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Prevalence and Types of Chromosomal Abnormalities among Infertile Patients from a Single Fertility Centre in India

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Abstract **Original Research Article**

Chromosomal aberrations either structural or numerical can contribute to infertility affecting 10-15% of couples of reproductive age. Chromosomal aberrations cause meiotic disturbances, leading to the formation of abnormal gamete, which upon fertilization may either cause miscarriage or development of abnormal offspring. Therefore, detection of chromosomal abnormalities based on karyotyping can be used as a preliminary diagnostic test. In this retrospective study, the prevalence and type of chromosomal aberrations was estimated over a period of 4.3 years from July 2015 to October 2019. A conventional cytogenetic study was performed on 4204 infertile individuals using peripheral blood lymphocyte cultures by the standard procedure of GTG banding. The prevalence and type of various structural and numerical aberrations among infertile individuals were evaluated and the overall rate of chromosomal aberrations were 1.55 % (65/4204). Among these cases, structural aberrations were observed in 36 (55.4%) individuals and numerical aberrations in 25 (38.4%) individuals. Reciprocal translocations (38.4%) were the most prevalent structural aberrations observed. Rare genetic conditions such as disorders of sexual development (4.6%) and chimerism (1.6%) were also detected in our study. The high prevalence of chromosomal abnormalities observed in our study highlights the importance of karyotyping prior to fertility treatment for infertile individuals. This helps in genetic counselling on how to manage the risks of birth defects or genetic disorders by providing various alternative approaches for successful pregnancy through assisted reproductive technique.

Keywords: Karyotyping, Chromosomal aberrations, Translocation, Mosaicism, Azoopsermia.

INTRODUCTION

Infertility is a health condition affecting 10-15% individuals of reproductive age around the world, who are attempting to achieve a successful pregnancy [1]. Infertility is caused due to several factors, such as environmental, endocrinological, nutritional, immunological, or genetic factors. Worldwide, 2-8% couples, suffering from recurrent pregnancy loss are found to have chromosomal abnormalities.

Chromosomal aberration is one of the important factors causing infertility. Gametes with abnormal chromosomal constitution have a very low chance of undergoing successful fertilization. Also, natural selection prevents the development of abnormal zygotes with major chromosomal aberrations, through spontaneous abortions [2]. The chromosomal aberrations are broadly classified into structural and numerical abnormalities. A structural aberration is the loss of genetic material or a rearrangement in the location of genetic material, which includes deletions, duplications, inversions, balanced or unbalanced translocations. Generally, those who are carriers of balanced translocations are clinically normal, but are at high risk of producing unbalanced gametes which may either result in spontaneous abortions or development of chromosomally abnormal offspring [3]. The previous studies have also reported that 50-60% spontaneous abortions are due to chromosome abnormalities [4]. A numerical abnormality is the change in the number of chromosomes, caused by non-disjunction during meiosis and results in trisomy, monosomy, and polyploidy of chromosomes [5].

Genetic testing is done for three main purposes in reproductive medicine: for identifying the cause of infertility, to identify and prevent the disorders which are

genetically transmissible to their offspring, and to increase the success rate of Assisted Reproductive Technology (ART) with better approaches [6, 7]. Conventional cytogenetic analysis, through karyotyping, provides the chromosomal constitution of an individual, giving important information about presence of aneuploidies and structural alterations.

This study aims at retrospectively analysing the prevalence and type of chromosomal aberrations found by cytogenetic analysis in infertile patients undergoing assisted reproductive techniques.

MATERIALS AND METHODS

In this study, patients who visited the infertility clinic of Gunasheela Surgical and Maternity Hospital, from July 2015 to October 2019 were referred for the karyotyping test. In total, 4204 individuals underwent conventional cytogenetic studies before starting assisted reproductive technique.

This retrospective study includes individuals who were referred for a karyotyping test with a clinical suspicion of an underlying chromosomal abnormality. The indication for cytogenetic evaluation included infertile couples with a history of recurrent pregnancy loss and recurrent implantation failure, males with severe oligospermia or azoospermia, couples with failed in-vitro fertilization (IVF) and those having a family history of genetic abnormalities. The referred cases were examined by taking case histories in prepared proformas. The case history covered reproductive failure details, laboratory investigation reports, family history of any genetic abnormality, age and number of miscarriages for all individuals of the study.

