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Does a Heterochromatic variant affect the Human Reproductive outcome?

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Abstract

To study the association of chromosomal polymorphic variations with recurrent miscarriage. Recurrent miscarriage becomes a problem that affect an increasing number of couples with the frequency of about 1% in the couples who want to conceive. This study is based on comparison of chromosome Heteromorphism in the karyotypes of two groups. The first group with 400 individuals with the history of more than two miscarriages and no live birth and as control group 200 individuals with one or more than one normal child. The study revealed that the frequency of chromosomal abnormalities and variations leading to recurrent miscarriage in couples was 18% Chromosomal rearrangements constituted 27.78% of the cases while heterochromatic variations constituted 72.22% of the chromosomal cause for recurrent miscarriages. In the present study, pericentric inversion of chromosome 9 and heteromorphism of chromosomes 1 were the most common findings. Present study indicates that there is need to evaluate the known heterochromatic variants as these variants play an important role in pregnancy loss.

Keywords: Chromosomal variations, infertility, recurrent miscarriage.

Introduction

Recurrent miscarriage is a common problem with approximately 15% of all clinically recognized pregnancies resulting in pregnancy failure. The causes of recurrent miscarriages are parental chromosomal anomalies, maternal thrombophilic disorders and structural uterine anomalies, maternal immune dysfunction and endocrine abnormalities and different kind of infections.

Chromosomal changes include normal polymorphic variants in addition to major chromosomal abnormalities. The term heteromorphism is also known as normal variant or polymorphism is used when there are any variations at chromosomal regions observed. Identification of more specific categorization of the already known variants as well as new variants has become much easier with the availability of new banding techniques[1]. Polymorphic variations are known to occur in the general population. They include varying sizes of heterochromatin blocks, satellite or repeat sequence regions and inversions[2,3]. Cytogenetic heteromorphisms have been characterized using multiple chromosome banding techniques. The common banding techniques are Giemsa, reverse, nucleolus organizing region, quinacrine. Common cytogenetic polymorphisms detected by GTG banding technique are considered as heteromorphisms which includes heterochromatin regions of chromosomes 1, 9, 16 and Y and also prominent acrocentric short arms, satellites and stalks[4].

Variations of the heterochromatic regions are individually stable and frequent in the normal population. Most polymorphic variants are familial and follow Mendalian inheritance from one generation to other with a low mutation rate[1]. De novo polymorphic chromosomal variants are rarer and appear, possibly as a result of an unequal crossover between heterochromatic regions of homologous chromosomes in meiosis. It is possible due to conjugation of repeated DNA sequences. De novo heterochromatic variants are considered to be large in size and to be associated with clinical conditions.

However, Madon et al reported the increased frequency of variants in association with different clinical conditions such as reproductive failure, recurrent spontaneous abortions and even psychiatric disorders[5].

Because of the need for more data on the knowledge of the recurrence risks involved in case of heterochromatic variations, the present study was undertaken with the objective of investigating the role of heteromorphic variations and chromosomal anomalies on spontaneous abortions and reproductive failures in the humans.

Material and Methods

Study Group and karyotype analysis: This study is based on comparison of chromosome Heteromorphism in the karyotypes of two groups. The first group with 400 individuals of 200 couples (age range 19 to 50, mean 32), with the history of more than two miscarriages and no live birth and as control group 200 individuals of 100 couples with one or more than one normal child (age range 20 to 50, mean 32), recruited simultaneously during the study at PreventiNe Life Care, Navi Mumbai.

Chromosome investigations were conducted by analysis of G-banded chromosomes using 2 mL heparinized peripheral blood sample. Metaphase spreads were made from phytohemaglutinin stimulated peripheral lymphocytes using standard cytogenetic techniques. Cultures were harvested and Karyotyping was performed on G-bands produced with trypsin and Giemsa (GTG)-banded chromosome preparations[6]. The metaphases were karyotyped using a Zeiss microscope (Carl Zeiss Light Microscopy, Germany) and MetaSystems software (Meta Systems, Germany).

