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Original Article

# Chromosomal Analysis Of Couples With Bad Obstetric Histoty

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## ABSTRACT

***Background and Objective:*** **Pregnancy termination and recurrent abortion are one of the common complications during pregnancy and in patients with a bad obstetric history.**

***Materials and Methods:*** **In this study, a total of 154 individuals including 75 couples and four single women from different communities and with various incomes were investigated for chromosomal abnormalities using blood culture and chromosomal banding technique.**

***Results:*** **Chromosomal analysis of these patients revealed three abnormal karyotypes (3.8%) in three women and two abnormal karyotypes in conceptions. Two of these couples had consanguineous marriage and the remaining women included one isochromosome for X [46, x,I (xq)], two translocations [45, xx, t (15:21)] and [46, xx, t (7:14)], one trisomy '21' (47, xx, +21), and a ring chromosome (46, xx, r(X). In addition, 27 conceptions had been reported for these five couples. These included 23 abortions with 18 of them within first trimester (78.26%) and four of them had abortions within second trimester (21.74%), one had a normal child, three had abnormal children, and one with stillbirth.**

**Conclusion:** **It was found out that abnormal karyotype is present in 3.8% of patients with a bad obstetric history. There was also a close relationship between number of deliveries and abortions and this relation was statistically significant ($p<0.01$). In addition, consanguinity was also related with number of abnormal children ($p<0.05$). There was also a significant relationship between consanguinity and first trimester abortions ($p<0.05$). Therefore, in couples with more than three abortions, especially within first trimester, chromosomal evaluation can have a diagnostic value.**

**Key words:** **Obstetric history, Karyotype, Chromosomal abmormalities, Abortion**

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## Introduction

Recent studies indicate that various factors including chromosomal abnormalities, immunologically related feto-maternal mismatch, hormonal changes, and anatomical factors are involved in provoking recurrent pregnancy loss (RPL). There are 30 genes showing different levels of expression among normal and RPL patients (1). In addition, other research groups have also identified a number of genes that are expressed aberrantly in women with pregnancy failure (1). Congenital malformations significantly contribute to the enormous public health problems of prenatal morbidity and mortality and to the medical problems at childhood. Despite these facts, the knowledge base about the etiologies and diagnoses has grown geometrically in the past few decades.

Early spontaneous abortion is a common phenomenon among more than 50% of early cases showing chromosomal abnormalities. Triploidy is a frequent chromosomal abnormality found in 12% of all spontaneous first trimester abortions. Triploidy can result from various mechanisms. In this regard, failure of segregation during meiosis I or II may lead to the formation of gametes with an extra haploid set of chromosomes or abnormal fertilization by two spermatozoa. The possibility of the prevention and treatment of triploidy in humans is very limited (2). According to Gita Arjun, factors involved in recurrent early pregnancy loss can be divided into genetic, anatomic, immunologic, endocrine, infectious, and environmental factors (3).

The birth of a normal child before or between spontaneous abortions is not a reason to cancel such investigations. Karyotyping of the couples should be done when there is a history of three consecutive early pregnancy losses or if there has been a history of an abnormal fetus or infant in addition to abortion (3). A report suggested the importance of cytogenetic analysis in phenotypically normal parents with a pervious bad obstetric history (4). Balanced rearrangements are rarely identified clinically unless a carrier with an unbalanced chromosome gives birth to an abnormal child (4). In such cases, chromosomal analysis of the couple can help in determining the origin of the abnormal chromosome. Unbalanced rearrangements are likely to come to clinical attention for unusual conditions. A familial chromosomal translocation t (6q; 7q) with habitual abortions was described by Zhang et al (5) with no phenotypic abnormality in the carriers. Similar familial translocation t (10; 21) (q22; q22) was observed by Delicado et al (6) in a family with several miscarriages and two siblings with multiple congenital malformations. In a recent report, Hou (7) presented a rare disorder in a boy with developmental delay and multiple anomalies. The statistical analysis of a computerized database generated from the literature on cytogenetic studies in couples experiencing repeated abortions showed a relationship between the distribution of the chromosomal abnormalities and the number of abortions. In addition, an uneven distribution of the chromosomal structural rearrangements according to the sex of the carrier was also found. Overall, 4.7% of the couples ascertained for two or more spontaneous abortions included one carrier. It also appeared that only translocations (both reciprocal and Robertsonian) and inversions are associated with a higher risk of abortion (8).

