Chromosomal Abnormalities Associated with Recurrent Spontaneous Abortions in Iraqi Women

Saad Merza Husain Alaraji
College Of Medicine, University of Babylon, Hilla, Babylon, Iraq.

Abstract

Chromosomal analysis is an important etiological investigation in couples with repeated spontaneous abortions as it helps in genetic counseling and deciding about further reproductive options.

Abortions ,specially first trimester abortions is a very common complication and a matter of concern for couples planning pregnancy. Balanced chromosomal rearrangements in either parent is an important cause of recurrent pregnancy loss particularly in the first trimester. In this study there is an evaluation of the contribution of chromosomal anomalies in causing repeated spontaneous abortions.

To understand the cytogenetic causes in the couples with spontaneous abortions ,cytogenetic study was done on 61 couples (122 individuals) with recurrent spontaneous abortions who were examined for chromosomal abnormalities and aberrations. Women who had at least two abortions, or spontaneous abortions preceded or followed by fetal deaths or birth of a malformed child. We have found 9.83% of all cases carrying chromosomal aberrations and abnormalities included 7.37% of all cases were structural aberrations & 2.45 of all cases were numerical anomalies.

دراسة التشوهات الكروموسومية في حالات الإجهاض المتكرر في النساء العراقيات

الخلاصة

يعتبر فحص الكروموسومات و إجراء التحليلات الكروموسومية من عوامل البحث المهمة في فحص الأزواج الذين يعانون من حالاتِ الإجهاض التلقائيةِ المتكرّرةِ والني تساعد في الاستشارة الوراثية و البحث في الخيارات المساعدة في حل هذه المشكلة . وتعتبرحالات الإجهاض و خصوصا في الثُلثِ الأول من الحمل من الحالات الشائعة في المجتمع . وتعتبر التشوهات الكروموسومية لدى الأب أو الأم من الأسباب الرئيسية للإجهاض التلقائي المتكرر . و من هنا جاءت هذه الدراسة لبحث دور العوامل الفسلجية الوراثية و الزيغ الكروموسومي في إحداث الإجهاض التلقائي المتكرر .

أجريت دراسة التحليلات الكروموسومية على واحد و ستون زوجا وزوجة من الذين يعانون من الإجهاض التلقائي المتكرر. وكانت النساء اللواتي شملتهن الدراسة لديهن مالا يقل عن اجهاضين تلقائيين متكررين , أو موت للأجنة أو ولادة طفل مشوه . وأظهرت الدراسة أن نسبة وجود التشوهات الكروموسومية الكلية قد بلغت 9.83% من كل العينة وكانت النسبة 2.45% تمثل التشوهات الكروموسومية العددية , فيما بلغت نسبة التشوهات الكروموسومية التركيبية 7.37% من الحالات الكلية. فيما لم يظهر تأثير للعمر على نسبة وجود الاضطرابات الكروموسومية.

Introduction

Abortion is defined as the termination of pregnancy before 20 weeks of gestation. Early pregnancy loss in the first trimester is the most common complication affecting at least 18% of the pregnancies [1]. These losses however, are those recognized pregnancies which are confirmed usually 4 to 5 weeks after

conception. There is now evidence that the pregnancy loss rate before this period i.e., during the 2 to 3 weeks following conception, may be as high as 50 % . Recurrent spontaneous abortion is defined as two to three or more consecutive pregnancy losses before 20–22 weeks of gestation [2]. Clinical studies have shown that in a patients with a history of two miscarriages, the subsequent risk of pregnancy loss rises to about 25%, whereas three abortions raise the risk of a fourth miscarriage to 33 – 40 % [3]. It is not unusual for perfectly healthy couples to experience three consecutive spontaneous pregnancy losses, each for a different reason and it has been seen that more than half of recurrent abortions are due to nonrecurrent causes [4]. Determining the cause of recurrent spontaneous abortion are extremely difficult.

The main causes of recurrent spontaneous abortion can be often related to factors associated with implantation, genetics, autoimmunity, endocrine abnormalities, infection, alloimmunity , anatomic uterine defects, anatomic factors and environmental factors[5] .

Chromosomal studies must done on abortus , and both husband and wife. Abortus study is more likely to be contributory as far as cause for that abortion is concerned , howevere in recurrent abortions, the recurrence risk may be more realistically assessed if we do the chromosomal studies of both husband and wife.

Chromosomal abnormalities considered to be significant and contributing to recurrent spontaneous abortions are Triploidy(69 ; XXX, 69 ; XXY) , Tetraploidy (92 ; XXXX , 92 , XXYY), Monosomy X , Structural abnormalities , Sex Chromosal polysomy (47 ; XXX , 47 ; XXY), Autosomal monosomy (G Chromosomes), Autosomal Trisomy (Chromosomes1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,19,20,21,and 22) , Double Trisomy and Mosaic Trisomy[9-11].

