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"Identification of Chromosomal Alternations in Parents and Their Products of Conceptions with Recurrent Miscarriage in Mosul"

Hussein Fathi Abood and Muwaffaq Khalil Hassan

Mosul University \ Science Collage \ Biology Department

Corresponding authors: hussainalabood91@gmail.com & Mufsbio62@uomosul.edu.iq

Abstract:

Recurrent pregnancy loss (RPL) is defined as two or more pregnancies losses occurring before 20 weeks of gestation and affecting 1-3% of the couples. Chromosomal abnormalities, uterine defects, thrombophilia, immunological factors, endocrine and metabolic factors are the known risk factors involved in the causation of recurrent pregnancy loss in 50% of the cases.

The current study was carried out at the labs of the Al-Batool teaching hospital, after obtaining approval from the Ministry of Health (Nineveh Health Directorate) to collect samples from the date ([1/Nov/2022- 30/June/2023). samples were collected from the peripheral blood for 40 couples with recurrent pregnancy loss as patient group and 2 healthy couples with no fetal loss as a control group, and 40 samples of their products of conception after dilation and curettage from the mothers with RPL to detection of chromosomal alternations.

40 couples with recurrent pregnancy loss (80 samples) founded that 4 cases (10%) of abnormality chromosomes, which are in 2 males (5%) & 2 females (5%). Additionally, 2 couples (4 samples) as control group which were normal karyotype. Also have studied 40 samples of product of conception (POC) immediately after miscarriage and found 35 sample (87.5%) with no metaphase which were excluded, 3 samples (7.5%) were clumped and 2 samples (5%) have get metaphase and karyotyped.

Key words: Chromosomal alternations**,** recurrent pregnancy loss, Products of conceptions

Introduction:

National Centre for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), and World Health Organization (WHO) define abortion as pregnancy termination prior to 20 weeks gestation or with a fetus born weighing less than 500 grams. Recurrent miscarriages occur in only 1% of women. Despite their rarity, the effects of recurrent miscarriages on subsequent reproductive outcomes have dominated the literature. By contrast, a single miscarriage has not traditionally been perceived as a major clinical problem (1).There are many factors related to spontaneous abortion, such as genetic abnormalities and infections (2).

Recurrent Pregnancy Loss (RPL) affects between1% to 5% of women of reproductive age. Existing guidelines describe RPL as two or more miscarriages documented by ultrasonography or histopathology, or three or more consecutive pregnancy losses before 20 weeks of pregnancy. It is widely believed that RPL is a complex disorder that is influenced by chromosomal abnormalities, genetic mutations, uterine anatomic deformity, endocrine dysfunction, immunologic factors, infections, and the environment. Thrombotic disorders are a frequent cause of RPL, accounting for almost half of all cases; however, in the rest of the cases, the cause of RPL remains unclear. (3)

About 15% of clinically recognized pregnancies result in spontaneous abortion in the first trimester and the vast majority of these are the result of chromosome abnormalities. Studies of chromosomal constitutions of first trimester spontaneous abortions have revealed that at least 50% of the abortions have an abnormal karyotype. Detection of chromosomal abnormalities in spontaneous abortion materials is very important to clarify the causes of loss of pregnancy. (4)

The types of chromosomal abnormalities include numerical abnormalities and structural abnormalities. The majority of chromosomal abnormalities are numerical abnormalities in spontaneous abortion, and the most common chromosomal abnormality is trisomy 16. Structural chromosomal abnormalities account for about 6–10% (5), including translocation, inversion, deletion, duplication, etc. Accurate cytogenetic identification of a pregnancy loss can provide important information for reproductive counseling. For those patients without chromosomal abnormal fetuses, the treatment should focus on other factors that influence the ongoing pregnancy, such as intrauterine malformations and endocrine diseases. (6)

Aim of the study

The importance of this research comes from the recent increase in the number of miscarriages and recurrent pregnancy loss among women in the city of Mosul. Therefore, this study was proposed to shed light on the some types of causes of miscarriage. Therefore, this study aims to:

1- Detection of chromosomal alternations in parents and its relation with causes of miscarriage

2- Detection of chromosomal alternations in product of conception to assisted in prognosis of causes of miscarriage.

Materials and Methods

Blood Sample Collection

Brought a piece (about 1 cm^3) of placenta tissues residues of mothers in transport media (RPMI without fetal bovine serum) from operation department after Dilation & Curettage (D & C).