## Materials and Methods

**Preparation of culture bottle (9)**

About 8 to 10 ml of filtered culture medium (R-PMI 1640) was taken to a 20 ml culture tube and then FCS (20%; Fetal calf serum), phytohaemaglutinin (2%; 0.2 ml for each 10 ml of culture medium), 1% glutamine, antibiotic, and 0.5 to 1 ml of heparinized venous blood was added. The culture tube was incubated at 37° C for 72 hours. The mitosis was stopped by adding colchicine and incubated again for 2 hours at 37°C. The culture material was then taken into a centrifuge tube and centrifuged for 5 min at 1000 RPM. The supernatant was discarded and about 10 ml of potassium chloride was added (hypotonic treatment) and incubated for 20 min. The sample was centrifuged again and the supernatant was discarded. The RBC portion was destroyed by adding fixative (3:1 methanol acetic acid) for 2 or 3 times and centrifuged clear WBC cells were then taken on the slide. The slides were then stained with Giemsa and metaphases were observed under

microscope. G-banding was done by seabright (10) and then photographs were taken and karyotype was prepared for each patient. In this respect, 20 metaphases were analyzed for each patient and the results were reported.

## Results

75 couples and four single women were studied cytogenetically for detection of abnormal karyotype in patients with a bad obstetric history. Five abnormal karyotypes were detected in these couples. These abnormalities belonged to three (3.8%) of the parents (wives) including two translocation and one isochromosome and two abnormal pregnancy outcome, i.e. one leading to abortion in a normal couple and another one in a 2-year old child with normal parents. In this investigation, six abortions were presented by the couples in which one abnormal karyotype (ring X) was found. In addition, we considered delivery as all types of pregnancy outcome, whether it is a normal child, abnormal child, stillbirth, abortion, or early abortion.

Table 1 shows the outcome of 27 deliveries for these five abnormal couples. The patients with a translocation of chromosomes 15 and 21 had 7 deliveries, all ended at the first trimester. The second woman with 4 deliveries had one normal, one abnormal, and one stillbirth child. Each of the 3rd and 4th couples had 5 and 7 deliveries respectively with no stillbirth and normal child. Although in the 3rd couple it was the wife who had an abnormal karyotype (isochromosome X), but in the 4th one, the normal parent had a Down syndrome child. The last couple with five deliveries had four abortions and one abnormal child. In total, 27 deliveries including one normal child, three abnormal children, one stillbirth, 18 abortions in the first and 4 abortions in the second trimesters were recorded for these five couples.

Table 2 gives a brief history of these abnormal cases. Two of these couples had consanguineous marriage, one with first cousins consanguineous marriage and the other one with second cousins marriage. Mean age of these chromosomally abnormal patients were higher than the mean age of the remaining couples participating in the study. The mean age for the wives of chromosomally abnormal couples was 28.6 and for their husbands it was 33.4, which is much higher than the mean age of normal couples in this group (24.1 and 28.6 respectively).

**Table 1: Cases with chromosomal abnormality and outcome of their deliveries**

| No. | Affected case | Marriage duration | No. of deliveries | No. of normal children | No. of abnormal children | No. of Stillbirth | No. of abortions in trimester | | karyotype |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 1st | 2nd | |
| 1 | Wife | 7 | 7 | 0 | 0 | 0 | 7 | 0 | 45•XX, t(15:21) |
| 2 | Wife | 3 | 4 | 1 | 1 | 1 | 1 | 0 | 46•XX,t(7:14) |
| 3 | Wife | 4 | 5 | 0 | 0 | 0 | 3 | 2 | 46•X,i(Xq) |
| 4 | Child | 8 | 6 | 0 | 1 | 0 | 4 | 1 | 47,XY,+21 |
| 5 | Abortus | 9 | 5 | 0 | 1 | 0 | 3 | 1 | 46•X,r(X) |
| Total | 5 | | 27 | 1 | 3 | 1 | 18 | 4 | |