Chromosome analysis was performed as per the standard GTG banding protocol with slight modifications [8]. Peripheral blood samples collected in sodium heparin vacutainers were cultured in sterile 15mL culture tubes, using 8ml of RPMI medium 1640 (1X) (Gibco by *life* technologies^{TM}) and 150µl of Phytohemagglutinin (PHA) (M form) (Gibco by *life* technologies^{TM}). The tubes were incubated in a carbon dioxide incubator at 37°C for 72 hours, at the end of which 60µl of KaryoMAX COLCEMID (10µg/ml) (Gibco by *life* technologies^{TM}) was added to arrest the dividing cells at the metaphase stage. The tubes were further incubated in the CO_2 incubator for 40 minutes and centrifuged at 1000rpm for 10 minutes. The cells were then treated with the hypotonic solution (0.075M KCl) for 10 minutes in a 37°C water bath and fixed with Carnoy's fixative (3:1 ratio of methanol and glacial acetic acid). Following the pre-fixing step, the cells were given four subsequent washes with Carnoy's fixative to remove the cell debris. The cell pellet suspension was dropped on prechilled slides and dried at 42°C on a slide warmer. Then the slides were aged at 90°C in a hot air oven for an hour. Following which, standard GTG-banding using 0.5% Trypsin-EDTA (10X) (Gibco by *life* technologies^{TM}) and Giemsa's stain for microscopy (Merck) was done. Banded chromosomes were captured using Olympus BX53 microscope and karyotyped using computerized image analysis software - Applied Spectral Imaging (ASI). Routinely, ≥20 metaphases of proliferating lymphocytes from peripheral blood were analysed in each individual. In case of any suspected mosaicism or abnormal karyotypes, ≥50 metaphases were examined. Analysis was performed on GTG banded metaphase chromosomes with the resolution of 350 - 550 bands. The karyotypes were analysed and reported as per the guidelines provided by International System for Human Cytogenetic Nomenclature (ISCN).

The waver of consent was obtained for this retrospective study from the institutional ethics committee.

RESULTS

A total of 4204 infertile individuals were investigated by karyotyping, among which 4139 (98.45%) patients had normal karyotype, whereas 65 (1.55%) showed chromosomal aberrations. The distribution of these chromosomal aberrations and their corresponding karyotypes have been tabulated in Table 1 and 2. In our study it was observed that these chromosomal aberrations were almost equally distributed amongst male (49.2%) and female (50.8%) patients.

The chromosomal aberrations are broadly classified into structural and numerical abnormalities. Structural aberrations were found in 36 patients (55.4%), of which balanced reciprocal translocation (38.5%) and Robertsonian translocation (7.7%) were the most frequent structural abnormality observed. Other structural aberrations such as inversions were observed in 6.2%, and deletions in 3% of patients. Numerical aberrations were found in 25 patients (38.4%), of which Klinefelter's syndrome (21.5%) was the most prevalent numerical abnormality, followed by mosaic cell lines (12.3%), Turner syndrome (3%), and small supernumerary marker chromosome (1.6%). Disorders of sexual development (DSD), and chimerism are very rare chromosomal abnormalities. In our study we found three patients (4.6%) with sexual development disorders, of which two patients were phenotypic females with a male chromosome complement, and one was a phenotypic male with a female chromosome complement. One patient (1.6%) was a phenotypic male identified with chimerism having both male and female chromosomal complement. The distribution of chromosomal abnormalities and prevalence of each abnormality in infertile individuals is depicted in a pie chart below in Figure-1.

Chromosomal polymorphisms were detected in 33 individuals, among which inversion 9 was the most prevalent polymorphic variant found in 19 patients. Heterochromatin regions of chromosome 1 (1qh+), satellites on short arms of acrocentric chromosomes and pericentric inversion of Y were also observed. Chromosome polymorphisms such as Inversion 9, pericentric inversion of Y, satellites, and other variants were reported as normal variations and not investigated in this study. These polymorphic variants have been tabulated in Table-3.