Heteromorphisms were reported according to International System for Chromosome Nomenclature ISCN 2009[3]. Visualized polymorphic variations in the length of the centromeric heterochromatin on the long arms of chromosomes 1, 9 and 16 (1qh+/-, 9qh+/- and 16qh+/-) were documented. Distinct polymorphic variants of the size of satellites (ps+) and lengths of stalks (pstk+) of the acrocentric chromosomes (13, 14, 15, 21, 22) were also recorded. The pericentric inversion of chromosomes 9 was considered as a heteromorphism. For classification of variants, there should be at least twofold increase in the size of the corresponding region on the other homolog. This works as an internal control during chromosomal analysis.

Results and Discussion

Results: The incidence of heterochromatic variation on our sample is shown in table-1. Cytogenetic evaluation of 200 couples (400 individuals) with recurrent miscarriage revealed chromosomal anomalies and variations in 36 cases (18%). Chromosomal rearrangements constituted 27.78% of the cases while heterochromatic variations constituted 72.22% of the chromosomal cause for recurrent miscarriages. The heterochromatic variations associated with recurrent miscarriage included qh+/qh-, ps+/pstk+ and inversions. The three most common variants observed in this study constituted 38.46% of qh+/qh- followed by ps+/pstk+ which constituted 34.62% and the inversions constituted 26.92% respectively. In our control group of 200 individuals, chromosomal variations were observed in 9 individuals (4.5%).

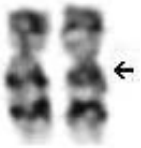
Inversion: The most common heterochromatin variants in the recurrent miscarriage group were heterochromatin variants within chromosome 9 (9qh+/9qh-/inv(9)) followed by chromosome 6. The 9qh+ observed in 2 females, 9qh- present in single female, 1qh+ found in 5 males as well as 1 female and 16qh+ also found in 1 female. The inversions constitute 26.92% of the heterochromatic variations. The most frequently observed inversion was chromosome 9 followed by inv(Y) and inv(6) of the heterochromatic variations.

Satellite variation: 13ps+ was found in single female. 15ps+ was found in 2 males. 15pstk+ was found in 2 females, 1 male and 21ps+ found in 1 female and 2 male.

Table-1
Frequency of heterochromatic variations in couples with recurrent miscarriages

Types of heterochromatic variations	Chromosome with heterochromatic variation	No. of Cases	Frequency in Recurrent Miscarriage Group (%)	
Variations of 'q' Heterochromatin	1qh+	6	23.07	38.46%
	9qh+	2	7.69	
	9qh-	1	3.85	
	16qh+	1	3.85	
Presence of satellite on Short Arm 'p'	13ps+	1	3.85	34.62%
	15ps+	2	7.69	
	15pstk+	3	11.54	
	21ps+	3	11.54	
Inversion	inv(6)	1	3.85	26.92%
	inv(9)	5	19.22	
	inv(Y)	1	3.85	

Table-2
Heterochromatic variations in couples with recurrent miscarriages

Variations of 'q' Heterochromatin	 **1qh+**	 **9qh+**	 **16qh+**
Presence of satellite on Short Arm 'p'	 **13ps+**	 **15pstk+**	 **21ps+**
Inversion	 **Inv(6)**	 **Inv(9)**	 **Inv(Y)**

