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Original Article

A Case-control Study Screening the Correlation of Chromosomal Alterations with Male Infertility in 180 Infertile Men from North Maharashtra

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Abstract

Purpose: To examine the association between chromosomal abnormalities and male infertility.

Objectives: To find out the frequency and types of chromosomal alterations; major chromosomal abnormalities and polymorphic chromosomal variants in infertile males and to assess its impact on male infertility.

Design: It is a case-control study, carried out on peripheral blood lymphocytes with standard G-banding technique in infertile males versus control fertile males. It is a type of observational study and comes under the analytical study because in this type of study comparison (control) group is used.

Patient(s): 180 infertile men and 60 control fertile men.

Result(s): To indentify these chromosomal alterations, the karyotyping of 43 azoospermics and 137 oligozoospermics including 106 severe oligozoospermics; total 180 infertile men were carried on peripheral blood lymphocytes with standard G-banding technique. In this study, total chromosomal abnormalities had found 16.3% (n=7/43) in azoospermics and 9.5 % (n=13/137) in oligozoospermics with an overall 11.2% (20/180) in infertile males. No major chromosomal abnormality had found in control fertile group ($P<0.05$). While the occurrence of polymorphic chromosomal variants was high (31.1%) in infertile men, but remained similar (35%) as in fertile men of control group ($P>0.05$).

Conclusion(s): Study shows that the frequency of chromosomal anomalies is very high in infertile men and inversely related to the sperm count. It is one of cause of male infertility and also linked with the genetic risk for next generation if the infertile couples are helped with Assisted Reproduction Techniques (ART).

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Introduction

Infertility is one of the most common disorders seen in medical practice worldwide. It is defined as the inability of a couple to conceive after 1 year of

unprotected sexual intercourse. The number of infertile couples in the general population is increasing and the recent studies show that about 15 to 20 % of couples in their reproductive age are unable to have their own child is presenting an almost unsolvable challenge to the health service. Infertility is a problem of immense importance due to its social, emotional and religious nature. Furthers more it exhausts the couple psychologically, socially, and also drains their financial resources (1, 2).

Chromosomal abnormality is one of the important causes of male infertility because it disrupts genes which are involved in the genetic control of spermatogenesis can leads into the abnormal semen parameters like non- obstructive azoospermia or severe oligozoospermia with asthenozoospermia or teratospermia or both oligo-astheno-teratozoospermia. The most frequent chromosomal abnormalities found in infertile men are translocations and sex chromosome abnormalities (3, 4). The effect of chromosomal abnormalities on male infertility is very high and inversely related to the sperm count. Major chromosomal abnormalities found with the range of 2.6-16.5% in infertile men compared to the incidence in normal male population 0.3-0.4%; in which azoospermia are at high frequency from 11.6%-23.8%, oligozoospermia the incidence is 2.3-6.6% while in severe oligozoospermia it is about 10.4%. Chromosomal abnormalities can be easily diagnosed by performing G banding using trypsin and Giemsa (GTG) karyotyping (5, 6,).

Polymorphic chromosomal variants also have been well studied both in control fertile group and in infertile males. These variations of chromosomes are known to occur in the general population. However, higher frequencies of these variants have recently been reported in infertile and subfertile individuals as compared to normal population are associated with poor spermatogenesis (6, 7).

Therefore, the present study was undertaken to find out the frequency and types of chromosomal alterations; major chromosomal abnormalities and polymorphic chromosomal variants in infertile males and its association with infertility and subfertility.

Hypothesis

1. Chromosomal abnormalities are high in infertile males than general population.
2. It is one of the causes of male infertility because it disrupts the spermatogenesis.
3. There is an inverse correlation between chromosomal abnormalities and sperm count.
4. The frequency and types of these chromosomal anomalies can be easily diagnosed by GTG karyotyping method.

Research Questions

1. What is the association between chromosomal abnormalities and male infertility?
2. What is the effect of chromosomal abnormalities on semen parameters?
3. Can these anomalies be diagnosed easily by GTG karyotyping method?

Aims and Objectives

To find out the frequency and types of chromosomal alterations, its impact on male fertility and its correlation with impaired semen parameters.