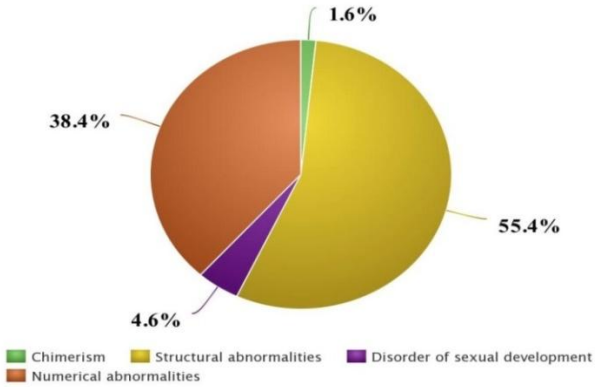

Fig-1: Prevalence of chromosomal abnormalities identified in infertile individuals

Table-1: Karyotype of all infertile individuals with structural chromosomal abnormalities and its prevalence

Structural abnormalities				
Abnormalities		**Karyotype**	**No.**	**%**
Inversion		46,XX,?inv(17)	**4**	**6.2**
		46,XY,inv(5)(p15.1q31)		
		46,XY,inv(21)(q11.2q22.3)		
		46,XX,inv(10)(p11.2q21.2)		
Deletion		46,X,del(X)(p11.2)	**2**	**3**
		46,X,del(Y)(q11.23)		
Translocation (carriers)	Robertsonian translocation	45,XY,der(13;14)(q10;q10)	**5**	**7.7**
		45,XX,der(13;14)(q10;q10)		
		45,XY,der(13;14)(q10;q10)		
		45,XX,der(13;14)(q10;q10)		
		45,XX,der(13;15)(q10;q10)		
	Reciprocal translocation	46,XX,t(11;22)(q23;q12)	**23**	**38.5**
		46,XY,t(2;14)(p23;q24)		
		46,XX,t(10;14)(p13;q24)		
		46,XX,t(6;7)(q25;q22)		
		46,XX,t(8;12)(p11.2;q24.3)		
		46,XX,t(20;22)(q11.2;p11.2)		
		46,XY,t(2;20)(q22;p13)		
		46,XY,t(4;11)(p14;q13)		
		46,XX,t(2;9)(p13;p22)		
		46,XX,t(1;18)(q32;p11.2)		
		46,XY,t(2;17)(q11.2;q25)		
		46,XY,t(2;17)(q31;p13)		
		46,XX,t(11;22)(q23;q11.2)		
		46,XX,t(1;6)(p36;q13)		
		46,XX,t(1;6)(p36;q13)		
		46,XY,?t(18p;22p)		
		46,XY,t(6;11)(p21;q23)		
		46,XX,t(5;8)(q31;q22)		
		46,XX,t(2;4)(q31;q31)		
		46,XX,t(7;13)(p13;q22)		
		46,XY,t(10;12)(q22;q22)		
		46,XY,t(8;12)(p11.2;q24.3)		
		46,XX,t(11;13)(q13;q14)		
	Double translocation	45,XX,der(13;14)(q10;q10),t(4;7)(q25;p15)	**1**	
	Complex translocation	46,XX,t(8;9;14)(q13;q13;q24.3)	**1**	

Table-2: Karyotype of all infertile individuals with numerical and other chromosomal abnormalities and its prevalence

Numerical abnormalities			
Abnormalities	**Karyotype**	**No.**	**%**
Mosaic Cell Lines	mos 45,X[2]/46,X,i(X)(q10)[18]	**8**	**12.3**
	mos 45,X[18]/46,XX[12]		
	mos 47,XY,+mar[14]/46,XY[36]		
	mos 45,X[5]/46,X,del(X)(q25)[7]/46,XX[38]		
	mos 45,X[2]/46,X,del(X)(q25)[16]/46,XX[32]		
	mos 45,X[9]/46,XX[31]		
	mos 45,XY,der(14;14)(q10;q10)[11]/46,XY[3]		
	mos 45,X[42]/46,XY[8]		
Klinefelter's syndrome	47,XXY	**14**	**21.5**
Turner syndrome	45,X	**2**	**3**
small Supernumerary Marker Chromosome	47,XX,+mar	**1**	**1.6**
Other chromosomal abnormalities			
Disorder of Sexual Development	46,XX (Male with female chromosome complement)	**1**	**4.6**
	46,XY (Female with male chromosome complement)	**2**	
Chimerism	chi 46,XY[31]/46,XX[18]	**1**	**1.6**