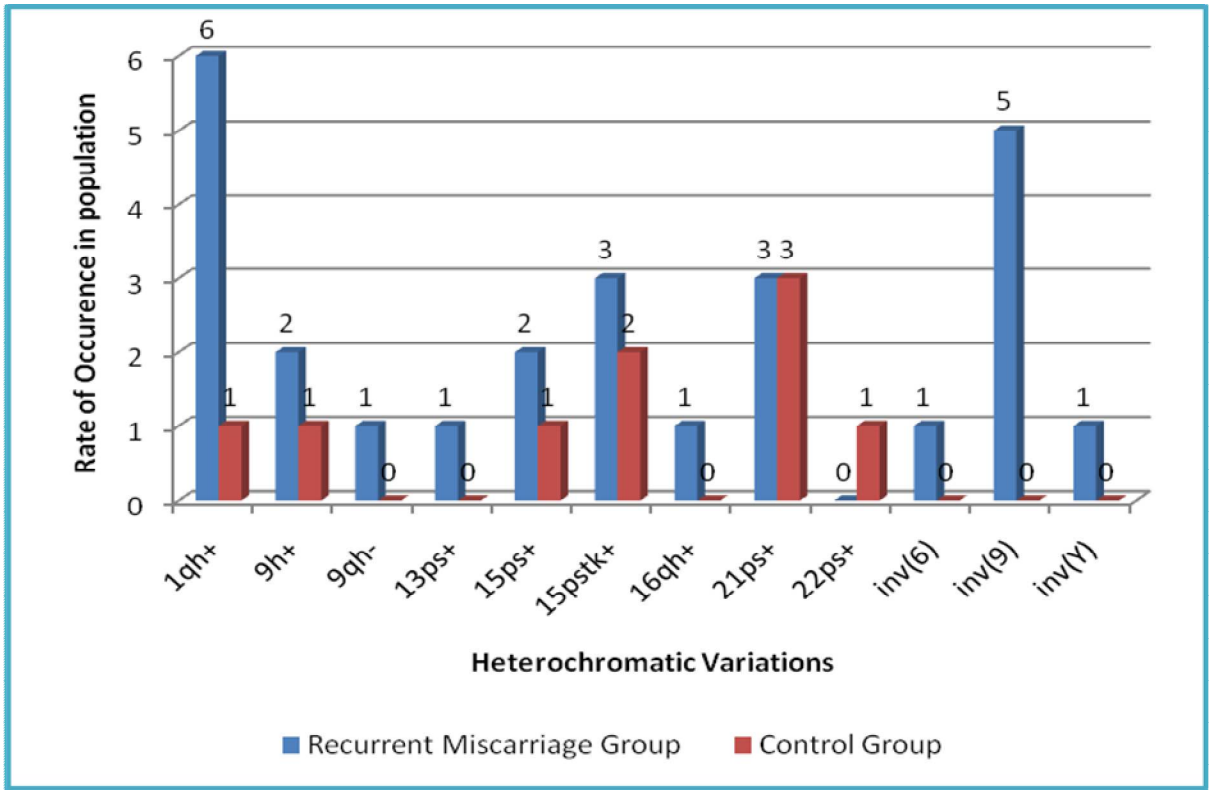


Figure-1
Occurrence of heterochromatic variations in Recurrent Miscarriage Group as compared to the Control

Discussion: The role of polymorphic chromosomal variations in infertility has been studied previously for many authors and despite of being over represented in infertile couples, no consistent data was found to correlate these variations with infertility. This subject continues to be an intriguing question.

The results of Madon et al. evaluated 842 individuals attending an IVF clinic with primary infertility or repeated miscarriages, showed polymorphic variants in 28.82% of males and 17.19% of females[5]. Hong et al. studied the effect of polymorphic variants in the outcome of in vitro fertilization on 1978 couples and found 182 males presented with chromosomal variations (9.2%). There was no any difference found among observed implantation rates but the incidence of first trimester pregnancy loss was higher compared to couples with normal karyotype[7]. Minocherhomji et al. in the case-control study identified a highly statistically significant increase in the frequency of total chromosomal variants in infertile women (28.31% vs. 15.16%) and infertile men (58.68% vs. 32.55%) as epigenetic alterations associated with the infertility phenotype[8]. Brothman et al. concluded that common cytogenetic variants were considered to be heteromorphisms without clinical significance[9]. Chopade et al. studied recurrent miscarriages, twenty nine individuals (16 males and 13 females; 9.06%) were found to have chromosomal heteromorphisms in the acrocentric chromosomes and in males the frequency of heteromorphism was 10% and in the females 8.12%[10]. Mau et al. reported chromosomal polymorphism in 13 out of 150 infertile male (8.7%)[11].

