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Heterochromatin Variations in Infertile Men

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ABSTRACT To correlate the association between heterochromatin variations and infertility in men, 70 infertile men (16 azoospermic men and 54 oligozoospermic men) and 35 fertile men were studied. Karyotype analysis using Giemsa (GTG) banding technique was performed. Total chromosome aberrations were observed in 28.5 percent of infertile men. It included variations in the heterochromatin region in chromosome 1, 9 and 16, presence of satellite, inversion in chromosome 9 and variation in the Y chromosome. In conclusion, chromosomal abnormalities found with a high frequency in infertile males are a major cause of male infertility, and they justify the requirement of cytogenetic analysis in every infertile man and genetic counseling prior to IVF treatment to rule out the carrier status.

INTRODUCTION

Infertility is the couple that has never been able to conceive a pregnancy, after at least 1 year of unprotected intercourse (Izzo et al. 2015). Nearly 25 percent of couples do not achieve pregnancy within 1year, 15 percent of the couples seek medical treatment for infertility and less than 5 percent remain childless without any cause. Both men and women are affected by infertility. 50 percent causes of these couples are due to male infertility. It is caused by many factors including change in the sperm count, morphology, motility, hormone and chromosome abnormalities (Chen et al. 2010) and tubal damage extra. Research is still pursuing in the area of reproduction and its association with the secondary constriction regions of chromosomes (Balkan et al. 2010).

Heterochromatin region has no coding potential and nucleolar organizing regions (NOR) contain genes coding for rRNA (Madon et al 2005). Hence, the variant is considered to be of no clinical significance. However, recent studies prove that chromosomal polymorphisms are related to infertility and recurrent abortions (Bhasin et al. 2005; Madon et al. 2005; Minocherhomji et al. 2009; Jaganathan et al. 2015). In 60-75 percent of cases, no causal factor is seen (idiopathic male infertility). These men present with no previous history associated with fertility issues, and are normal on physical examination and endocrine laboratory testing.

Semen analysis shows absence of spermatozoa in the semen (azoospermia), reduction in the number of spermatozoa (oligozoospermia), low motility (asthenozoospermia) and many abnormal forms on morphological examination (teratozoospermia). When these abnormalities usually occur together and it is described as the oligoastheno-teratozoospermia (OAT) syndrome. Several factors for male infertility include stress, global warming and endocrine abnormalities due to environmental pollution, reactive oxygen species and genetic causes. Andrological examination is required if the semen analysis shows abnormalities. Appropriate treatment is carried out based on the semen analysis and complete laboratory work up is standardized.

For the couples seeking fertility treatment because of male infertility, correct advice can be

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given by the urologist working in Andrology, who has vast knowledge of genetic abnormalities in infertility. Men with very low sperm counts can be given a reasonable chance of paternity by means of in vitro fertilization (IVF), ICSI (intracytoplasmic sperm injection) and TESE (testicular sperm extraction). However, the sperm of infertile men shows an increase in aneuploidy, other genetic abnormalities and DNA damage because the possibility of passing genetic abnormalities on to the next generation is increased. Screening of peripheral blood samples is the routine clinical practice, although there are prospects for screening of sperm (Johnson 1998). Standard chromosome analysis should be carried out in all men with damaged spermatogenesis who are seeking fertility treatment with IVF/ICSI.

Objectives

The main purpose of this study was the investigation of the possible cytogenetic causes of azoospermia and oligozoospermia among infertile men.

MATERIAL AND METHODS

Infertile men attending the genetic clinic with non-obstructive azoospermia and severe oligozoospermia, in the age group from 20 – 50 years were enrolled in this study. Out of 70 patients, 54 had severe oligozoospermia and 16 had non-obstructive azoospermia. For each patient a detailed medical history, including pedigree analysis was procured. Routine physical examination was carried out. As a control group, 35 age matched males with normal reproductive function were included. For each patient 5 ml of peripheral blood was taken.

Chromosomal Analysis

Standard protocol was followed for the chromosomal analysis of peripheral blood lymphocytes. RPMI-1640 media along with all the supplements was used for culturing the peripheral blood lymphocytes. Colchicine was added after a period of 72 hrs incubation, and this was followed by hypotonic treatment and fixation. GTG (Giemsa Trypsin) banding technique was used for banding metaphase chromosomes prepared. For each patient, at least, 25 well spread metaphases were scored.

RESULTS

The Cytogenetic analyses of 70 infertile men showed chromosomal aberrations in 20 infertile men(28.5%), which included variation in the heterochromatin region of 1,9,16 chromosome, Y chromosome and satellite region of chromosome 22. Normal karyotype of 46, XY was observed in all the controls.