Approximately, 2ml of whole blood was collected by sterile syringe from venous blood and placed into heparin tube and mixed gently for couples with recurrent pregnancy loss (RPL) and incubated at 37°C for chromosomal study.

Chemicals and Solutions in chromosomal analysis:

1-Culture Media

Lymphoprime complete media (100 mL) for acculturation of peripheral blood lymphocytes LymphoPrime Medium is designed for the short-term culture of lymphocytes from the peripheral blood for chromosomal analysis. The medium built on a basic medium with L-Glutamine, fetal bovine serum, and antibiotics added. (Gentamicin) and phytohemagglutinin-M (PHA-M). The culture media was provided by Capricorn firm/ Germany. It is delivered as a frozen medium, which is ready to use after thawing.

2-Colchicine

Capricorn Company provided the colchicine solution at a concentration of (10 g/mL). A concentration of 1 g/mL, as advised by the supplier, was used to bind the tubulin protein and prevent the growth of spindle fibers, and it was stored frozen (-20 ℃) until use.

3-Hypotonic Solution (KCL)

The potassium chloride (KCl) solution was made by dissolving 5.587 grams of potassium chloride in one liter of distilled water (D.W) and adjusting the pH to (7.2).

4-Working Giemsa Stain (25%)

The working stain was prepared by mixing 2 volumes of stock with 4 volumes of Phosphate Buffer.

5-Fixative Solution

A mixture of three volumes of methanol and one volume of glacial acetic acid was used, And the solution was ready to use right away.

6-Trypsin Solution

Trypsin-EDTA in DPBS at 0.05% (1X) The product was separated into sterile aliquots and stored at (-20°C) until use after being sterile-filtered. 0.05% of EDTA in DPBS trypsin (1X) the trypsin solution was supplied by Capricorn Company and contained red calcium and magnesium without phenol. (7)

Methods

Short term culture

At 37ċ add 0.3ml of PHA and then add 0.3 ml heparinized blood to 5ml sterile RPMI 1640 prepared medium and incubated for three days, and then add 0.05 ml of colchicine solution and incubated at 37ċ for 1 hour.

Long term culture

Each specimen of products of conception delivered to the laboratory was immediately placed in a sterile Petri dish, rinsed using normal saline solution, and dissected from blood clots and endometrial tissue. Long-term cell cultures (7–14 days) were established from the processed fetal tissue using Lymphoprime completed media in T-25 cell culture flasks at 37°C and 5% CO2, according to standard protocols.(8)

Cell Harvesting

After colchicine treatment centrifuge the culture tube at 1500rpm for 10min. Aspirate the supernatant leave about 0.5 ml over the pellet then mix very well gently, add 10ml of the 37ċ pre warmed hypotonic solution (KCL) drop with mixing to 10ml then incubated at 37ċ for 25min. And then centrifuge at 1500rpm for 10min. The supernatant was discarded. The residual cell pellet was shaken gently and then freeze freshly prepared fixative was added drop-wise with initial mixing, to give a total volume of 5ml. The cell were gently suspended and refrigerated at 4ċ for 1 hour or one day. The tubes were again centrifuged at

1500rpm for 10 min and the supernatant discarded, 5ml of fresh fixative was added to the residual cell pellet. The cell were suspended and centrifuged at 1500 rpm for 10 min. Three other consecutive washes with the fixative were made. 1ml of fixative was added to the cell pellet after the last wash.

Slide Preparation and Staining

The cells were suspended and then dropped using a Pasteur pipette on labeled microscope slide that had been pre-dipped in cold distilled water. Slides were air-dried, stained with Giemsa stain for 2min and examined with a light microscopic.

Results and Discussion

Spontaneous pregnancy loss is a common clinical occurrence. Many studies have demonstrated that 50% of all fertilized eggs die and spontaneously abort. (9)

Among the pathogenic factors leading to miscarriage, chromosomal abnormalities are the most common, accounting for approximately 50% of first trimester fetal loss. Cytogenetic analysis of products of conceptions can be performed to identify the genetic cause of miscarriage, as well as to estimate the recurrence risk, providing valuable information for genetic counseling and reproductive planning. (10)

Table (1): Distribution of results of cytogenetic analysis for 126 cases

Sample	No. of Cases	Results obtained
Couples with RPL	40 Couples (80 samples)	Each cases metaphase with good enough chromosomes
Couples as control group	2 Couples (4 samples)	Each cases metaphase with good enough chromosomes
POC afterMiscarriage	40 samples	35 cases No metaphase, No cell in division
		3 cases clumped (un spread) metaphase