The research project was planned with following objectives:

1. To analyze the semen samples in infertile and control fertile males.
2. To classify them based on sperm count.
3. To find the frequency and types of chromosomal alterations in infertile and control fertile males.
4. To assess the impact of these chromosomal alterations in male infertility.
5. To assess the correlation between these chromosomal anomalies and impaired semen parameters in male infertility.

Materials and Methods

Infertile men (n=180) in the age group of 20 to 40 years with affected semen parameters, who approached for their help to our infertility center were included in this study. Written informed consent, confirmed by the Ethics Review Committee was taken from every participant. All participants had given verbal as well as written information about the procedure. Every patient also referred for physical examination and consulted for their medical histories and reproductive problems (4).

Inclusion criteria

a. Age groups range of patient and controls were similar.

b. History of infertility for more than one year was obtained.

c. No history of psychological dysfunction.

d. No findings suggestive of any abnormality in female partner.

Exclusion criteria

a. Obese patients are excluded.

b. Patients above 40 years of age excluded.

Semen samples were collected after the period of at least 7 days of ejaculatory abstinence. Semen analyses were performed according to the manual of World Health Organization (WHO) (6, 8). Semen analyses were carried out at least twice for each patient before a diagnosis of azoospermia or severe oligozoospermia. Blood samples had collected and stored for cytogenetic analyses. Patients with obstructive azoospermia were not included in this study (6, 7).

Control group included 60 fertile males with the same age groups and underwent the same examinations and analyses as in the infertile study group. Each participant in the control group had fathered at least one child (4, 5).

Chromosomal analyses were carried out in peripheral blood lymphocyte culture using the G-banding technique. Lymphocytes were cultured in RPMI 1640, phytohaemaglutinin and fetal bovine serum and treated with colcemid after the 72 hrs of incubation period. Then G-banding of metaphase chromosome was performed. For each participant, minimum 10 metaphases were analyzed by karyotyping (7). Chromosomal alterations: major chromosome abnormalities and polymorphic chromosome variants were analyzed and classified as per the International System for Human Cytogenetic Nomenclature (6, 9).

Chromosomal analysis by GTG banding karyotyping method (9)

1. Sterilization of glassware
2. Preparation of culture medium (stock solution)
3. Preparation of serum
4. Leucocytes micro culture
5. Harvesting of culture
6. Preparation of slides
7. Chromosome Staining (Banding technique)
8. Karyotyping- arrangement of chromosomes according to international convention

Statistical analyses

Statistical analysis was done by Z-test, chi-square test and correlation coefficient technique. The differences between the compared groups considered statistically significant in all cases at $P<0.05$.

Results

Among the 180 infertile males, 43 were non-obstructive azoospermics and 137 were oligozoospermics including 106 severe oligozoospemics. The frequency and types of chromosomal alterations found in infertile males are summarized in Table I, II, III & IV. Major chromosomal abnormalities found in infertile males was 16.3% (7/43) in azoospermics and 9.5% (13/137) in oligozoospermics with an overall 11.2% (20/180) in infertile males (Table I).

In total 20 (11.2%) cases of chromosomal abnormalities, numerical abnormalities found in 6 (3.3%) cases in which 4 (9.3%) were in azoospermics and 2 (1.4%) were in oligozoospermics while structural abnormalities found in 14 (7.7%) cases in which 3 (6.9%) were in azoospermics and 11 (8%) were in oligozoospemics (Table II).

All 6 cases of numerical abnormalities were Klinefelter's syndrome; 4 cases were with classic pattern 47,XXY karyotype and 2 were with mosaic forms 47,XXY/46, XY. However, out of 14 cases of structural abnormalities; 7 were autosomal translocations, 3 were inversions, 2 were deletions and 2 cases had of supernumerary marker chromosomes (sSMC) (Table III). No major

TABLE III : The type of some chromosomal aberrations found in infertile males.

