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CHROMOSOMAL ABNORMALITIES CAUSING RECURRENT PREGNANCY LOSS IN FEMALES IN MEERUT REGION

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Abstract

Recurrent pregnancy loss is a devasting event affecting 1-5% of couples in their reproductive life. Chromosomal rearrangements are considered to be an important cause of recurrent miscarriages with prevalence of translocation in either of the parent. Purpose of present study, is to detect the presence of chromosomal abnormalities in the females suffering from recurrent pregnancy loss in Meerut region. A total of 300 female subjects with two or more consecutive pregnancy loss were evaluated for the presence of chromosomal translocation through blood lymphocyte culture. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study. In our present study, chromosomal aberrations were found in twelve females with six having reciprocal translocation, four with inversion, one case with Robertsonian Translocation and one case of Balanced Complex Chromosomal Rearrangement. Early recognition of presence of any chromosomal abnormality will be helpful in providing genetic counselling and fetal monitoring in subsequent pregnancies.

Keywords: *Spontaneous abortions, chromosomal aberration, translocation, genetic counselling, CCR.*

Introduction

Miscarriages which are considered as most common complication of pregnancy, affects approximately 15% to 20% of all clinically recognized pregnancies in the general population with recurrent pregnancy loss (RPL) occurring in about 1% to 2% of this same population. The traditional definition of RPL included those couples with 3 or more spontaneous abortions. The ASRM (American Society of Reproductive Medicine) has defined RPL as "a distinct disorder defined by 2 or more failed clinical pregnancies". Several studies also recently indicated that the risk of recurrent miscarriage after 2 successive losses is similar to the risk of miscarriage in women after 3 successive losses; thus, it is reasonable to start an evaluation after 2 or more consecutive spontaneous miscarriages to determine the cause of their pregnancy loss, especially when the woman is older than 35 years of age, or when the couple have had difficulty conceiving.

Various factors that are considered to be associated with recurrent pregnancy loss include parental and fetal chromosome abnormalities, uterine abnormalities, antiphospholipid syndrome, some thrombophilias, autoimmune disease and endocrinological disorders such as polycystic ovarian syndrome and untreated diabetes .Among these well established one are genetic, uterine anomalies and thrombophillia .However studies reveal that in 4% to 8% of couples with recurrent pregnancy loss there is presence of chromosomal aberration in at least one of the partners which may be either numerical or structural .

This study was conducted to determine the role of chromosomal abnormalities in causing recurrent pregnancy loss. Identification of the underlying factors is crucial not only for the development of more successful treatment but also in the improvement of the outcome of future pregnancies in women experiencing Recurrent Miscarriages (RM). Genetic factors causing RM are, however, difficult to study because the foetus is lost at an early stage of development and is therefore difficult to examine. Consequently, most of the studies and the present study is based on studying the couples experiencing the miscarriages.

Materials And Methods

This study included 300 females with occurrence of two or more recurrent pregnancy loss from Meerut region who visited different gynecological centers between October 2010 and December 2012. Blood samples of these subjects were referred for cytogenetic studies at the Dr Lal

Pathology Lab, Delhi The medical history of the patients was also taken in consideration.

Chromosome preparation and GTG banding

For standard cytogenetic analysis, heparinized human whole blood (0.4 mL) was cultured a 37° C for 72 hr in 10mL Chromosome Medium (RPMI 1640). Cell division was arrested by colchicine (10μg ml^{-1}) for 30 minutes. Chromosome preparations were made by incubating the cell suspension in 0.075m KCL for 13 minutes followed by a fixation step in a freshly prepared mixture of 3:1 methane : acetic acid at -20° C .GTG banding was performed by incubating glass slides in a 0.05% trypsin solution at 37° C for 15 second followed by rinsing the slides in phosphate buffered saline and staining in 5% Giemsa stains for 8 minutes. Then slides were rinsed with water and air dried. Chromosomes were analyzed using image software Cytovision 4.2. Karyotypes were reported as per ISCN 1995.

Results

A total of 300 females with history of repeated abortions were examined. The age of the females ranged from 21 to 37 years.