Sample	No. of Cases	Results obtained
		with short chromosome(Not analyzed)
		2 cases metaphases with good enough chromosomes
POC after birth	2 samples	Metaphases with good enough chromosomes
Total	126	88 sample was reading karyotype

As shown in table (1) result process of chromosomal analysis was carried out on peripheral blood in 40 couples with recurrent pregnancy loss (80 samples) founded that 4 cases (10%) of abnormality chromosomes, which are in 2 males (5%) & 2 females (5%). Additionally, 2 couples (4 samples) as control group which were normal karyotype. Also have studied 40 samples of POC immediately after miscarriage and found 35 sample (87.5%) with no metaphase which were excluded, 3 samples (7.5%) were clumped and 2 samples (5%) have get metaphase and karyotyped.

Chromosomal aberrations in morphology in couples with recurrent pregnancy loss, in (4) samples as shown in table (2):

Table (2): Karyotyping findings in the couples with history of recurrent pregnancy loss.

No. of Case	Code of Case	Maternal age	No. of Miscarriage	Male karyotype	Female karyotype
1	5(A,B)	29	3	46,XYchromosomes with reciprocal translocation of 7,8	46,XX chromosomes

2	9(A,B)	30	3	46,XY chromosomes	46, XXchromosomes with deletion in ch. 5 & ch. 16
3	12(A,B)	28	3	46, XX chromosomes with Robertsonian translocation 22,14 chromosomes	46,XX chromosomes
4	36(A,B	37	4	46,XY chromosomes	46, XX chromosomes with deletion in ch 13

the first case showed reciprocal translocation as chromosome aberration in male with code 5A as shown in figure (1) and present karyotype in chromosome (46,XYchromosomes with reciprocal translocation of 7 ch. & 8 ch.)

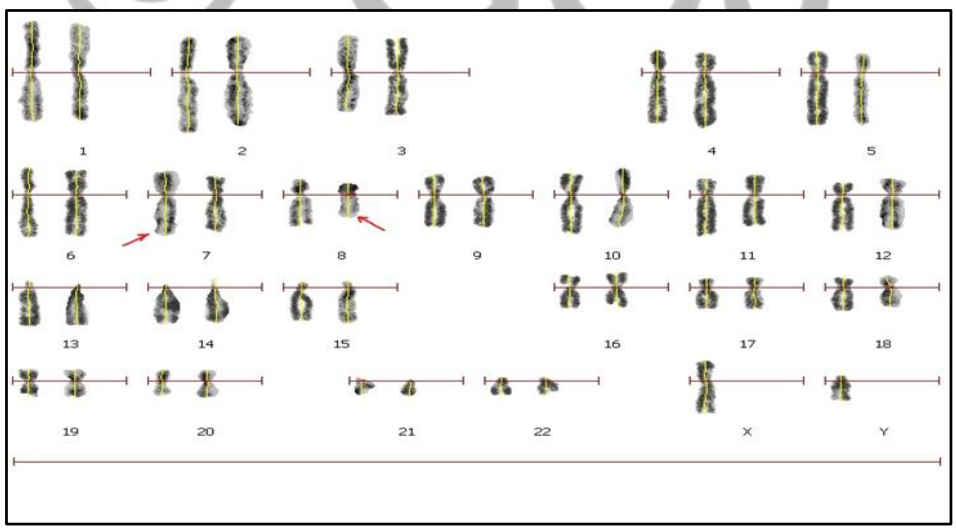

Fig. (1): Chromosomal aberration of male with RPL case code (5A), Karyotype (46, XY chromosomes with reciprocal translocation of 7 ch. & 8 ch.)

The second case founded chromosomal aberration in female with RPL case code 9B as shown in Figure (2) and present karyotype in the chromosomal (46, XX chromosomes with deletion in ch. 5 & ch. 16)

