To Study The Polymorphic Chromosomal Variants and Male Infertility.

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Abstract :

Objectives : To find out the frequency and types of polymorphic chromosomal variants and its impact in male infertility. **Design :** A case control study was carried on peripheral blood lymphocytes with standard G-banding technique in infertile males versus control fertile males. **Patient (s):** 180 infertile men and 60 control fertile men. **Intervention (s) :** Semen analysis, Karyotyping by G-banding technique.**Main Outcome measure(s) :** Polymorphic chromosome variants in infertile males prior to the use of assisted reproduction technique like ICSI versus control fertile males. **Result (s) :** To indentify these chromosomal variants, the karyotyping of 180 infertile men including; 43 azoospermics and 137 oligozoospermics (106 severe oligozoospermics) were carried on peripheral blood lymphocytes with standard G-banding technique. In this study, the occurrence of polymorphic variants was high (31.1%) in infertile men, but remained similar (35%) in fertile men of control group ($P>0.05$). **Conclusion(s) :** Since the genetic risk to the next generation, the occurrence of Polymorphic chromosomal variants with other chromosomal abnormalities among these infertile men strongly suggests the genetic testing and counseling of infertile couples prior to the use of assisted reproduction technique and it should be mandatory in all infertility clinics.

Key words : Male infertility, Polymorphic Chromosomal variants, Karyotyping, Azoospermia, Oligozoospermia

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]

Due to increased number of infertility centers, the diagnostic and therapeutic facilities are coming within the reach of these infertile couples and can be easily helped by the use of assisted reproduction techniques. Even, Intrauterine Insemination (IUI) and Intracytoplasmic Sperm Injection (ICSI) by using the donors semen has received tremendous acceptance by these infertile couples. The practice of using ICSI technique by these severely infertile men carries the risk of passing on genetic disorders to their children including chromosomal abnormalities.[3]

Chromosomal alterations; major abnormalities and polymorphic variants, is one of the important cause of male infertility because it disrupts genes which are involved in the genetic control of spermatogenesis can leads in to abnormal semen parameters like non-obstructive azoospermia or sever oligozoospermia with asthenozoospermia or teratospermia or both oligo-astheno-teratozoospermia. The common chromosomal abnormalities found in infertile males are chromosomal aneuploidy, structural and numerical abnormalities and Y chromosomal microdeletions. If these are present with polymorphic variants creates severe problem for fertility. Heterochromatic polymorphic chromosome variants 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.[4] Chromosomal variants can be easily diagnosed by performing G banding using trypsin and Giemsa (GTG) karyotyping. Therefore, karyotyping study is definitely a mandatory test in the diagnostic center of any infertile male.[4]

Therefore, the present study shows the importance of cytogenetic screening of such patients prior to the use of Assisted Reproduction Technique (ART), as it is important to prevent the chromosomal alterations being passed on to their children.

Aim and Objectives : To find out the frequency and types of polymorphic chromosomal variants and its impact in male infertility, the following objectives are intended:

1. To analyze the semen samples in infertile and control fertile males.
2. To find the frequency and types of polymorphic chromosomal variants in infertile and control fertile males.
3. To assess the impact of these chromosomal variants in male infertility.

Material 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 was also underwent a physical examination and consulted for their medical histories and reproductive problems. 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 [5]. Semen analyses were carried out at least twice for each patient before a diagnosis of azoospermia or sever oligozoospermia. Blood samples were collected and stored for cytogenetic analysis. Patient with obstructive azoospermia were not included in this study. Control group included 60 fertile males with the same age groups underwent the same examinations and analyses as in the infertile study group. Each participant in the control group had fathered at least one child.

Chromosomal analyses were carried out in peripheral blood lymphocyte culture using G-banding technique. Lymphocytes were cultured in RPMI 1640, phytohaemaglutinin and fetal bovine serum and treated with colcemid after the 72 hr of incubation period. Then G-banding of metaphase chromosome was performed. For each participant, minimum 10 metaphases were analyzed by karyotyping. Polymorphic chromosome variants were analyzed and classified as per the International System for Human Cytogenetic nomenclature.[5,6]

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 variants found in these infertile males are summarized in (Table 1 & 2).

Polymorphic chromosomal variants were found in 56 infertile males (31.1%), this incidence was similar in fertile men 21 (35%) from the control 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 2).