Different type of chromosomal aberrations were seen in 7 men with azoospermia (10 %) and 13 men with oligozoospermia (18.6%) was indicated in Table 1. The most observed were polymorphic variations in chromosome 9 and Y.

Table 1: Different type of chromosomal aberrations

S. No.	*Age*	*Gender*	*Provisional diagnosis*	*Karyotype*
1.	31 yrs	M	Azoospermia	46,XY,16qh+
2.	30 yrs	M	Azoospermia	46,XY,inv9
3.	30 yrs	M	Azoospermia	46,XYp-
4.	28 yrs	M	Azoospermia	46,XYqh+
5.	39 yrs	M	Azoospermia	46,XYqh-
6.	31 yrs	M	Oligozoospermia	46,XY,22ps+
7.	34 yrs	M	Oligozoospermia	46,XY,9qh+
8.	46 yrs	M	Oligozoospermia	46,XY,9qh+
9.	37 yrs	M	Oligozoospermia	46,XY,9qh-
10.	37 yrs	M	Oligozoospermia	46,XYqh-
11.	35 yrs	M	Oligozoospermia	46,XYp-
12.	30 yrs	M	Azoospermia	46,XYqh-
13.	32 yrs	M	Oligozoospermia	46,XY,1qh+
14.	31 yrs	M	Oligozoospermia	46,XY,9qh+
15.	20 yrs	M	Oligozoospermia	46,XY,9qh+
16.	45 yrs	M	Azoospermia	46,XY,22ps+
17.	27 yrs	M	Oligozoospermia	46,XYqh+
18.	31 yrs	M	Oligozoospermia	46,XYqh+
19.	36 yrs	M	Oligozoospermia	46,XY,9qh-
20.	31 yrs	M	Oligozoospermia	46,XY,9qh+

The frequency of chromosome aberrations was given in Table 2 showed heterochromatin variation in chromosome 1 (1.4%), 16 (1.4%) and inv (9) (1.4%). Aberrations in the heterochromatin region of the Y chromosome, chromosome 9 were the most frequently identified polymorphism in 8 infertile men (11.4%) and in 7 infertile men (10%) respectively. Presence of satellite in chromosome 22 was seen in 2 patients (2.8%)

Table 2: Frequency of chromosome aberrations

Karyotype	*Frequency*	*Percentage*
46,XY,16qh+	1	1.4
46,XY,9qh+	5	7.1
46,XYqh+	3	4.2
46,XYp-	2	2.8
46,XYqh-	3	4.2
46,XY,22ps+	2	2.3
46,XY,1qh+	1	1.4
46,XY,inv(9)	1	1.4
46,XY,9qh-	2	2.8

In azoospermic males the most prevalent were the heterochromatin variation in the Y chromosome (46, XYqh+), (46, XYqh-) and deletion in the Y chromosome (46, XYp-) were also identified (Figs. 1, 2 and3). Heterochromatin variation in chromosome 16, presence of satellite in chromosome 22 and inversion in chromosome 9 was observed as shown in Figures 4, 5 and 6.

In oligozoospermic men, polymorphic variations in chromosome Y, 1, 9, deletion in Y chromosome and presence of satellites in chromosome 22 was identified (Figs. 7 and 8).

The azoospermic and oligozoospermic men showed both sex chromosomal variations in 8 infertile men (11.4%) and autosomal chromosomal variations in 12 infertile men (17.1%) as shown in Table 3.

DISCUSSION

In male infertility with abnormal semen parameters, chromosomal abnormality played a

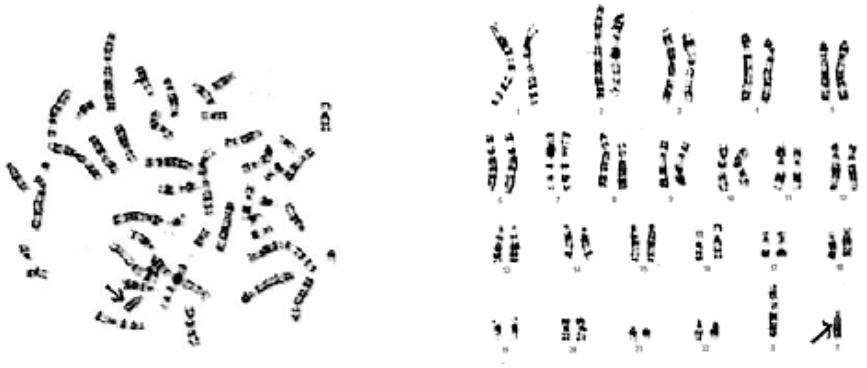

Fig. 1. Heterochromatin variation in Y chromosome (Yqh+)