Numerical abnormalities	
47, XXY,3qh+,9qh+	Azoospermia
Mos47,(XXY)[92]/46,XY[8],Yqh+	Severe Oligozoospermia
Mos47,(XXY)[86]/46,XY[14]	Severe Oligozoospermia
Structural abnormalities	
Translocations	
45,XY,rob t(21;21)(q10;q10)	Azoospermia
46,XY,t(Y;9)(q12;q11)	Azoospermia
46,XY,t(14;12)(q32;q24)	Severe Oligozoospermia
46,XY,t(15;17)(p13;q26)14ps+,Yqh+	Severe Oligozoospermia
Inversion & Deletion	
46,XY,del(Y),(q11),Inv(9)(p13;q13)	Azoospermia
Marker Chromosome	
mos47,XY,+mar[22]/46,XY[78]	Severe Oligozoospermia

TABLE I : Chromosome alteration in male infertility and in the control fertile group, n (%).

Patients/controls	*Autosomal chromosome abnormalities*	*Sex chromosome abnormalities*	*Major chromosomal abnormalities*	*Polymorphic Chromosomal variants*	*Total chromosome alterations*
Infertile males (n=180)	10(5.6)	10(5.5)	20(11.2)	56(31.2)	70(38.9)*
Azoospermics (n=43)	1(2.3)	6(13.9)	7(16.3)	14(32.5)	20(46.5)**
Oligospermics (n=137)	9 (6.5)	4 (2.9)	13(9.5)	42(30.6)	50(36.5)***
Control group (n=60)	—-	—-	—-	21(35)	21(35)

*Six infertile males have both major chromosomal abnormalities and polymorphic variations; 1 in azoospermics** and 5 in oligospermics***

TABLE II : Frequency and types of major chromosomal abnormalities in infertile males, n (%).

Type of Abnormalities/ Normal	*Total infertile males n=180 (%)*	*Azoospermic males n=43 (%)*	*Oligozoospermic males n=137 (%)*
46, XY (normal)	160 (88.8)	36 (83.7)	124 (90.5)
Numerical abnormalities	6 (3.3)	4 (9.3)	2(1.4)
47, XXY	6 (3.3)	4 (9.3)	2(1.4)
Structural abnormalities	14 (7.7)	3 (6.9)	11(8)
Translocation	7 (3.8)	2 (4.6)	5 (3.6)
Inversion	3 (1.67)	—	3(2.2)
Deletion	2 (1.2)	1 (2.3)*	1(0.7)*
Marker chromosome	2 (1.2)	—	2(1.4)
Total abnormalities	20 (11.2)	7 (16.3)	13(9.5)

*Two patients of Y chromosome deletion also found with inversion of chromosome 9.