Table-3: Karyotype of infertile individuals with polymorphic variants and its prevalence

Chromosomal polymorphisms		
Normal variation	**Karyotype**	**No.**
	46,XX, 15p+	**3**
	46,XX, 15ps+	**2**
	46,XX,1qh+	**1**
	46,XX,inv(9)(p12q13)	**19**
	46,X,inv(Y)(p11.31q11.23)	**3**
	46,X,Yqs	**2**
	46,XX,22ps+	**2**
	46,X,Yqh-	**1**

DISCUSSION

Our study results show the frequency and distribution of various chromosomal aberrations found among the infertile patients studied. It is observed that 1.55% of infertile individuals (65/4204) had chromosome aberrations. Out of these, structural chromosomal aberrations were found with a prevalence of 55.4% (n = 36), numerical aberrations with an incidence of 38.4% (n = 25) and other chromosomal aberrations accounting for 6.2% (n = 4).

As of structural aberrations, our data shows that a high number of infertile couples are affected by balanced reciprocal translocation with a prevalence of 38.5% (n=25). The translocations which we observed are divided into balanced reciprocal translocations and Roberstonian translocations. In balanced reciprocal translocation, usually there is an exchange of chromosomal segment between two non-homologous chromosomes, the carriers of such balanced translocations produce gametes with unbalanced chromosomal complement with duplications and/or deletions. Such imbalances are lethal to the developing embryo or fetus, which may cause spontaneous abortion. Couples experiencing 2-3 recurrent spontaneous abortions are suspected to be carriers of such translocations. The exact risk depends on the specific chromosomes involved, size of the segment involved in the rearrangement, genes contained in the segment, sex of the transmitting parent, family history, and mode of ascertainment [9]. The following reproductive outcomes are expected from the individuals carrying balanced reciprocal translocation between two chromosomes, 25% of offspring would be chromosomally normal, 25% would have the reciprocal translocation, and 50% would be chromosomally unbalanced [10].

Complex chromosomal rearrangements (CCRs) are defined as reciprocal exchanges between three or more chromosomes [11]. Such chromosomal rearrangements are quite rare, but for individuals who have reciprocal translocations between three chromosomes, the following reproductive outcomes would be expected: 12.5% of offspring would be chromosomally normal, 12.5% would have the reciprocal translocation, and 75% would be chromosomally unbalanced [10]. In our study, we found one female patient (1.6%), with complex translocation, involving chromosome 8, 9, and 14. Another female patient showed double translocation (1.6%), that is, Robertsonian translocation between chromosomes 13 and 14, and balanced reciprocal translocation between

chromosome 4 and 7. Both patients were diagnosed with recurrent pregnancy loss. The interpretation of complex translocation by conventional GTG banding alone is not sufficient, especially when other chromosomal aberrations such as insertions, deletions or inversions are present along with the reciprocal translocations [11]. In such cases the patients are referred for Spectral Karyotyping (SKY) to explore chromosome rearrangements in greater detail.

Robertsonian translocations result from the fusion of the entire long arm of two acrocentric chromosomes. The incidence of Robertsonian translocations is estimated to be 1/1000 live births. Although all acrocentric chromosomes are capable of participating in Robertsonian translocations, their occurrence in general population is not so random, with der(13q14q) occurring most commonly, having a frequency of 85% and the rest of the Robertsonian translocations accounting for only 15% [12]. Our data also showed similar results with higher frequency of der(13q14q), and one patient with der(13q15q), with a total prevalence of 7.7% (n=5). Such carriers may give birth to infants with Patau syndrome (trisomy 13). However, most of these conceptions are observed to result in early pregnancy loss [13]. Carriers do not show any abnormal phenotypes and remain undetected until they attempt to reproduce. The male carriers experience infertility associated with oligospermia, whereas females experience miscarriage or infertility [12].