There are certain chromosomal abnormalities seen in couple which are considered to be significant cause of Recurrent Spontaneous Abortion(1. Numerical abnormalities : a. 45 , X / 46 , XX ; b. 45 , X / 46 , XY; c. 46 , XX / 47, XXX & d. 46 , XY / 47 , XY + fragment and 2. Structural abnormalities: Inversion 9 , 1 q +, 9q+&Yq+ , 16 q + inversion 9, 14 p +, 15 p + , 22 p +, Y q +, . Inversion Y, fragile sites 3 p 14 & 6 q and Translocations : Robertsonian translocation , Reciprocal translocation & Multiple translocation)[9,12, 13].

The importance of such studies is to estimate the prevalence of chromosomal aberrations among couples with Recurrent Spontaneous Abortion and identification of the treatable causes with reducing the chances of pregnancy failure. Also, it is helpful in introducing such women in the assisted fertilization program and improves their pregnancy outcome[15, 16].

Materials and Methods

In the present study 61 couples (122 individuals) with not less than two spontaneous abortions were analyzed for cytogenetic study to determine the chromosomal aberrations. These couples were categorized as:

(1) Couples only with repeated spontaneous abortions ,

(2) Couples with repeated spontaneous abortions preceded by stillbirth or malformed child , and

(3) Couples with repeated spontaneous abortions and normal live issue/s.

All the couples were in the age group ranging from 22 to 46 years and

number of abortions ranged from 2 to 4.

Cytogenetic analysis was done according to Yassin [17] and Alaraji [18]. Five ml (5 ml) of peripheral blood was collected in heparin washed syringes. For every subject whole blood (0.5 ml) cultures was set up in 5 ml RPMI 1640 media containing 10 - 15% human plasma of AB+ blood group , antibiotic mixture of penicillin and streptomycin and phytohemagglutinin (PHA) were incubated at 37°C for 72 hours .Cultured cells were harvested by adding 20µL colcemid for 20 minutes, followed by hypotonic KCl solution for 5 minutes and fixation using standard 3:1 methanol - acetic acid fixative. Microscopic examination of 5-10 metaphases per case were done after standard Giemsa-Trypsin G-banding staining technique [17,18].

Results

Among 61 couples (122 cases) studied, chromosomal abnormalities and aberrations were found in 12 subjects(9,83%) including eight females(13.1% of females) and four males(6. 54% of males) (Table:1 &2). Among 12 subjects, 9 (75% of cases showed chromosomal aberrations&7.37% of the total No. of all couples only with repeated spontaneous abortions) showed structural aberrations, and 3 (25% of cases showed chromosomal aberrations &2.45% of the total No. of all couples only with repeated spontaneous abortions) carried numerical abnormalities. The Among structural abnormalities, translocations were seen in 6 subjects (66.66%), which involved chromosomes, 2;11, 3;11; 3;17 ,11;12,16;X and 21;22 (Table :3). Three subjects (33.33%) showed deletions, one each in chromosome #2 , #3 and #11 (Table :3). The deleted portions of these chromosomes were present in all the metaphases appearing as marker. Since both these subjects were clinically normal, it was assumed that there was no loss of chromatin following deletions and these markers were actually the deleted part of the chromosomes which otherwise was quite evident from their banding pattern . Numerical chromosomal aberrations in couples with repeated spontaneous abortions showed . one male with Mosaic :47,XXY : 46,XY and two females with Mosaic :46,XX : 45,XO and Mosaic :47,XXX : 46,XX(Table :4).

Table 1 Number and percentage of structural and numerical chromosomal abnormalities according to the total No. of all couples only with repeated spontaneous abortions.

Chromosomal Aberration	Male N= 61	%	Female N= 61	%	Total N= 122	%
Numerical	1	1.63%	2	3.27%	3	2.45%
Structural	3	4.91%	6	9.83%	9	7.37%
T0TAL	4	6. 54%	8	13.1%	12	9.83%

Table 2 Number and percentage of Structural and Numerical chromosomal abnormalities according to the No. of Couples with repeated spontaneous abortions who showed chromosomal aberrations.

Chromosomal Aberration	Male N= 61	Female N= 61	Total N= 122	%
Numerical	1	2	3	25%
Structural	3	6	9	75%
T0TAL	4	8	12	%100

Table 3 Structural chromosomal abnormalities in couples with repeated spontaneous abortions who showed chromosomal aberrations.