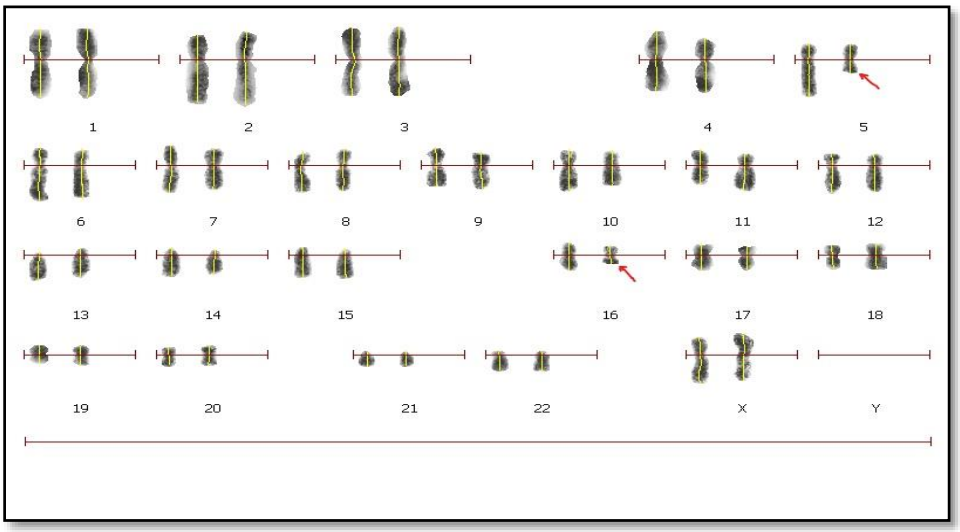

Fig. (3 A): Chromosomal aberration of female with RLP case code (9B), Karyotype (46, XX chromosomes with deletion in ch. 5 & ch. 16)

Third case with Robertsonian translocation as chromosome aberration in male with code 12B as shown in figure (3) and present karyotype in chromosomes (46, XY chromosomes with Robertsonian translocation 22, 14 chromosomes).

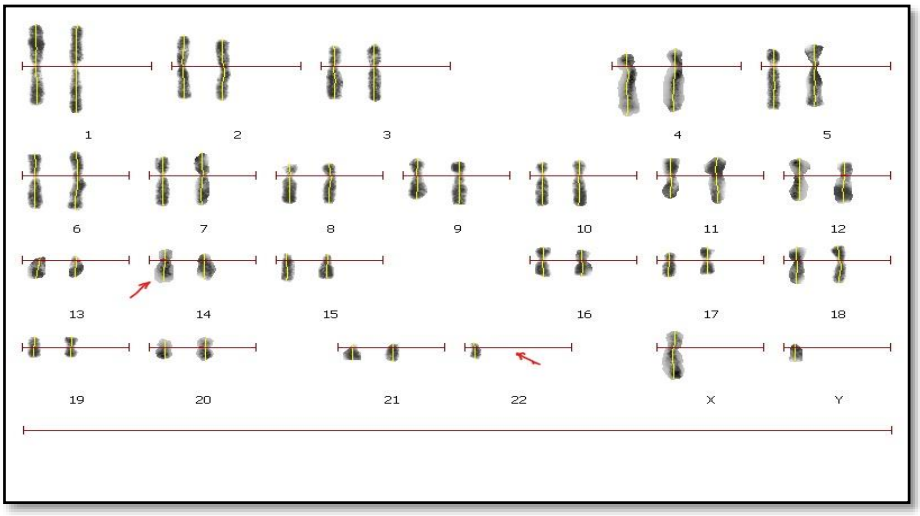

Fig. (3): Chromosomal aberration of male with RPL case code (12A), Karyotype (46, XY chromosomes with Robertsonian translocation 22, 14 chromosomes).

The fourth case showed Chromosomal deletion as chromosomal aberration in female with RPL case with code 36B as shown in figure (4) and present karyotype in chromosome (46, XX chromosomes with deletion in ch.13)

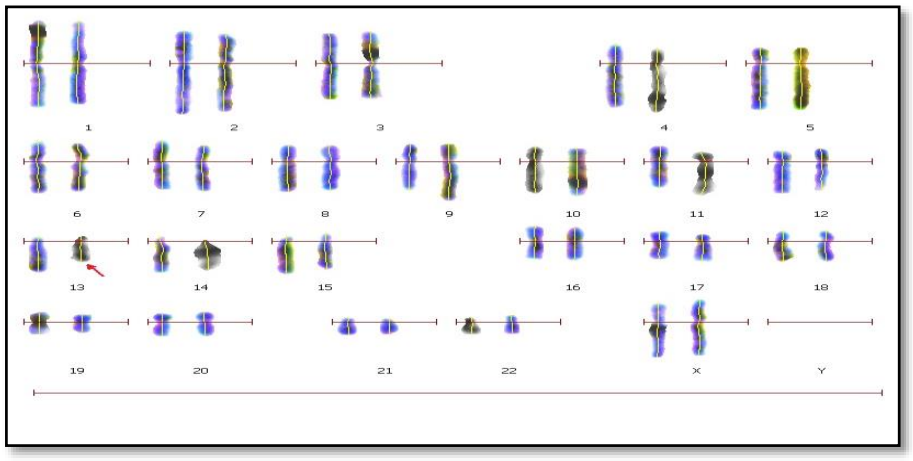

Fig.(5 A): Chromosomal aberration of female with RLP case code (36B),

Structural chromosomal abnormalities were detected in 4 patients with RPL. The presence of structural chromosomal abnormalities and lack of numerical abnormalities which founded in this study was not predictable. Structural chromosomal abnormalities can lead to unbalanced gametes depending on the specific recombination and segregation patterns during meiosis. Some experts considered a significant impact on the pregnancy outcomes for couples with abnormal karyotypes whereas others did not. (11)