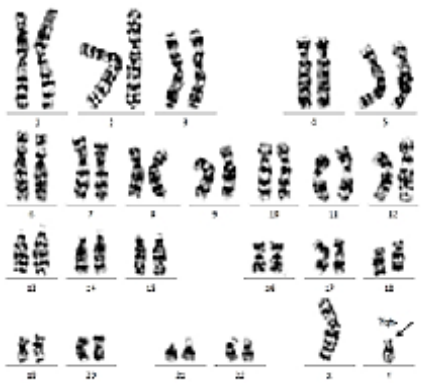

Fig. 2. Heterochromatin variation in Y chromosome (Yqh-)

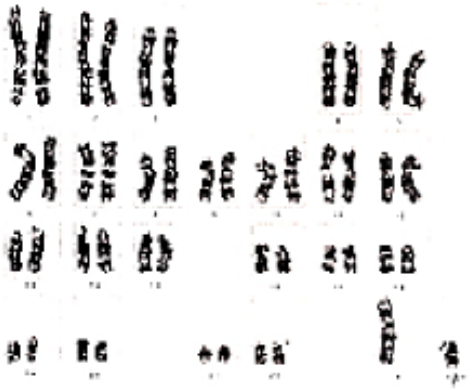

Fig. 3. Presence of Y p deletion

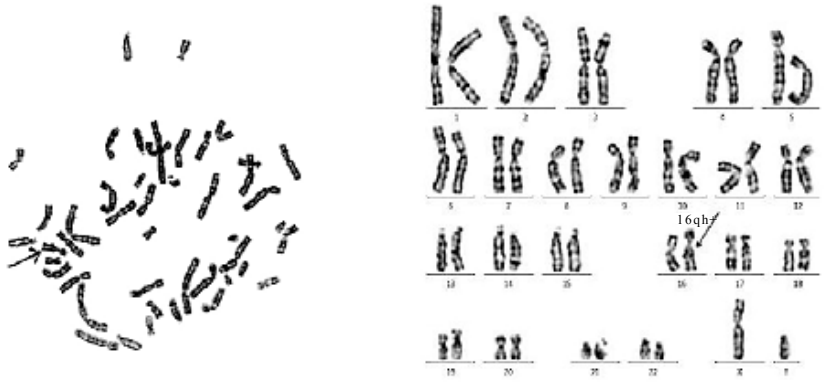

Fig. 4. Heterochromatin variation in chromosome 16 (16qh+)

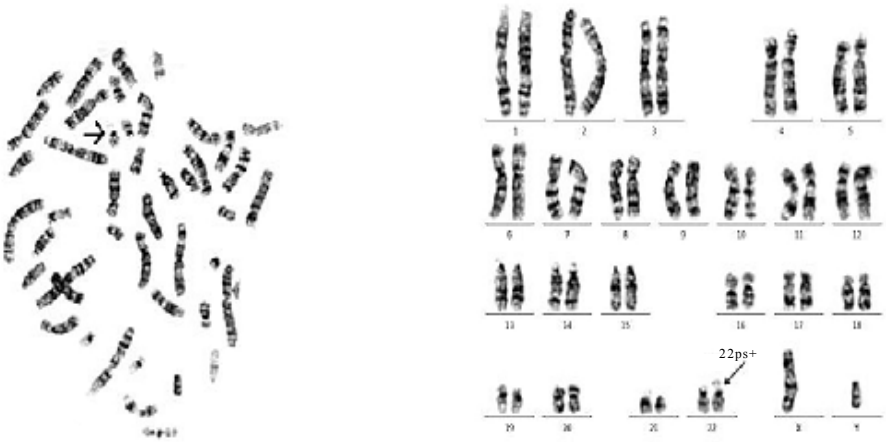

Fig. 5. Presence of satellite in chromosome 22 (22ps+)