**Table 2: Cases with chromosomal abnormality and some common variables**

| No | Affected couple | Marriage duration | Age H/W | consanguinity | Blood group H/W | karyotype |
|---|---|---|---|---|---|---|
| 1 | Wife | 7 | 40/30 | 2nd | O/A | 45•XX,t(15:21) |
| 2 | Wife | 3 | 30/27 | Absent | A/A | 46•XX,t(7:14) |
| 3 | Wife | 4 | 32/28 | 1st | A/B | 46•X,i(Xq) |
| 4 | Child | 8 | 36/31 | Absent | B/A | 47,XY,+21 |
| 5 | Abortus | 9 | 29/27 | Absent | A/B | 46•X,r(X) |

Blood groups in BOH patients were also considered to be one of the factors for increased rate of abortion as compared with normal control group of the same population (Data have not been shown). These couples (BOH) significantly had a higher prevalence of A and AB blood groups as compared with normal populations.

## Cases reports

1. This case was the wife of the family and married 7 years ago. She was 30 years old and married to her second cousin. During her 7 years of marriage, she had 7 deliveries, all with first trimester abortions (Table1). The type of abnormality found was a Robertsonian translocation between chromosomes 15 and 21 [45, xx, t(15:21)] (Figure. 1). Phenotypically, she was normal. The parents' ABO blood group was recorded as 'A+' for the wife and '0+' for the husband.

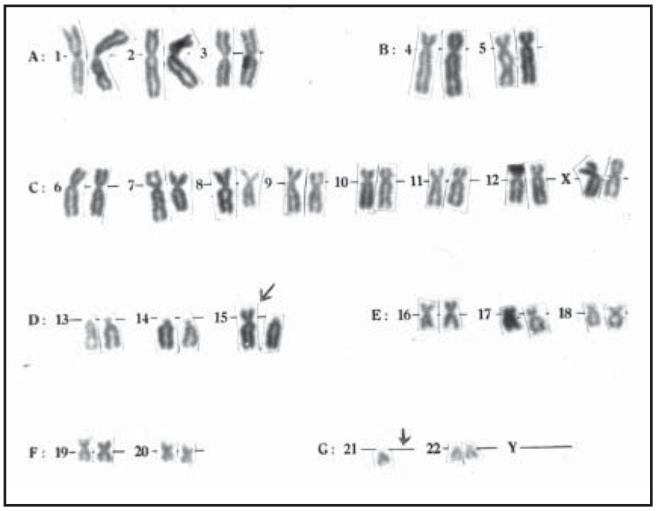

**Figure 1:Translocation 15:21**

2. The second abnormal karyotype belonged to a wife who got married 3 years ago. She was 27 years old at the time of presentation and had 4 deliveries with one normal child, one abnormal child, one stillbirth, and one abortion. Cytogenetic investigations using G-banding techniques revealed a translocation between chromosomes 7 and 14 [46, xx, t(7:14)](Figure 2). In this regard, both parents were A+ following blood grouping (Table 2).

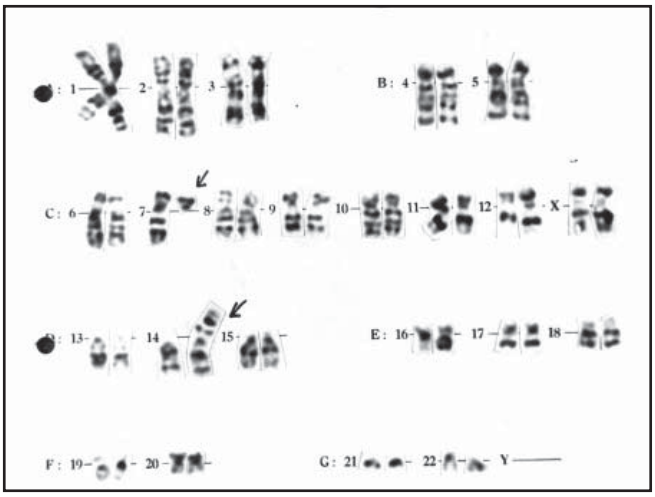

**Figure 2: Translocation 7:15**

3. The abnormal karyotype here belonged to mother of a family, who had experienced 5 abortions since she has married with her cousin four years ago. She was 28 years old at the time of presentation and had miscarried 5 deliveries. Out of these deliveries, 3 abortions occurred within first trimester and 2 abortions came about within second trimester (Table 1). Cytogenetic analysis of this couple revealed a normal karyotype for the husband and an isochromosome (i, Xq) karyotype

for the wife (Figure 3).