Among the studied subjects, twelve females with overall incidence of 4% were found to have abnormal karyotype (Table 1). Among twelve subjects, abnormalities included six balanced Reciprocal Translocation (50 %.) with overall incidence of 2%, four inversions (33.3%) with overall incidence of 1.4%, one case of Robertsonian Translocation (8.33%) with overall incidence of 0.3% and one case of Balanced Complex Chromosomal Rearrangements i.e., CCR (8.33%) with overall incidence of 0.3% (Bar Chart 1& 2).

Discussion

The evaluation of patients with a history of repeated spontaneous abortions requires careful consideration of potential genetic, anatomic, endocrine, infectious, and immunologic factors. Assigning proper etiological role to each of these contributing factors is often unclear, however the specific information about the cytogenetic makeup of the couples and if possible of the abortus, still remains a primary focus during evaluation of such cases. In this study, the incidence of chromosomal abnormalities among the females with RSA was 4% The frequency reported in literature varies from 2.9 to 5% except few studies in which higher frequencies have been reported but the number of subjects studied were less. The incidence of chromosomal abnormality in couples with recurrent abortions reported in one cumulative study was 2.86% on an

average, which is less than that of the present study.

The finding of this study is in agreement with the study of Dubey *et al.* on 742 couples, which showed 2% risk of chromosomal abnormalities in subjects with recurrent spontaneous abortions, of which structural abnormalities formed the largest group. The findings of this study are also in agreement with the study of Sheth *et al.* on 415 couples, which showed 3.5% risk of chromosomal abnormalities in subjects with recurrent spontaneous abortions.

In the present study, Translocations constituted about 50% of all the chromosomal abnormalities, which makes it the most common chromosomal abnormality. The findings of Sheth et al. (2012), are also in agreement of our study who reported the presence of 3.5% chromosomal abnormalities.

In a balanced translocation, a person usually has all the genetic material necessary for normal growth — a piece of a chromosome is merely broken off and attached to another one. Carriers of balanced chromosome rearrangements, although phenotypically normal, experience recurrent adverse pregnancy outcomes as a consequence of formation of unbalanced gametes and another important concern in couples with a partner having structural chromosomal abnormality is the risk of giving birth to an abnormal child. Their risk of giving birth to an abnormal child with chromosomal imbalance is very low. The exact risk depends upon the specific chromosome involved, size of segment(s) involved in the rearrangement, sex of the transmitting parent and the mode of ascertainment.

Only one case of Robertsonian translocations was reported with t(14q;21q). It is a common and significant type of chromosome rearrangement that is formed by fusion of the whole long arms of two acrocentric chromosomes (chromosomes with the centromere near the very end). One in about 900 babies is born with a Robertsonian translocation making it the most common kind of chromosome rearrangement known in people. Balanced Roberts onian translocation only matters when a couple tries to have a baby. Studies indicate that when the Robertsonian translocation is maternal, there is greater risk that fetus will exhibit an unbalanced phenotype. In the present case, Robertsonian translocations between 14 and 21 may result in trisomy 21 (Down syndrome).

Pericentric inversion of chromosome 9 was noted in four out of twelve cases in which breakage and reunion have occurred at bands 9p11 and 9q13. Though pericentric inversions

have been detected in all chromosomes with varying frequencies, chromosomes 2, 5, 7, 9 and 10 are statistically more prone to rearrangements. As many as 1:100 people exhibit such inversions in the heterochromatic region of chromosome 9, which is considered to be a population variant. This study showed that inversion of chromosome 9 is the most common variant. It was seen in four of all females in this study and its relative high prevalence suggested that inversion of chromosome 9 is the most common chromosomal alteration associated with repeated pregnancy loss but it does not confirm any strong relationship with spontaneous abortions. Further more studies need to be done to prove any strong association.

Three way translocations involving reciprocal exchange are not so common, one case of reciprocal translocations in which three chromosomes were involved in a particular translocation event was observed in the present study. This case involves long arm of chromosome 4 and short arm of chromosome 16 and 20. As the woman on whom this rearrangement had been identified was entirely healthy, it was concluded that three way translocation was balanced. Ascertainment was done because of recurrent spontaneous abortions. Although no cytogenetic study of the abortuses of this subject was conducted, it was reasonable to associate her spontaneous abortions due to unbalanced karyotype in fetus. The proband conceived after this evaluation. The couple did not give consent for any prenatal testing. She delivered a full-term normal male baby without any malformations. The karyotype of the baby was done for balanced rearrangement and the baby was found to have 46XY karyotype.