The increase in the length of the secondary constriction in the long arm of chromosomes 1, 9 and 16 is also common in chromosome variations. The repeat segments may cause clinical symptoms because of increased highly repetitive DNA sequences.

The heterochromatin regions contain a significant amount of repetitious DNA, the repetitious DNA of these heterochromatin regions is heterogeneous. Chromosomal variants are an expression of morphological variability chromosome-related changes in the amount of heterochromatin. It is believed that the presence of chromosomal variant increases the risk of the non-disjunction of chromosome segregation. Heterochromatin has a specific role and behavior in the synapsis of human homologous chromosome[12].

Changes in structural element of the centromere due to polymorphic, heterochromatin may lead to the defective chromosome segregation.

Variants of Chromosome 1: The polymorphism of 1qh+ have been reported in the relation with recurrent miscarriage or malignant disease by some authors. In inversion, inverted segment may cause synapsis failure, including asynapsis or early desynapsis, and pairing abnormalities of homologes leading to male infertility[1]. In general, inversions of heterochromatic regions are considered not to cause phenotypic abnormalities. In present study 6 cases with 1qh+ were found.

Variants of Chromosome 9 and infertility: The mechanisms of origin of inversions 9 are highly complex. The inv(9) is said to be common in the general population and it is inherited in a Mendalian fashion[13,14]. A small pericentric inversion of chromosome 9 is a most common inversion seen in human chromosomes with the incidence of 1-3% in the general population[14-16]. Pericentric inversion 9, especially complete inv(9)(p11q13) has been reported in association with recurrent miscarriages, infertility and congenital anomalies[4,5,13,17-22]. Inversion 9 has been considered to play significant role in chromosomal non-disjunction, and have variable effects on spermatogenesis, from azoopspermia to severly altered sperm morphology, motility and meiotic segregation.

During meiosis I, a loop will be formed in chromosomes with inversion and that can lead to production of abnormal and unbalanced gametes. Carriers of such inversion are at risk of having an offspring with unbalanced karyotype. It is suggested that inv (9) might have also some inter-chromosomal effect leading to a higher incidence of mitotic disturbances and it is known to be associated with aneuploidy such as mosaic Trisomy 21[5].

Our study revealed that chromosome 9 showed the maximum variations which included qh+ (11.54%) and inv(9) (19.23%). In the present study, heterochromatic variations of chromosome 9 were significantly more frequent as compared to the control individuals.

Polymorphic variations in acrocentric chromosomes: D/G-genome chromosomes are the common heteromorphisms show increased heterochromatin at the chromosome telomere, the short arm, and the nucleolar organizing region (NOR). Heterochromatin located in centromeres has an essential role in spindle attachment and chromosome movement, meiotic pairing and sister chromatid cohesion. Chromatin variation in these regions causes defects in centromere function and kinetochore assembly, difficulty in homologous chromosome pairing, and impacts on cell division, thus affects gamete formation [7].

No specific functions have been reported to be associated with the satellite segments (ps+). However such variations in the couple may make the fetus susceptible to translocations which may lead to fetal wastage[23].

Conclusion

The high incidence of heterochromatic variants of chromosome 1 and 9 (qh+ and inversion) is detected in the recurrent miscarriage cases. Therefore the study suggests the significantly higher incidence of pregnancy losses in those who are carriers of heterochromatic variations of chromosome 1 and 9. For the carriers there is a risk of transfer of abnormal chromosome

which could result in chromosomally unbalanced gamete and formed a malformed offspring or spontaneous fetal death. As the variants play an important role in reproductive failure, it is suggested that the cytogeneticists should not ignore these variants. Carriers of an abnormal karyotype should be counseled thoroughly to avoid unnecessary reproductive wastage. Pre-conceptional prenatal genetic testing is also indicated. Molecular cytogenetics may increase the number of variants, leading to the detection of new forms of polymorphisms in the human genome which are not detectable by previous methods. As we gain more insight into the human genome, the identification of chromosome variation will apparently receive new association.

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