	P	P	P	P	P	P	P	P	P	P	P	P
44	OLI 44-1	30	†	†	–	> 9	P	P	P	P	P	P	P	P	P	P	P	P
45	OLI 45-1	29	†	†	–	17	P	P	P	P	P	P	P	P	P	P	P	P
46	OLI 46-1	37	–	–	†	> 6	P	D	D	P	D	P	P	P	P	P	P	P
47	OLI 47-1	31	†	†	–	16	P	P	P	P	P	P	P	P	P	P	P	P
48	OLI 48-1	28	–	–	†	> 11	P	P	P	P	P	P	P	P	P	P	P	P
49	OLI 49-1	29	†	–	–	14	P	P	P	P	P	P	P	P	P	P	P	P
50	OLI 50-1	30	†	–	–	17	P	P	P	P	P	P	P	P	P	P	P	P
51	OLI 51-1	34	†	–	–	> 4	P	P	P	P	P	P	P	P	P	P	P	P
52	OLI 52-1	29	†	–	–	> 10	P	P	P	P	P	P	P	P	P	P	P	P
53	OLI 53-1	18	†	–	–	14	P	P	P	P	P	P	P	P	P	P	P	P
54	OLI 54-1	20	†	–	–	16	P	P	P	P	P	P	P	P	P	P	P	P
55	OLI 55-1	31	†	†	–	> 8	P	P	D	P	D	P	D	P	P	D	P	P

P, Present; †, Exposed; SMO, smoking; E, Chemical exposure; D, Deleted; –, unexposed; ALO, Alcoholics.

concern about the possible deleterious effects of environmental factors on sperm quality. Amongst others, cigarette smoke is one factor which can theoretically influence male fertility in several ways. Suggested mechanisms include mutagenic effects of aromatic hydrocarbons (Kier et al., 1974), toxic effects of heavy metals such as cadmium (Ostergaard, 1977), reduced availability of haemoglobin due to carbon monoxide (Kaufman et al., 1983), accumulation of radio-active particles in the testes (Ravenkolt, 1982) and toxic effects of nicotine (Mattison, 1982). Significantly elevated leukocytes have been reported in the peripheral blood of smokers (Parry et al., 1997). Leukocytes are the major source of reactive oxygen species (ROS) in the ejaculate (Sharma and Agarwal, 1996). Elevated leukocytes may impair fertility by formation of ROS (Ochsendorf, 1999). ROS are harmful to sperm DNA (Shen et al., 1999) and membrane phospholipids (Kim and Parthasarathy, 1998) because of oxidation. The effects of excessive oxidation on sperm function have been suggested as detrimental. The role of ROS, however and whether ROS concentrations were elevated in the semen of smokers, has not been studied yet. Unfortunately, the results of several studies in this area have been contradictory. In some studies, no relation could be demonstrated between tobacco consumption and sperm quality whilst in other reports, an association has been described between smoking and low sperm count a relatively higher proportion of abnormal spermatozoa and reduced sperm motility. In one study, enhanced sperm movement has been associated with smoking, at least within the first hour of