Inversion is the rearrangement of a single chromosome within itself. The complications caused by the inverted chromosome depends on the size of inverted segment and the chromosome involved, which may either cause recurrent miscarriage or increase risk of giving birth to a child with congenital defects [14]. We observed 4 cases of inversion with a prevalence of 6.2%, of these, one case was found with pericentric inversion of chromosome 10. According to the study conducted by Morag N. Collinson *et al.,* inv(10)(p11.2q21.2) can be regarded as a variant analogous to the pericentric inversion of chromosome 2 [inv(2)(p11q13)]. Also there has been no recorded instance of a recombinant chromosome 10 arising from this inversion and no excess of infertility or spontaneous abortion among carriers of either sex. In another observed case the individual had inversion 5 where to such carriers would be expected to produce gametes with (a) a normal chromosome, (b) a pericentric inversion, (c) a partial deletion of short-arm material and partial duplication of long arm material, and (d) a partial duplication of short-arm material with partial deletion of long-arm material [15]. Also, one patient with paracentric inversion of chromosome 21 was seen - a rare chromosomal inversion. To our knowledge this inversion has not been reported till date and clinical significance of such condition is not known. The referred patient was a male with oligospermia. Further molecular studies are required to learn the impact of such inversions.

Deletions involve loss of a chromosome segment, resulting in chromosome imbalance. A chromosomal deletion produces monosomies that are usually associated with significant pathology due to haploinsufficiency. In our study we observed two cases with allosomal deletions, with a prevalence of 3%. One individual with 46,X,del(Y)(q11.23), this region on chromosome Y contains genes encoding azoospermic factors (AZF) and any deletions in this region cause azoospermic, or oligospermic conditions [16, 17]. Hence, any individual suspected with such deletions during conventional studies are referred for molecular analysis for Y-microdeletions for further confirmation. The other individual was identified with 46,X,del(X)(p11.2), affecting the short arm of the X chromosome at band p11.2. Such deletions, are considered as variant of Turner syndrome and were observed to cause ovarian failure in about half of the women, and the other half experienced menstrual irregularities. Even if menstruation occurs, fertility is rare. If the deletion occurs more distally, such as at band p21, patients usually display a milder phenotype with normal menarche, even though secondary amenorrhea or infertility is common. Most women with Xp deletions are short, even if ovarian function is normal. [18].

Numerical chromosomal aberrations are those that cause a change (addition or deletion) in the number of chromosomes, and were observed in 38.4% (n=25) of the patients. Of these, a high frequency of individuals (21.5%) were detected with Klinefelter's syndrome (with an additional X chromosome - 47,XXY) in 14 cases. It is found to be the most prevalent sex chromosomal aberration affecting 1 in 660 newborn males [19]. According to literature, an extra X chromosome is the result of meiotic non-disjunction during parental gamete formation, the chances of which increase with both maternal and paternal age [20, 21]. This sex chromosomal abnormality is associated with severe spermatogenic failure causing a reduction in testicular size and are usually azoospermic resulting in infertility [22]. A study conducted by Lissitsina *et al.,* showed that the prevalence of Klinefelter's syndrome among infertile men is high in those having azoospermia, as opposed to those with oligospermia [23]. Our study showed the similar result, with higher incidence of azoospermic patients having Klinefelter's syndrome.

Turner syndrome (TS) is a sex chromosome aberration in females characterised by partial or complete loss of one X chromosomes. 45,X karyotype was found in 2 cases with a prevalence of 3%. It occurs with an incidence of approximately 1 in 2200 new born females [24]. Spontaneous puberty occurs in 5–30% of Turner's syndrome individuals and fertility rates vary

from 5–10% [25]. A case report by Cools M *et al.*, described a 45,X karyotype individual with three pregnancies, her first daughter had normal 46,XX karyotype; her second pregnancy ended in miscarriage and the third pregnancy resulted in a girl child with 45,X karyotype [26]. According to previous literature reviewed by Gorduza *et al.*, spontaneous pregnancy in Turner syndrome patients with 45,X karyotype is rare, and in case of mosaicism, probability of pregnancy is high when compared to Turner syndrome. But chromosomal abnormality in foetus and miscarriage rate is high in both pure and mosaic karyotype [27]. Therefore, these patients should be monitored carefully during pregnancy. Spontaneous pregnancy in Turner syndrome patients are still unclear. Therefore, further research in this area is required in order to clarify the actual mechanism of spontaneous conception in this genetic condition.