Conclusion

Cytogenetic analysis should be performed in all couples with more than two spontaneous abortions as it is an important part of the etiological investigation. Physicians should bear in mind that at least 5% of the couples they examine, chromosomal abnormality is the cause of abortion. Those cases have to be detected as early as possible to arrange for adequate genetic counseling and to allow parents to make an informed reproductive decision regarding subsequent pregnancies

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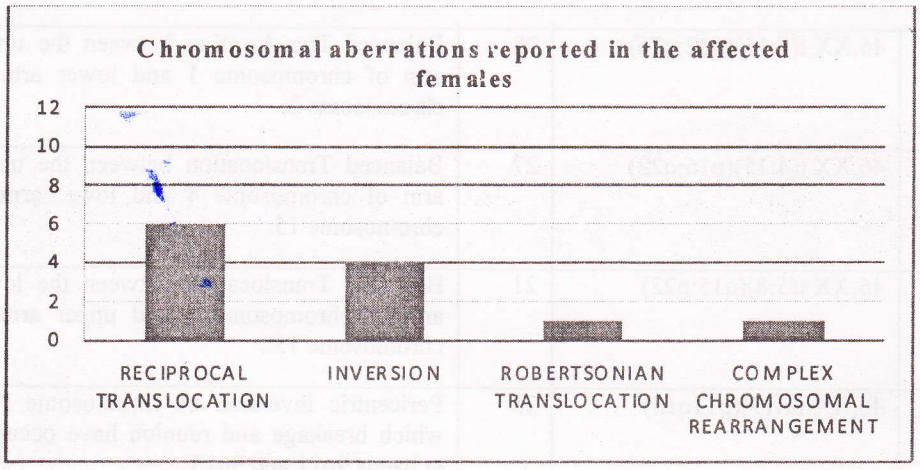
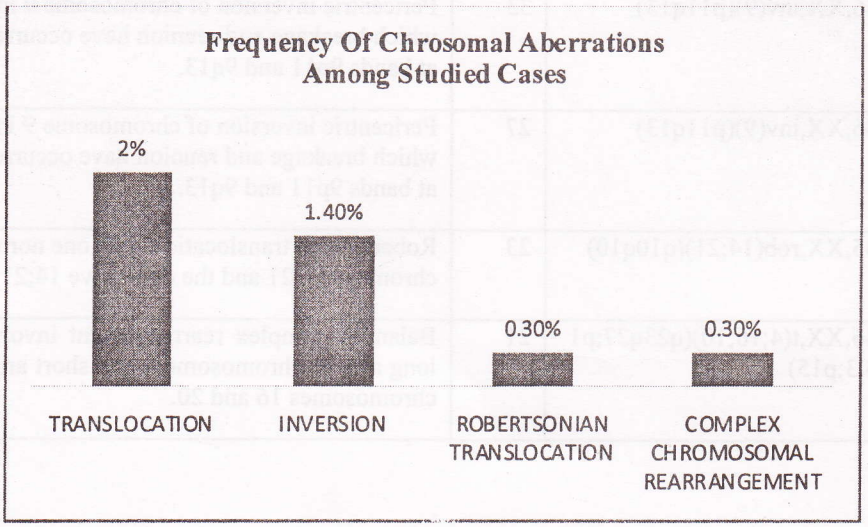
Table I : IDENTIFIED CHROMOSOMAL ABNORMALITIES

S. NO	KARYOTYPE	AGE	DESCRIPTION
1	46,XX (2;15)(p16;q26)	37	Balanced Translocation between the upper arm of chromosome 2 and lower arm of chromosome 15.
2	46,XX (4;15)(p16;q32)	30	Balanced Translocation between the upper arm of chromosome 4 and lower arm of chromosome 15.
3	46,XX(11;22)(p23;q11))	24	Balanced Translocation between the upper arm of chromosome 11 and lower arm of chromosome 22.