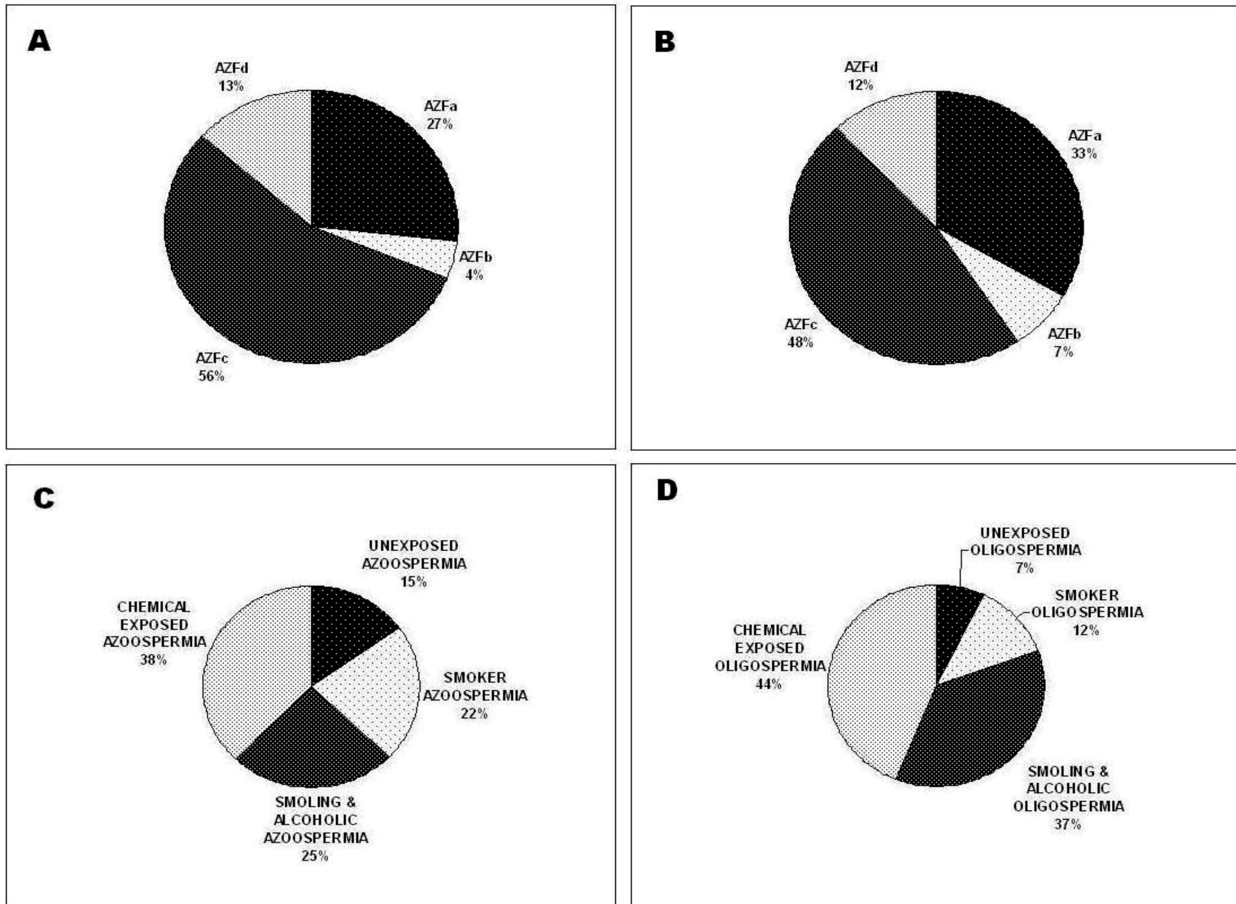

Figure 2. Pie diagram showing the percentage of Y chromosome deletion observed in AZF regions of azoospermia and oligospermia. (A) In azoospermia the microdeletion events in four critical regions of the long arm of the Y chromosome, namely azoospermia factor (AZFa, AZFb, AZFc and AZFd). 56% of azoospermia men with Y chromosomal microdeletions to have AZFc deletions as compared with 27% with AZFa, 4% with AZFb and 13% with AZFd deletions. (B) In oligospermia the microdeletion events in four critical regions (AZFa, AZFb, AZFc and AZFd) showed 48% of oligopermia men with AZFc deletions as compared with 33% with AZFa, 7% with AZFb and 12% with AZFd deletions. (C) In this new ethnic population the percentage of microdeletion observed in unexposed azoospermia had 15%, smoker azoospermia 22%, smoker and alcoholic azoospermia 25% and chemical exposed azoospermia had 38%. (D) The percentage of microdeletion observed in unexposed oligospermia had 7%, smoker oligospermia 12%, smoker and alcoholic oligospermia 37% and chemical exposed oligospermia had 44%.