Mosaic cell lines were observed in our study with the prevalence of 12.3 % (n=8). They are characterised by the presence of two or more cell lines with different chromosomal constitutions such as 45,X/46,XX. In our study we identified 6 individuals with mosaic variants for Turner syndrome. Mosaic Turner syndrome individuals are more likely to experience normal pubertal development, regular menstrual cycles and achieve a spontaneous pregnancy when compared to Turner syndrome [28]. Spontaneous pregnancies are more frequent in individuals with mosaic Turner syndrome [29]. Tarani *et al*., reviewed and studied 6 cases of women with Turner syndrome having mosaic karyotype and analysis of these patients with spontaneous pregnancies, had a high risk of miscarriage, still-birth and malformations in offspring [30]. But they are more likely to be fertile than those with Turner syndrome. Our study has also detected low level sex chromosome mosaicism. Carriers with low level sex chromosome mosaicism can have an increased risk of chromosomal segregation error leading to an abnormal pregnancy [31].

We have detected one individual with 45,X/46,X,i(Xq) mosaicism, a variant of Turner syndrome (TS), has the short arm of the X chromosome lost, while the long arm is duplicated. In this case, 10% of cells were found with 45,X chromosome complement and the rest 90% of cells with 46,X,isochromosome Xq. Genes located on the p-arm of the X chromosome are important for normal ovarian function. This suggest the pathogenesis of gonadal dysgenesis occurred in Turner females [32]. The major band p11 on X chromosome is usually not inactivated and could be the position of the Xp gonadal determinant. The absence of Xp determinant could account for infertility [33]. The 45,X/46,XY mosaicism represent a wide spectrum of phenotypes, from Turner females to phenotypically normal males with varying degrees of genital ambiguity. The presence of Y chromosome material in female individual with TS are at increased risk of developing gonadoblastoma and estimated to be 15-20%. Gonadal dysgenesis and infertility can occur in Turner patients with 45,X/46,XY mosaicism [34]. We have also detected one male patient with 45,XY,der(14;14)/46,XY mosaicism. To our knowledge, such a case has not been reported till date and its clinical significance is not known.

Small Supernumerary Marker Chromosome (sSMC) is a very small segment of unidentified chromosome that occurs in addition to the normal set of 46 chromosomes. Only one case, with a prevalence of 1.6%, was detected with marker chromosome in all the cells analysed. Most of the cases occur as de novo and are usually derived from acrocentric chromosomes [35]. The role of such marker chromosome should be considered and further molecular cytogenetic study is required to know the origin of the marker chromosome.

Disorder of sexual development (DSD) is a rare genetic condition that includes a group of congenital disorders concerned with atypical development of external and internal genital structures. We found 3 such cases and its prevalence is 4.6% in our study. XX- male syndrome was observed in one individual who showed phenotypic male characteristic with female chromosome complement. Most of them have the SRY gene translocated to an X chromosome but they lack the important spermatogenic genes located on the q-arm (long arm) of the Y chromosome [36]. Normal male genitalia are seen in 46,XX males with SRY, and testicular DSD, but show arrest in spermatogenesis and develop azoospermia and severe testicular atrophy [37].The SRY gene encodes the critical testis-determining transcription factor which activates a number of downstream transcription factors involved in testes formation.

Two phenotypic female individuals were found with male chromosome complement in this study, with an incidence of 3%. The clinical spectrum of 46,XY DSDs is very much variable, along with their genetic background [38]. Androgen insensitivity and gonadal dysgenesis, are what mostly cause 46,XY females. In few cases, mutations are found in the SRY gene - sex determining region of chromosome Y [39]. In both the above cases of DSDs, X-Y rearrangements cannot be detected using conventional cytogenetic method. Further molecular cytogenetic studies are required to check the status of SRY gene, to correctly give the prognosis with respect to infertility.