Figure 1-a ↓

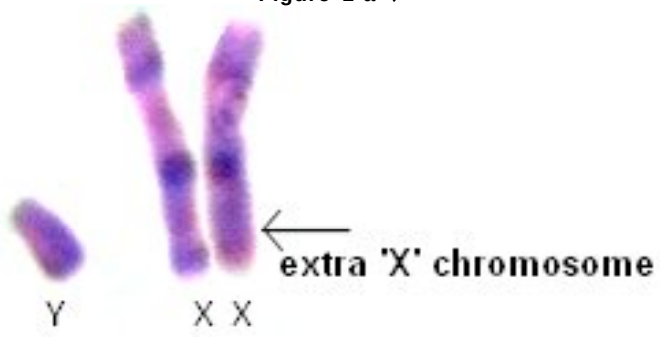


Figure 1-b ↓

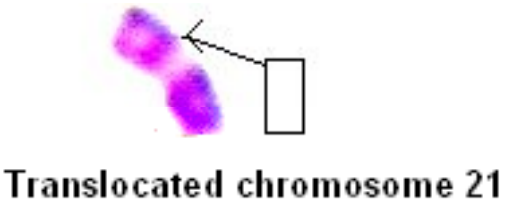


Figure 1-c ↓

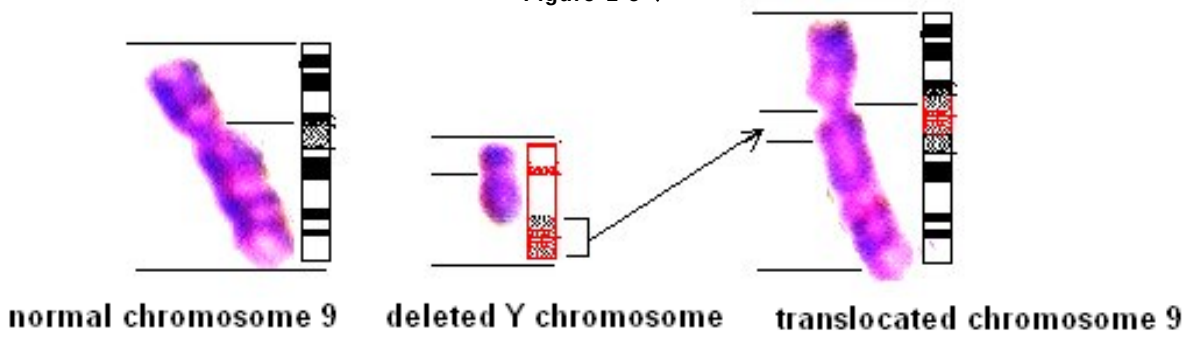


Figure 1-d ↓

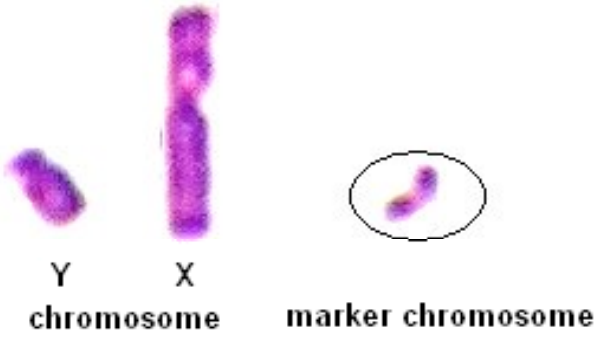


Figure 1-e ↓

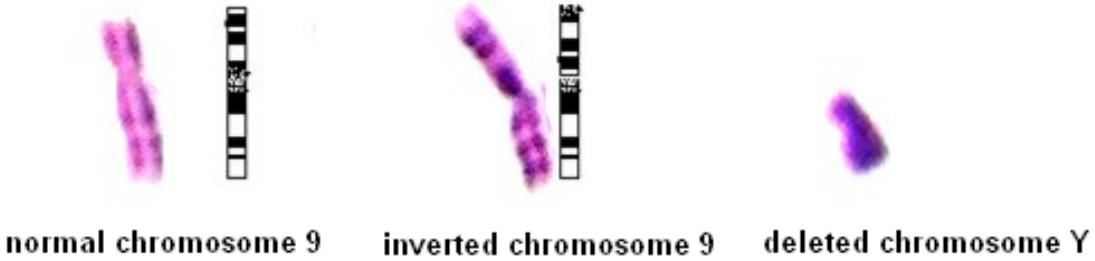
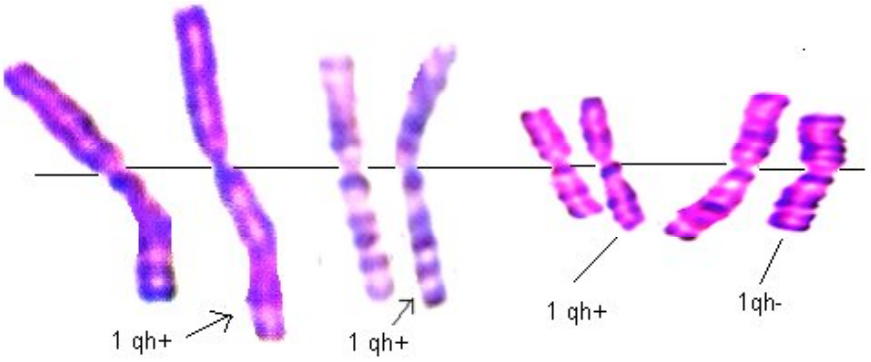


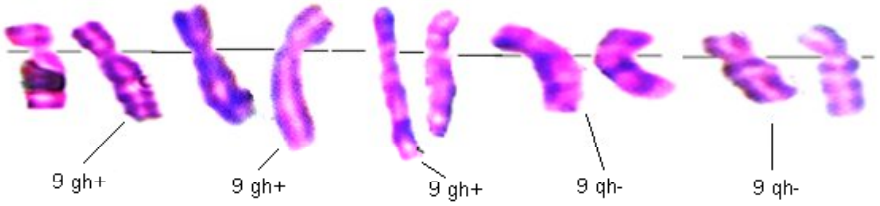
Fig. 1 : Examples of major chromosomal abnormalities found in infertile males; Partial karyotypes of
a. Klinefelter's Syndrome; 47,XXY,
b. Robertsonian translocation; 45,XY,rob(21;21)(q10;q10)t(21;21)
c. Translocation; 46,XY,t(Y;9)(q12;q11)
d. Small supernumerary marker chromosome; mos47,XY,+mar[6]/46,XY,[92]
e. Inversion of chromosome 9 and deletion of Y; 46,XY,del(Y)(q11),inv(9)(p21;q22)