4	46,XX t(7;15)(q22;q26)	28	Balanced Translocation between the upper arm of chromosome 1 and lower arm of chromosome 2.
5	46,XX t(4;15)(p16;q22)	27	Balanced Translocation between the upper arm of chromosome 4 and lower arm of chromosome 15.
6	46,XX t(5;8)(q15;p22)	21	Balanced Translocation between the lower arm of chromosome 5 and upper arm of chromosome 15.
7	46,XX,inv(9)(p11q13)	25	Pericentric inversion of chromosome 9 in which breakage and reunion have occurred at bands 9p11 and 9q13.
8	46,XX,inv(9)(p11q13)	24	Pericentric inversion of chromosome 9 in which breakage and reunion have occurred at bands 9p11 and 9q13.
9	46,XX,inv(9)(p11q13)	33	Pericentric inversion of chromosome 9 in which breakage and reunion have occurred at bands 9p11 and 9q13.
10	46,XX,inv(9)(p11q13)	27	Pericentric inversion of chromosome 9 in which breakage and reunion have occurred at bands 9p11 and 9q13.
11	45,XX,rob(14;21)(q10q10)	23	Robertsonian translocation with one normal chromosome 21 and the derivative 14;21
12	46,XX,t(4;16;10)(q23q27;p13.3;p15)	21	Balanced complex rearrangement involving long arm of chromosome 4 and short arm of chromosomes 16 and 20.