Tables : Table 1 Polymorphic Chromosome variants in infertile and control fertile group

Patients/ controls	Polymorphic Chromosomal variants n (%)
Infertile males (n=180)	56(31.2)
Azoospermics (n=43)	14(32.5)
Oligospermics (n=137)	42(30.6)
Control group (n=60)	21(35)

Table 2 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)****

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

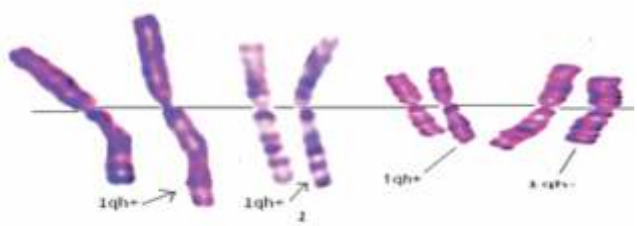

Figure 1-a

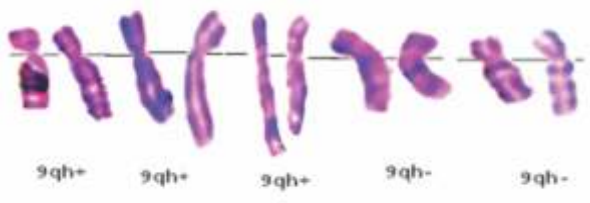

Figure 1-b

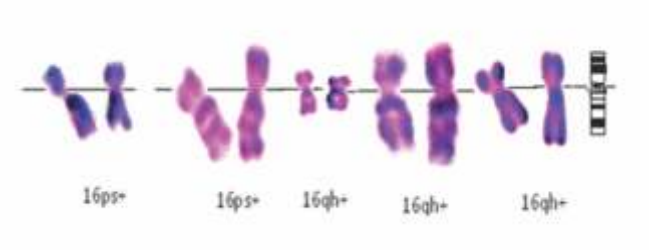

Figure 1-c

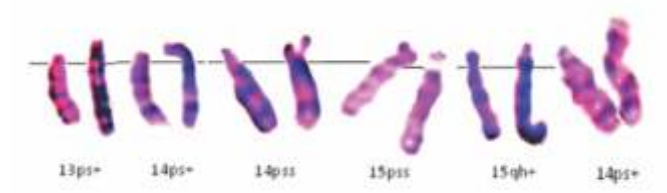

Figure 1- d

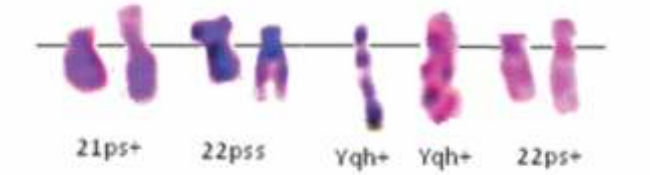

Figure 1-e

Figure 1: 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.

Discussion : Research of last few years has clearly shown that infertile men have higher occurrence of chromosomal alterations including major chromosomal abnormalities and polymorphic variants. Obviously these findings are further co-related with increased incidence of chromosomal alterations in newborns and fetuses born from the pregnancies conceived by 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. Major chromosomal abnormality is one of the important causes of male infertility but polymorphic variants also gaining importance because if it is present with major abnormality disrupts genes more severely involved in the genetic control of human spermatogenesis. [7,8]

The present study is mainly focused on heterochromatic polymorphic chromosomal variants in infertile males. Higher frequencies of these variants have recently been reported in infertile and subfertile individuals are shown to be associated with poor spermatogenesis.

A large heterochromatic block in the pericentrometric region of chromosome 1 (Fig. 1-a) affects the pairing of chromosomes may leads into meiotic arrest, death of germ cells and infertility. Variants of chromosome 9 (qh+) (Fig 1-b) 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%). [9,10]

Y chromosome polymorphic variants (Yqh+ and Yqh-) (Fig. 1-e) 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.[11] 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. In our study we found 'Y' chromosome variants in 5.5% in which all were with increased heterochromatin ('Y' qh+). [12,13]

Polymorphisms of acrocentric chromosomes D and G-groups (Fig. 1-d) are found both in the fertile 5% and in infertile men 6.1% (Table 2). 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 of infertility. [14]

Conclusion : The occurrence of polymorphic chromosomal variants in infertile males strongly suggests that the

genetic testing and counseling of infertile couples should be mandatory prior to the use ICSI treatment. It should be included in all infertility clinics for to minimize the risk of propagation of these chromosomal alterations into the next generation.

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