Figure 2-a →



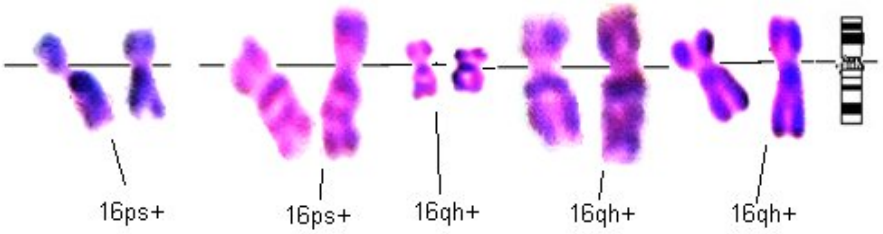
Variants of chromosome 1

Figure 2-b →



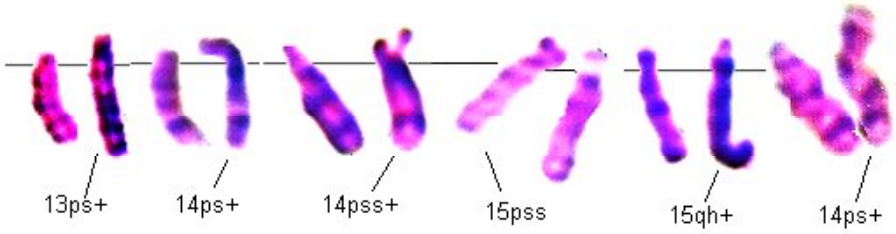
Variants of chromosome 9

Figure 2-c →



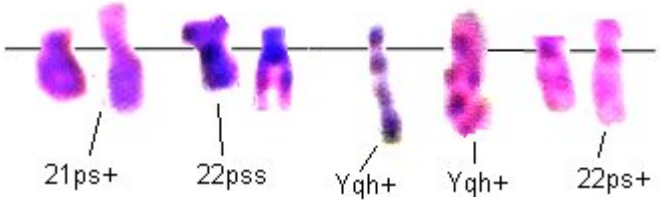
Variants of chromosome 16

Figure 2-d →



Variants of chromosome 13, 14 & 15

Figure 2-e →



Variants of chromosome 21, 22, and Y

Fig. 2 : Most commonly observed heterochromatin polymorphic variants;
a. Variants of chromosome 1, b. Variants of chromosome 9,
c. Variants of chromosome 16, d. Variants of chromosome 13, 14 & 15, and
e. Variants of chromosome 21, 22, and Y.

TABLE IV : Total polymorphic variants according to the types of chromosome in all groups, n (%).

Polymorphic variants	*Azoospermics n=43 (%)*	*Oligozoospermics n=137 (%)*	*Total infertile males n=180 (%)*	*Control n=60 (%)*
Total variants of chromosome 1	8(18.6)	19(13.8)	27(15)	11(18.3)
1 qh+	6(13.9)	17(12.4)	23(12.7)	9(15)
1 qh–	2(4.6)	2(1.4)	4(2.2)	2(3.3)
Total variants of chromosome 9	5(11.6)	11(8)	16(8.8)	7(11.6)
9 qh+	4(9.3)	9(6.5)	13(7.2)	6(10)
9 qh–	1(2.3)	2(1.4)	3(1.6)	1(1.6)
Total variants of chromosome 16	2(4.6)	6(4.3)	8(4.4)	2(3.3)
16 qh+	1(2.3)	5(3.6)	6(3.3)	1(1.6)
16 qh–	1(2.3)	0(0)	1(0.5)	0(0)
16 ps+	0(0)	1(0.7)	1(0.5)	1(1.6)
Total variants of chromosome 'Y'	3(6.9)	7(5.1)	10(5.5)	3(5)
Y qh+	3(6.9)	7(5.1)	10(5.5)	3(5)
Yqh–	0(0)	0(0)	0(0)	0(0)
Satellites of chromosome 13,14,15,21 & 22	3(6.9)	9(6.5)	11(6.1)	3(5)
Total Variants n (%)	14(32.5)	42(30.6)	56(31.1)*	21(35)**

18* infertile males and 5** fertile controls found more than one polymorphic variants.

chromosomal abnormality had found in control group (P<0.05).