ejaculation (Saaranen et al., 1987). Earlier studies have generally found an association between female smoking and pro-longed time to pregnancy (Howe et al., 1985; Alderete et al., 1995), but no association for male smoking (Baird and Wilcox, 1985; Suonio et al., 1990; Bolumar et al., 1996). Studies of caffeine use among women have found both decreased fecundability (Joesoef et al., 1990; Hatch et al., 1993) and no association (Joesoef et al., 1990; Florack et al., 1994), while studies examining male caffeine use have not found an association with fecundability (Florack et al., 1994). No associations between alcohol use and fecundability have been found (Weinberg et al., 1989; Florack et al., 1994). Limitations of some of these studies include failure to examine dose-response gradients and lack of control of spouse's behaviors. In this new ethnic Dravidian population the percentage of microdeletion observed in unexposed azoospermia had 15%, azoospermia smokers 22%, azoospermia smokers and alcoholics 25% and chemical exposed azoospermia had 38% (Figure 2C). The percentage of microdeletion observed in unexposed oligospermia had 7%, oligo-spermia smokers 12%, oligospermia smokers and alcoholics 37% and chemical exposed oligospermia had 44% (Figure 2D). The consumption of alcohol shows little effect on fecundability (Olsen et al., 1983). Using the endpoint of TTP (time to pregnancy, length of time to achieve pregnancy), Sallmen et al. (1998) found limited support for the hypothesis that paternal exposure to organic solvents might be associated with decreased fertility. Farmers and agricultural workers are exposed to a variety of potentially harmful chemicals. No association

was previously found between exposure to chemicals and infertility (Gerber et al., 1988). By contrast, occupational exposure to pesticides in fruit growers in The Netherlands (De Cock et al., 1994) was found. In an infertility-consulting population, environmental exposure, particularly to pesticides and solvents, is associated with dramatic changes in seminal characteristics (Oliva et al., 2001).

It was known that a comparison of the baseline characteristics of infertile and fertile men revealed that infertile men were more likely than fertile men to be Caucasian population, employed in blue-collar jobs, and less educated. Cases and controls were not evenly distributed among the clinical sites, and significant overall differences remained even when sites were categorized by geographic location. Among infertile subjects, socio-economic characteristics were similar for persons with malefactor infertility and persons with unexplained infertility (Gracia et al., 2005).

Further it has been reported that in a certain ethnic men with a particular haplotype (II) have a lower sperm concentration compared with haplotype (III) and (IV) and the frequency of haplotype (II) is more common in azoospermia men compared with normal men (Kuroki et al., 1999).Thus it seems that genetic contribution towards male fertility on the account of a decreased sperm concentration might be significant in some ethinic group. Encouraged by those observations, we planned to look into the azoospermia and oligospermia patients from a new geographical and ethnic Dravidian population, Chennai, South east India. The assumption was proved to be correct that the frequency of infertility is high in the new Dravidian population of Chennai, South east India.

Conclusion

The high frequency of Y microdeletions suggests that the Y chromosome is susceptible to spontaneous loss of genetic material. Aberrant recombination events occur between areas of homologous or similar sequence repeats between X and Y chromosome or within Y chromosome itself by unbalanced sister chromatid exchanges (Yen et al., 1990). The instability of the Y chromosome may be related to a high frequency of repetitive elements clustered along the length of the chromosome (Krausz and McElreavey, 1999). Although it is very clear that microdeletions in the AZF region are responsible for spermatogenic failure, further studies are worthwhile to delineate the exact function of the genes present in AZF region and their role in spermatogenesis and fertility. However, etiologies of a large number of azoospermic men are still unknown. Analyzing the remaining azoospermic men with additional Y chromosome STS, X chromosome, and autosomal markers would help in identifying the etiology of the remaining azoospermic individuals (Brandell et al., 1998; Wang et al., 2001). In light of this study, we believe that the etiology of male infertility may differ between ethnic populations. Therefore, researchers need to keep this in mind and define the strategies for analyzing infertile samples. This data will be useful for infertility clinics for genetic counseling by advising them to choose a female child in case of Y chromosome deletion and to adopt appropriate methods for assisted reproduction.

ACKNOWLEDGMENTS

The authors are indebted to patients and extremely grateful to Dr. Dhanapal and Dr. Sudhakar, Stanley Medical College and Hospitals, Chennai, India for providing us with the samples. The authors would also like to thank the management of VIT University for providing all the facilities to carry out this work.

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