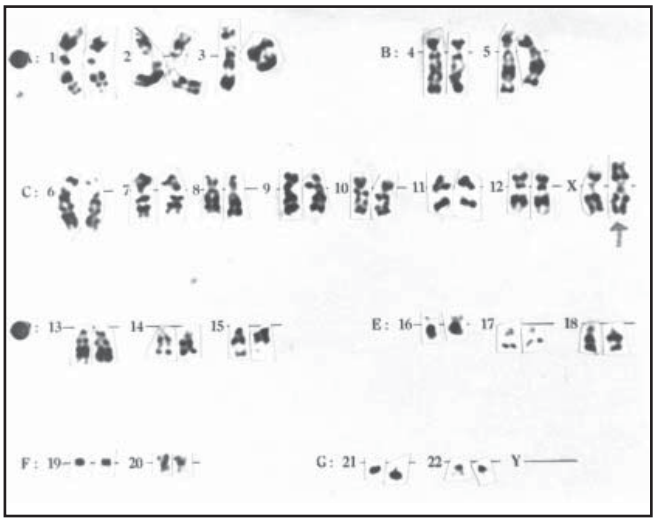

**Figure 3:Isochromosome X**

4. This case was diagnosed as Down syndrome (47, XY, +21) and the child of a couple with 6 deliveries, who got married 8 years ago. In her obstetric history, four first-trimester abortions and one second-trimester abortion was recorded (Table 1). Although the cytogenetic analysis of this couple showed a normal karyotype, but it is an unusual case and some translocation between chromosomes 21 or an extra material of chromosome 21 (duplication) could be present that was not detected (Figure 4). He was two years old and was the only child of these couples. The most notable anomaly with this patient was a characteristic face, hypotonia, broad square hands with shortened fifth finger, and mental retardation.

5. This case was an abortus from a BOH couple

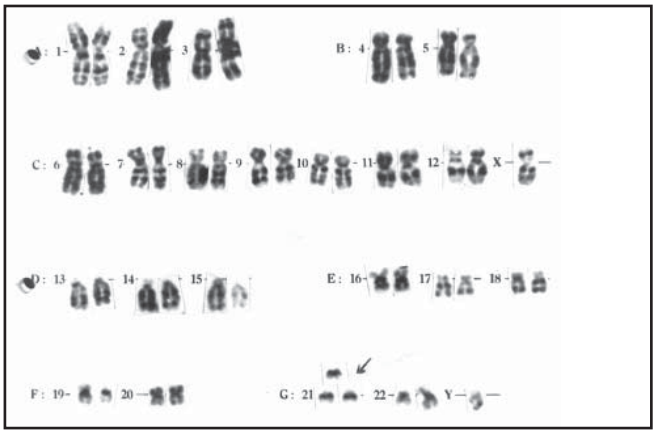

**Figure 4:Down syndrome**

referred for chromosomal culture because of complications during the pregnancy. This couple had married nine years ago and during this period the wife had experienced 5 deliveries (three abortions in the first-trimester and one abortion in the second-trimester) and one abnormal child (Table 1). The type of abnormality found was ring chromosome of X [46, X, r(X)], (Figure 5).