No.	Sex	Age Years	Chromosomal abnormalities
1	Female	39	Translocation 46,XX t(2;11)
2	Female	44	Translocation 46,XX t(3;11)
3	Female	29	Translocation 46,XX t(3;17)
4	Male	32	Translocation 46,XX t(11;12)
5	Female	29	Translocation 46,XX t(16;X)
6	Female	38	Translocation 46,XX t(21;22)
7	Male	36	Deletion 46,XYdel (2) + marker
8	Male	40	Deletion 46,XYdel (3)
9	Female	31	Deletion 46,XYdel (11) + marker

Table 4 Numerical chromosomal aberrations in couples with repeated spontaneous abortions who showed chromosomal aberrations.

No.	Sex	Age Years	Chromosomal abnormalities
1	Male	34	Mosaic :47,XXY : 46,XY
2	Female	24	Mosaic :46,XX : 45,XO
3	Female	27	Mosaic :47,XXX : 46,XX

Table 5 The mean of maternal and paternal ages of couples with repeated spontaneous abortions who carrying chromosomal aberrations.

Sex	Age /Years	Mean of Maternal and Paternal Ages
Male	32	35.5
	36	
	40	
	34	
Female	39	32.6
	44	
	29	
	29	
	38	
	31	
	24	
	27	

Discussion

The evaluation of patients with a history of recurrent spontaneous miscarriage 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.

Recent data on recurrent abortions is discussed in the framework of the selection failure hypothesis which states, 'Recurrent miscarriage is the result of failure of the 'poor quality' embryos to implant, and represent clinically as recurrent abortions. Thus, recurrent abortions is a failure of nature's quality control' [19]. Failure of implantation and/or poor feto-uterine interaction that caused by chromosomal and non-chromosomal factors, represent the main cause of abortions. Therefore, pre-fertilization factors (sperms and/or oocytes) or post-fertilization factors (mitotic and /or consequences of meiotic errors) might represent major problems that affect the fetal chromosome integrity [7 ,20].

In this study, the incidence of chromosomal abnormalities among the couples with recurrent spontaneous abortions was 9.83%, and chromosomal variants were detected .

Parental rather than fetal karyotyping is of clinical value for determining a chromosome abnormality with a recurrent risk of causing recurrent spontaneous miscarriage. In this study, twelve cases (eight women and four men) had chromosomal abnormalities, and the female: male ratio is 2 : 1. The predominance of females appears to be due to the fact that chromosomal abnormalities that are compatible with fertility in females may be associated with sterility in males[21]

The mean maternal and paternal ages of subjects carrying chromosomal anomalies were 32.6 and 35.5 years respectively (Table 5) . The age associated with increased incidence of meiotic non-disjunction, with increased abortion rate. Also, balanced translocations were commonly seen in this study (Table 3). Many authors [22

&16] mentioned that balanced translocations are commonly seen among couples suffering recurrent miscarriage, where the gene dosage is not affected by gain or loss, but just rearranged. Additionally, we have found a case with duplicated chromosome segment (imbalance rearrangement), i.e. gene dosage imbalance. The female partner was phenotypically normal while she gains a duplicated segment in the long arm of chromosome 6, thus this segment could contain no genes at all, few genes, or with gene dosage not critical. Such female carries abnormal chromosome in germline that could leads during meiosis to abnormal karyotyped fetus. Surprisingly, we have found a case with imbalance rearrangement within the normal control. Authors have published many cases who are apparently normal mentally and phenotypically but carry different forms of chromosomal anomalies such as deletion [23], and duplication [3].

There was no positive correlation of advanced maternal and paternal ages with the number of abortions observed in these subjects indicating that the chromosomal abnormalities could arise because of some reasons other than advanced maternal or paternal age.

According to this data the percentage of structural and numerical chromosomal abnormalities according to the total number of all couples only with recurrent spontaneous abortions were 9.83% . The recurrent spontaneous abortions percentage varies greatly according to the number of cases studied. For example, in a study performed by Al-Hussain and colleagues [16] comparing the percentage of recurrent spontaneous abortions in different countries using variable case groups, they have found a great variety in that ratio. For example; in a French study using 217 couples, the ratio was 2.6 %; in a Japanese study using 639 couples, the ratio was 4.5%; whereas in a Spanish study using 32 couples, the ratio was 17.8%. There was no increase in the rate of chromosomal abnormalities relative to the number of abortions in this study. This is in consonance with earlier reports. [23].

Therefore all the couples with chromosomal abnormalities should be strongly advised to monitor their future pregnancies by prenatal diagnosis to exclude the possibility of a chromosomally unbalanced zygote. Cytogenetic studies give considerable information about the genetic make up leading to recurrent spontaneous abortions and still remain an important tool.

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