Bar Chart 1 showing detected chromosomal abnormalities among affected females



Bar Chart 2 showing frequency of chromosomal aberrations among studied cases

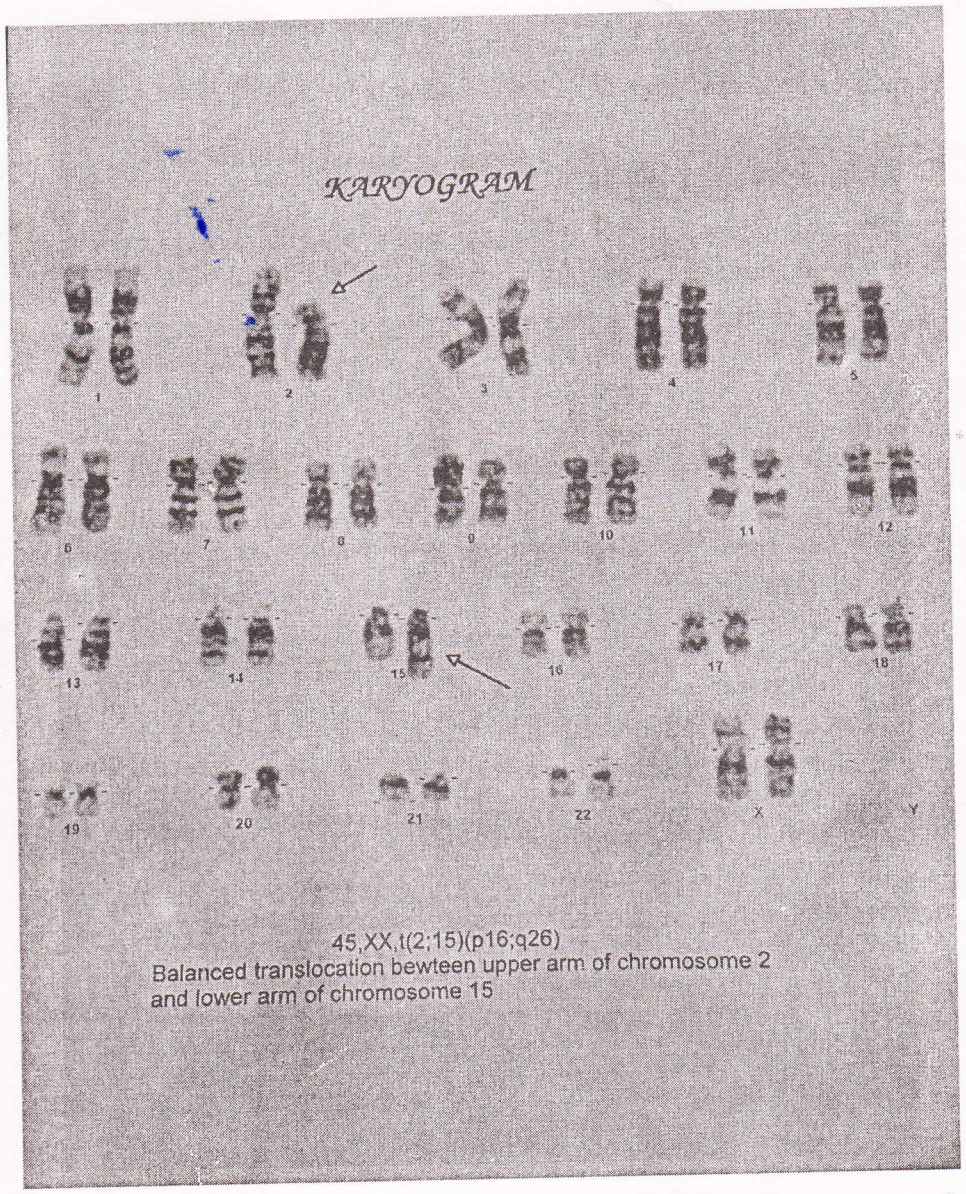


Fig 1: Case 1 showing Balanced Translocation between chromosome 2 and 15 .