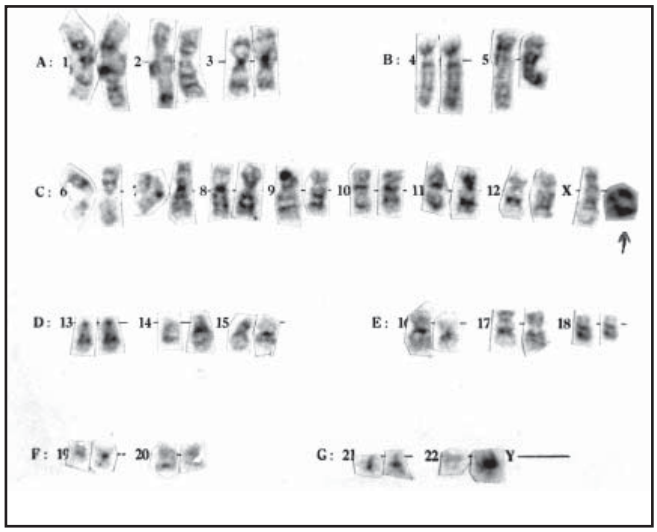

**Figure 5:Ring Chromosome X**

## Discussion

Chromosomal abnormality and its relation to the number of abortions (first and second) have been studied in many studies. In this respect, Sachs and Jahoa (11) found no apparent relation with the number of abortions. Meanwhile, Luigi et al (12) found an association between spontaneous abortion and ectopic pregnancies. In addition, Robert et al (13) showed that there exists an inverse relationship between spontaneous abortion rate and the prevalence at birth of neural tube defect. In this study, we found an increased frequency of abortions in patients with a bad obstetric history and with no apparent abnormal karyotype and even a relationship between patient with a bad obstetric history and abnormal karyotype. Many other studies found the same relationship between increased frequency of chromosomal abnormalities and increased number of abortions (14, 15). Some other studies however did not find this relationship (16).

Sant Cassia (17) in a study on 182 consecutive couples with two or more spontaneous abortions reported the presence of chromosomal abnormality in these patients as 4.67%. Another study on 500

couples with recurrent spontaneous abortions revealed abnormal karyotype in 50 partners (10%) (11). Meanwhile, Fryns et al (16) found an incidence of 5-7% for chromosomal abnormalities in couples with recurrent fetal wastage (RFW) and suggested chromosomal studies in couples with this issue. In addition, Osztovics et al (18) found an incidence of 4.78% for this abnormality in 418 couples. Many other studies (19, 20, 21, 22) reported different frequencies varying between 0 and 7.4%. In our study, an incidence of 3.6% for chromosomal abnormality among patients with a bad obstetric history was found out. There are many other studies reporting different frequencies for chromosomal abnormalities. This difference can partly be as the result of selection of patients in different studies. Of note, most of the studies with patients of more than three abortions found the frequency of this abnormality as 3-6%.

Due to selected group of patients, the cytological abnormality involved may be different. In our BOH patients, all kinds of abnormalities could be expected due to heterogeneity of the problem involved. In some studies with RFW patients, most of the chromosomal abnormalities were found to be autosomal reciprocal balanced translocation (16). In the present study, we observed an increase in the frequency of this abnormality in female carriers as compared to males. Liipman et al (19) reported similar findings in translocated carriers. A likely explanation for this finding can be the effect of these abnormalities on infertility in males and it may lead to disturbances in spermatogenesis.

A computerized database generated by Braekeleer et al (8) from the literature on cytogenetic studies in couples experiencing repeated pregnancy losses showed a relationship between the distribution of the chromosomal abnormalities and the number of abortions which is in agreement with our findings. An uneven distribution of the chromosomal structural rearrangements according to the sex of the carrier was also found, however, we did not reach this finding in BOH patients. It also appeared that only translocations and inversions are associated with a higher risk of pregnancy wastage (9), therefore, genetic counseling should be offered to these couples and investigations should be performed on their extended and related families. Information about the cause of pregnancy loss in these patients is important and not only can help the clinicians for the management of their patients, but also significantly reduce the psychological stress for these couples. In a study by Seible et al (23), a frequency of 71% was obtained for women experiencing spontaneous abortion. There are some accepted causes of recurrent abortion that are highly suitable for clinical investigation. These causes include anatomic uterine defects, endocrine factors, chromosomal abnormalities, lupus anticoagulant, and cervical incompetence. Although parental chromosomal anomalies are not a frequent cause of repeated abortion, they occur more commonly in couples affected by the problem than in the general population. In approximately 5% to 10% of couples with repetitive abortion, one parent will have a translocation or inversion.

## Conclusion

Therefore, cytogenetic examination of both partners may be helpful in predicting recurrence as well as forming a basis for genetic counseling.

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