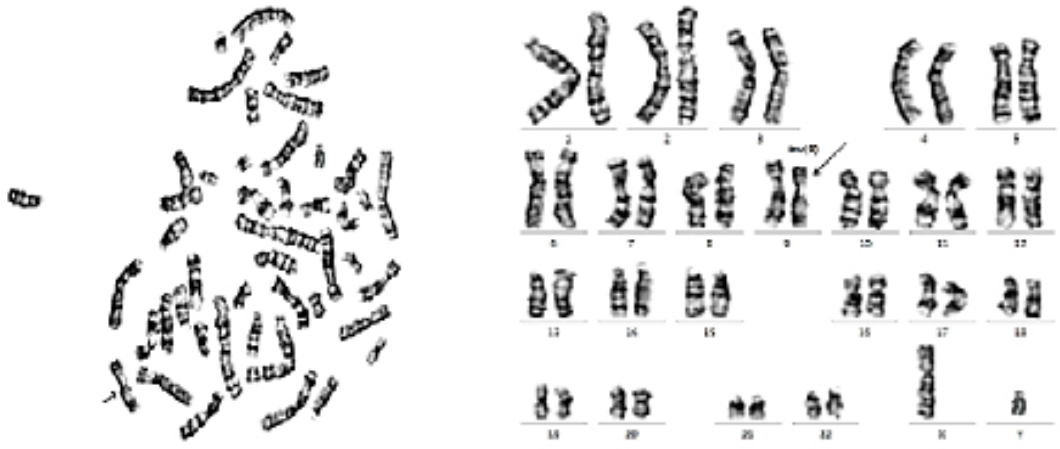

Fig. 6. Pericnteric Inversion in chromosome 9

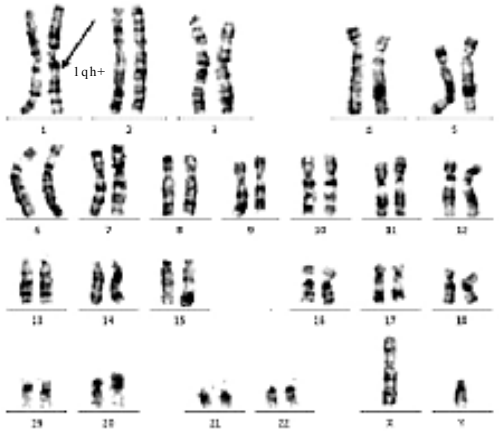

Fig. 7. Heterochromatin variation in chromosome 1(1qh+)

Table 3: Chromosomal polymorphisms in infertile men

Classification	*Karyotype*	*No. of men with aberrations*
Azoospermia	46,XY,16qh+	1
	46,XY,inv(9)	1
	46,XY,22ps+	1
	46,XYqh-	2
	46,XYp-	1
	46,XYqh+	1
Oligozoospermia	46,XYqh+	2
	46,XYp-	1
	46,XY,22ps+	1
	46,XY,9qh+	5
	46,XY,9qh-	2
	46,XY,1qh+	1
	46,XYqh-	1

principal role. It has been known for some 20 years that the prevalence of chromosomal abnormalities is higher in infertile men and is inversely related to the sperm count (Lissitsina 2006; Naasse et al. 2015). The results showed an inverse correlation between chromosomal anomalies and sperm count. Spermatozoa bearing abnormal chromosomes may cause abnormal embryonic development, which can in turn; cause early pregnancy loss (Oruganti et al. 2015) Chromosome abnormalities are responsible for at least half of spontaneous abortions or miscarriages and are an important cause of congenital malformations (Rimoni et al. 2002). The prevalence of chromosomal abnormalities is 13.8 percent in the general population (Duzcan et al. 2003). In different studies, the rates varied between 5.2 percent and 13.4 percent (Nagvenkar et al. 2005; Lissitsina et al. 2006; Pina-Neto et al. 2006).

The occurrence of chromosomal abnormalities among infertile men depends on a number of factors and the most important one is the sperm count. The present study shows 10 percent chromosomal abnormalities in males with azoospermia and 18.6 percent chromosomal abnormalities in men with oligozoospermia. In this study, karyotype determination was solely based on GTG-banding. The existence of qh variants was easily detected based on the location of heterochromatin.

Deletion in the long arm of the Y chromosome was seen in three patients. Studies have indicated that deletions of the long arm of the Y chromosome might lead to azoospermia and sometimes to severe oligozoospermia (Vijayalakshmi et al 2011). The frequency of 1qh+, 16qh+

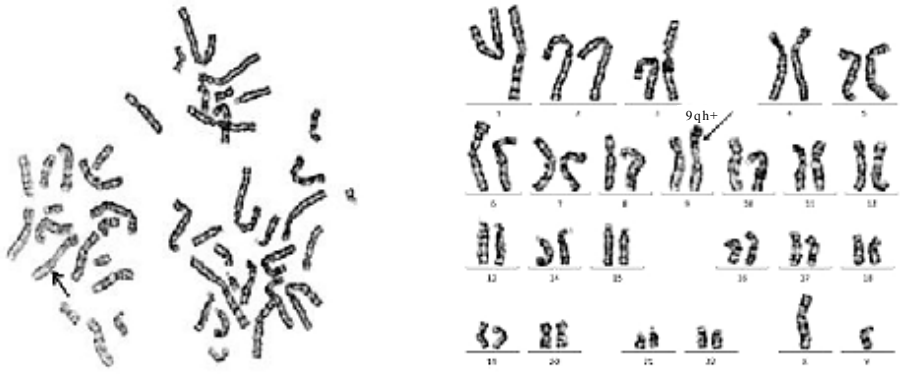

Fig. 8. Heterochromatin variation in chromosome 9 (9qh+)

and 22s+ is lower. Some reports observed a high frequency of 9qh+ in parents of chromosomally abnormal abortuses. It cannot be concluded whether the reproductive problem of the individual with this karyotype was caused by 9qh+.