Chimerism is a genetic condition, where an individual has more than one cell line. Chimeric patients, with a 46,XX/46,XY karyotype, are extremely rare and only one such case of a phenotypic male was seen in our study with a prevalence of 1.6%. Most of the chimeric individuals are infertile, however, there have been reported cases where chimeric patients with

azoospermia/oligospermia, fathered healthy offspring aided with successful in vitro fertilization [40, 41]. Thus, it can be said that chimeric individuals, though thought to be infertile, have the chance of bearing offspring through assisted reproductive techniques.

Chromosomal polymorphism or heteromorphism are structural chromosome rearrangements that are considered to have no apparent clinical consequences for the patients that carry them. The chromosomes that carry these rearrangements are referred to as normal heteromorphic or polymorphic variants [42]. Constitutive heterochromatin consists of highly repeated sequences of DNA that do not encode proteins, and variations in this region are considered as normal [43]. Brothman *et al.*, concluded from their survey, that common cytogenetic variants are considered to be heteromorphisms having no clinical significance [44]. Thus, there is no conclusive evidence reported in earlier studies, so it is difficult to conclude that chromosomal polymorphisms or hetermorphism can cause infertility. In our study, chromosomal polymorphisms were detected in 33 individuals, among which inversion 9 was the most prevalent polymorphic variant found in 19 patients. Heterochromatin regions of chromosome 1 (1qh+), satellites on short arms of acrocentric chromosomes (eg. 15ps+ and 22ps+), and pericentric inversion of Y were also observed. Chromosome polymorphisms were reported as normal variations and were not investigated in this study.

However, in recent years studies suggest that some polymorphic variants in heterochromatic and NOR regions could play a significant role in certain clinical conditions. According to the study conducted by Pokale, a higher incidence of chromosomal variants of chromosome 1 and 9 (qh+ and inversion) was detected in the recurrent miscarriage cases [45]. Another study carried out by Minocherhomji S. *et al.*, showed higher incidence of polymorphic variants in infertile men (58.68%) and women (28.31%) compared with fertile men (32.55%) and women (15.16%) [46]. These studies suggest that chromosome variants may play a role in infertility. The contribution of chromosome variants to infertility is still questionable. Future investigation on larger study population and analysis at molecular level is required to evaluate their role in infertility.

CONCLUSION

Our study highlights the prevalence and distribution of various chromosomal aberrations in couples experiencing recurrent miscarriage or infertility, and the necessity of conventional karyotyping for such couples. All couples with the history of two or more miscarriages, or those who are unable to achieve pregnancy are recommended to undergo parental cytogenetic analysis with karyotyping to rule-out the possibility of chromosomal rearrangement. In some cases, combined use of conventional and molecular cytogenetics is necessary to identify the chromosomal regions involved in specific rearrangements. Identifying the presence of chromosomal aberrations in a parent is useful because it not only explains the cause for miscarriages or infertility, but also provides information about the risk of child to be born with severe congenital abnormalities, and risk for future miscarriages. Such cases have to be detected as early as possible to arrange for adequate genetic counselling and to allow parents to make informed reproductive decisions regarding subsequent pregnancies. With the advent of technology, preimplantation genetic testing (PGT) for aneuploidies is now widely available and will be useful in such couples. Alternatively, the subsequent pregnancies must be monitored with prenatal diagnosis for the suspected chromosomal abnormality. Thus, karyotyping can be considered as a preliminary test for unexplained infertility prior to assisted reproductive Ttechniques.

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Declarations

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Authors' contributions

Jnapti Johnson: Performed and analysed karyotypes, wrote the manuscript, interpretation of the results.
Navya Shetty: Performed and analysed karyotypes, wrote the manuscript, interpretation of the results.
Ajay Kumar J.: Performed and analysed karyotypes, wrote the manuscript, interpretation of the results.
Rajsekhar Nayak: The main conceptual ideas and proof outline.
Devika Gunasheela: The main conceptual ideas, proof outline, clinical evaluation of patients and patient counselling.
Jayarama S Kadandale: Devised and supervised the project, conceptual ideas, evaluation and interpretation of the karyotype results, proof outlines.
Swathi Shetty: Conceptual ideas and proof outline.
All authors provided critical feedback and helped shape the research, analysis and manuscript.

Ethics Approval

A waiver of consent was obtained for the study from the institutional ethics members since this is a retrospective study.

Consent to Participate: Informed consent was obtained from all the participants

Consent for Publication: Not applicable.

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