Polymorphic chromosomal variants had found in 56 infertile males (31.1%), this incidence was similar 21 (35%) males in control fertile group (P>0.05). Autosomal chromosome variants were observed more frequently than sex chromosome variants. Alterations in the heterochromatin region of the chromosome 9 were the most frequently identified polymorphism in 16 (8.8%) infertile males; 5 (11.6%) men in azoospermics and 11 (8%) in severe oligozoospermics. Polymorphic variants were also found in chromosome 1 (n=27), chromosome 16 (n=8), Y chromosome (n=10) and in acrocentric chromosome 14, 21 and 22 called satellites (Table IV).

Discussion

Research of last few years has clearly shown that infertile men have higher occurrence of chromosomal abnormalities. Obviously these findings are further co-related with the increased incidence of chromosomal abnormalities in newborns and fetuses born from the pregnancies conceived by Intracytoplasmic Sperm Injection (ICSI). As also reported in literature, in half of the infertile couples with unsuccessful pregnancy, the cause of infertility is male related, in which about 30% are genetic factors with abnormal semen parameters should be considered. Chromosomal abnormality is one of the important causes of male infertility because it disrupts genes involved in the genetic control of human spermatogenesis (10, 11).

In present study, the incidence of chromosomal abnormalities in azoospermic group 16.3% was higher than in oligozoospermic 9.5% with an overall occurrence of 11.2 %. It has clearly demonstrated an inverse correlation between chromosomal anomalies and sperm count. Also these findings were comparable to the literature data varying from 2.2-22.6% (12, 13). No chromosomal abnormality had been found in control group in this study (P<0.05).

Sex chromosomal abnormalities (13.9%) were predominant in azoospermia over autosomal abnormalities (2.9%), while the autosomal abnormalities (6.5%) were predominant in oligozoospermia over sex chromosomal abnormalities (2.3%). All autosomal abnormalities were structural type while the sex chromosome abnormalities were found both structural as well as numerical types.

All numerical abnormalities in 6 cases were of Klinefelter's syndrome in which 4 patients had of classical form 47, XXY and 2 had of mosaic form 47,XXY/46,XY. Presences of Klinefelter's syndrome in males impair the spermatogenesis associated with severe oligozoospermia or azoospermia causing infertility. This is caused due to lethal dosage introduced into cells by an additional 'X' chromosome, which does not permit the development of Sertoli cells and survival of germ cells in the testis, resulting in azoospermia due to the advanced germ cell atresia and aplasia. This type of gonosomal mosaicism leads into severe oligospermia, may be a probable cause for the failure of assisted reproduction (5, 14).

In structural abnormalities of 14 (7.7%) cases, translocations had found in 7(3.8%) cases, inversions in 3(1.67%), deletions in 2(1.2%) and marker chromosomes in 2 (1.2%) infertile males. Such type of anomaly can results in a variety of sperm production phenotypes from normal spermatogenesis to an inability to produce spermatogonia.

Out of autosomal translocations, 5 had non-reciprocal while 2 had Robertsonian translocations. As the most common chromosomes involved in translocations of infertile men are acrocentric chromosomes 13, 14, 15, 21 & 22, they are more harmful for fertility of the carriers because of high tendency of these chromosomes to be associated with the X-Y body causing severe spermatogenic defects (15). Translocations can also cause the loss of genetic material at the breakpoint of genes, which can corrupt the genetic message and leads into infertility (16).

Reciprocal translocations form quadrivalents during the meiotic division which cause the impairment in segregation of chromosomes leads to infertility, birth with defects or spontaneous abortion, depending on the chromosomes involved. While the Robertsonian translocations form trivalents that influence the pairing of homologous chromosomes during I meiotic division and cause male infertility (5, 17, 18).