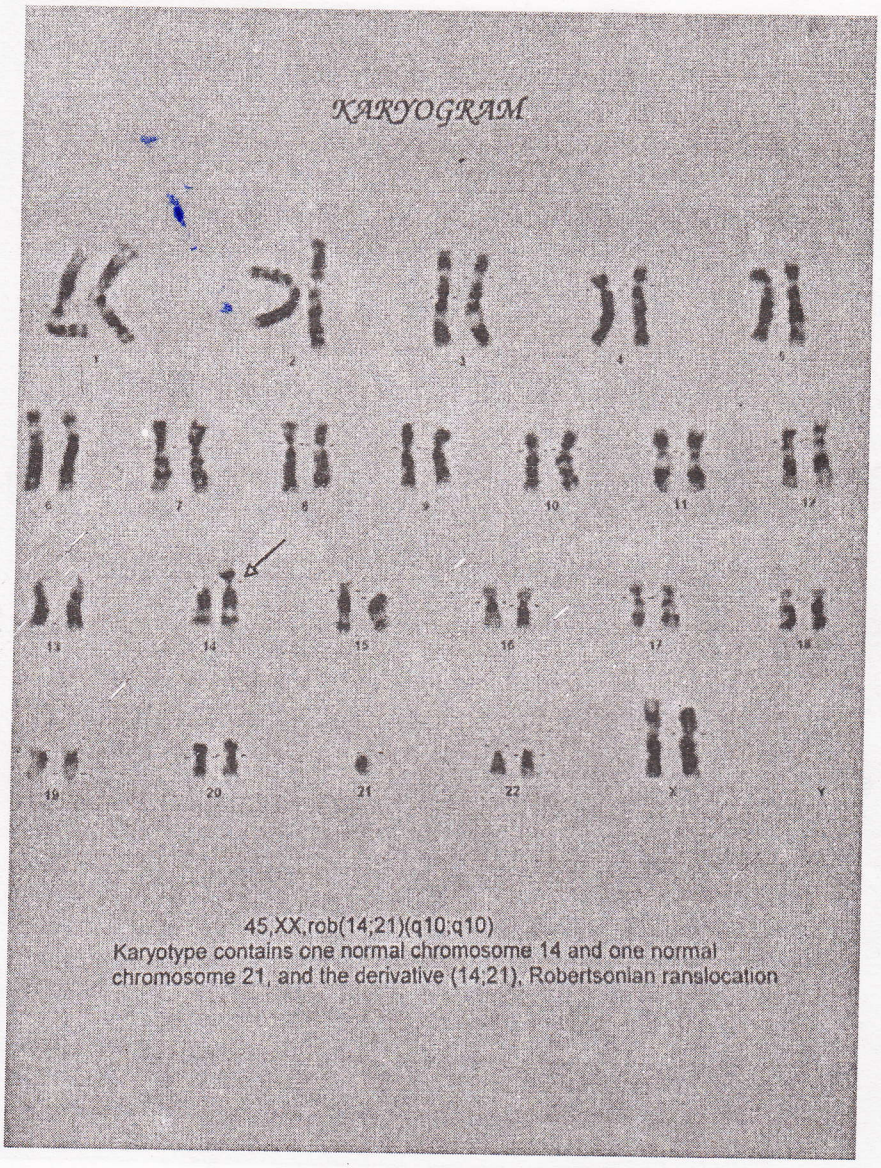


Fig 2 : case 11 showing Robertsonian Translocation

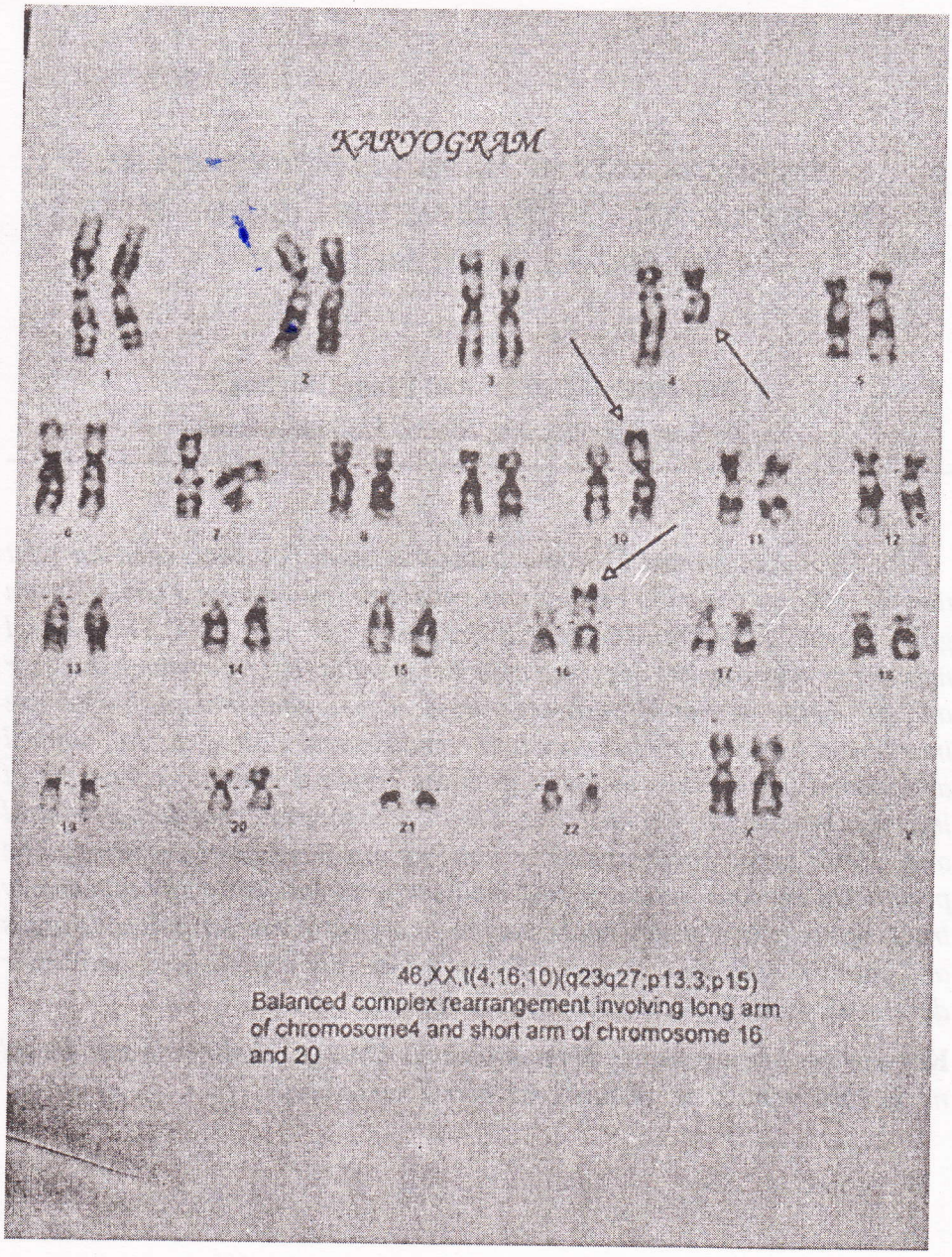


Fig 3: Case showing Balanced Complex Chromosomal Rearrangement