The results of this study showed that the incidence of chromosomal abnormalities in couples with recurrent miscarriage was 10 %. The present results consistent with previous studies have shown that the incidence of chromosomal abnormalities in the general population is less than 1% (12) and RPL population is 2–5% (13), indicating that parental chromosomal abnormalities rate increased assuredly in the miscarriage couples. Balanced translocation was the most common type. The balanced translocations and deletion will not affect the parents themselves in phenotype, but their unbalanced gametes

during meiosis may indeed be part of the cause of miscarriage. Similarly, Robertsonian translocation of parental chromosomes can also cause miscarriage, birth defects or mental retardation of offspring (14). However, all these studies could not demonstrate the explicit causality between aberrant chromosome and abortions. (11)

The 2 samples of POC which analyzed and get karyotype, one of them was normal karyotype (46XX chromosomes) while the anther was abnormal in karyotype and happened addition in chromosome 21 (Trisomy 21) in metaphase. The code of case was (6C) and 3 metaphases were analyzed and the karyotype (47 XY chromosomes trisomy 21) as shown in figure (5) while, his parents was normal according to karyotyping.

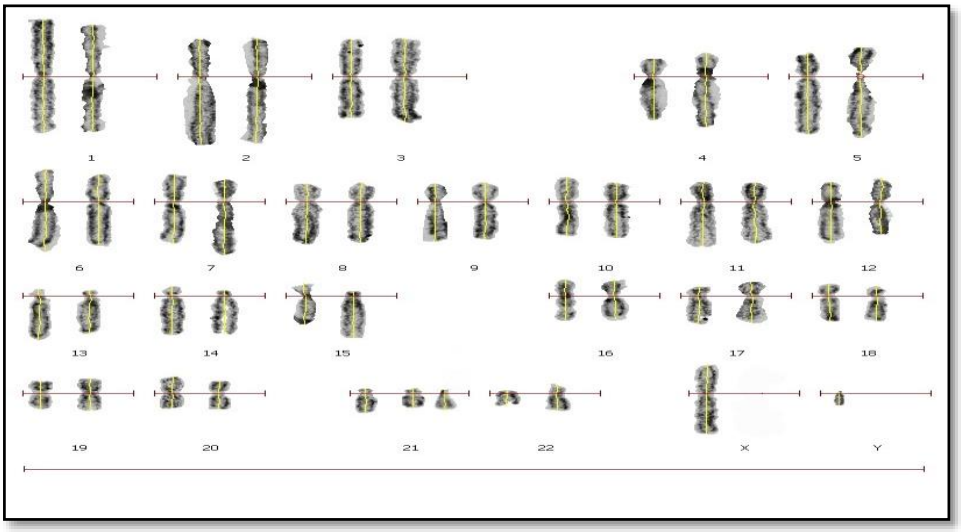

Fig. (4-7 A): Chromosomal aberration of POC case code (6C), Karyotype (47, XY chromosomes with Trisomy in Ch. 21)

Chromosomal analysis of POCs is an important to get a clue of the cause of fetal loss. This information can be used to estimate the risks of recurrence in future pregnancies. From India, a study by (15) showed majority of the cases of RPL was having balanced reciprocal translocations. Among chromosomal aberrations in fetuses, trisomy 9 was detected in a fetus (POC23) along with monosomy of

15q11.2. Trisomy 9 is a rare and often fatal chromosomal abnormality which occurs in approximately 2.4% of pregnancy losses. (16)

Chromosome analysis, which requires dividing cells, is the current gold standard for genetic evaluation of products of conception (POC) but has three limitations. First, a successful cell culture is required but failure occurs in 10–40% of cases due to microbial contamination or lack of viable dividing cells. Second, the results take approximately 4–6 weeks. And third, if the results suggest normal female karyotype (46, XX), a result that happens 55–80% of the time, it is unknown whether the tested sample was fetal or maternal in origin (17).

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