An association between inversions and infertility in the males has been reported. These inversions may cause a problem for mitotic divisions which will disrupt cell division and thus reduce spermatogenesis (19). Two cases of marker chromosomes (sSMC) had found in oligospemic group. Males carrying sSMC are often phenotypically normal. But sSMC may associate with the X-Y bivalent at meiotic prophase and cause male infertility. In this case, the infertility is caused due to the impairment of spermatogenesis by meiotic arrest resulting in maturation arrest on spermatocyte stage (20).

The incidence of deletions of Y chromosome had found in 2 patients (1.2%) in our study was lower than that given in literature (3-18%) (21). Y chromosome abnormalities, particularly the deletions involving long arm of the Y chromosome lead to azoospermia and male infertility. This type of deletion does not appear to impair spermatogenesis in some males but leads to infertility in others. The simple explanation for these observations is that there is a key locus (or loci) close to the boundary between genetically inert heterochromatin and Yq euchromatin. The removal of this locus in some males by more extensive deletion causes infertility. Such large structural changes to the Y chromosome might disturb normal pairing and segregation with the X chromosome during meiosis, is the cause of spermatogenetic failure in these males (5, 22).

Heterochromatic polymorphic chromosomal variants also well studied both in the infertile men and fertile control group. The frequency of these variants in infertile men was higher in our study (31.1%), but remained similar to the control group (35%) ($P>0.05$). It was also coincidental with the literature data in infertile males (4.7-56.8%) and fertile males (32.7%) (13). Polymorphic variants are usually considered as normal variants inherited from one generation to another with low mutation rate and without any direct harmful phenotypic effect due to the scarcity of protein-coding region in them. However, the increased polymorphic variants may have some clinical significance and associated with clinical anomalies. The detrimental effect of variants may be not direct to phenotype but indirect through the disturbing spermatogenesis and causing the death of germ cell resulting in infertility or children with

congenital anomalies (23, 24).

A large heterochromatic block in the pericentromeric heterochromatin region of chromosome 1 affects the pairing of chromosomes leads to meiotic arrest, death of germ cells and infertility (25). Variants of chromosome 9 (qh+) might be associated with spontaneous miscarriages, stillbirth, congenital abnormalities, and chromosomal anomalies in aborts and newborns. However, the result of our study and some of other authors do not support this report as of high incidence of 9qh+ found both in normal (10%) and infertile males (7.2%) (25, 26).

Y chromosome polymorphic variants (Yqh+ and Yqh–) have been seen more frequent in azoospermia and severe oligozoospermia. Long Y chromosome has seen to be associated with an increased risk of fetal loss. The variation in relative length of Y chromosome is said to be associated with male infertility. However, other study did not show any relationship between the size of Y chromosome and the risk of abortion (27). Genest and Genest also reported that short Y chromosome does not see to represent an increased risk of pregnancy loss. The contribution of Y chromosome variants to cause infertility is still a controversial topic and further studies are required to understand this (28,29). In our study, we found 'Y' chromosome variants in 5.5% in which all were with increased heterochromatin ('Y' qh+).

Polymorphisms of acrocentric chromosomes D and G-groups are found both in the fertile 5% and in infertile men 6.1% in this study. It is reported that higher frequencies of satellite variants have been found in patients with reproductive failure and spontaneous abortions. Very large satellites of acrocentrics have been reported in infertile males, but other studies have not shown them as a risk factor to infertility (5, 30).

Conclusion

The occurrence of major chromosomal abnormalities is very high in infertile males as compare to general population and inversely related to the sperm count. It is one of the important causes of male infertility because it disrupts genes which are involved in the genetic control of spermatogenesis and leads to infertility. While the polymorphic chromosomal variants are known to occur in general population but their effect on infertility is still a controversial topic and further studies are required to understand the facts.

Limitations and future perspectives

The limitations of the study are cost effective and time consuming procedures. Also participants were not easily agreed to give the samples particularly for semen analyses. Based on the present results, it would be relevant to continue this prospective study further to the molecular level for to find out the role of various genes involved regulation of spermatogenesis and interference of their mutations with man's fertility potential. This will help us to get the answers of most of idiopathic causes of male infertility.

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