In this study, there was another aberrant karyotype 46, XY, inv (9) (p11q13). The inv (9) reported to be associated with infertility and congenital anomalies (Nagvenkar et al. 2005; Jeong et al 2010). In 2.3 percent of the couples with a history of recurrent spontaneous miscarriages pericentric inversion of chromosome 9 was detected (Purandare et al. 2011). Nevertheless, most of the cytogeneticists believe that there was only one kind of breakpoint (p11q13) on the inversions of chromosome 9, which has no known deteriorated effect on the carriers and does not appear to be associated with a significant risk of miscarriage or unbalanced offspring. It was, therefore, generally considered as a normal chromosome variant. It has been suggested that phenotypes of inversion 9 may vary depending on the location of breakpoints.

Pericentric inversion of chromosome 9 [inv (9) (p11q13)] is the most common (1–3%) type of inversion in the general population (Tural et al. 2007). Although inv (9) (p11–q13) has been regarded as a normal familial karyotype variant, it has also been reported in various human diseases such as couples with congenital malformation, habitual abortus, mild growth retardation, malformations of the skull and facial (craniofacial) region, undescended testis, skeletal malformations, mental retardation, hermaphroditism and cardiac defects (Lourenco et al. 2007; Ramegowda et al. 2007).

Several reports on male infertility mentioned the presence of chromosomal variants or polymorphisms. Y chromosome polymorphisms have been preferentially seen in azoospermia and severe oligozoospermia (Hammami et al 2015). 'Long Y chromosome' (Yqh+) and 'short Y chromosome' (Yqh-) is known to exist (Gagare et al. 2012). The variation in the length of the Y chromosome is usually due to variation in the distal part of the long arm that is known to contain heterochromatin (Hammami et al 2015).

Autosomal polymorphisms were observed in the present study, which includes increases in the centeromeric heterochromatin in chromosomes 1, 9 and 16. Some workers have considered variations in heterochromatin in chromosomes 1, 9 and 16 to be associated with fetal wastage, recurrent abortions, and abnormal phenotypes (Sheth et al. 2013). Both studies indicated no significant difference in the heterochromatic regions between aborting and non-aborting couples, and also revealed that heterochromatic regions of chromosomes 1, 9, 16 and Y in children showed signs of embryonic development disorder (Purandare et al. 2011). Heterochromatin has a specific role and behaviour in the synapsis of human homologous chromosomes. An association of heterochromatic variation with bad obstetric history is also indicated in the studies (Minocherhomji et al. 2008; Sheth et al. 2013).

In this study, we have analyzed the effect of heterochromatin variations & other chromosome abnormalities such as satellite variations in patients who are infertile.

Presence of satellites in acrocentric chromosomes 22 was observed in two patients. Positive correlation existed between the frequency of acrocentric variants and sterility, due to an increase of risk satellite association and of non disjunction leading to aneuploidy in gametes (Moreau et al. 2009). The report was limited by only using cytogenetic detection methods, without confirmation by genetic testing.

Analysis at the molecular level like polymerase chain reaction, restriction fragment length polymorphism, if required DNA sequencing may be needed to unveil any relation between heteromorphisms and reproductive failure taking into consideration, that the heterochromatin have been regarded to have more crucial cellular roles than previously thought (Lissitsina et al. 2006) .

CONCLUSION

In conclusion, the occurrence of chromosomal anomalies among infertile men strongly suggests genetic testing like polymerase chain reaction and counseling prior to the ICSI treatment. Moreover, prenatal diagnosis in the case of abnormalities is of utmost importance. Such investigation is a pre-requisite to minimize the risk of propagation of chromosomal abnormalities into the next generation. Additionally, a thorough follow up of babies conceived through ICSI, in particular the male progeny is essential.

RECOMMENDATIONS

The frequency of chromosomal abnormalities is high among infertile men. So cytogenetic analysis and detection of Y chromosome microdeletion should be done to all the infertile men prior to IVF treatment.

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