

Histopathology and Cytogenetics study in aborted women

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Abstract

This study has been made to draw attention to some causes of abortion. One hundred forms were filled for women with unknown cause of abortion. These forms are compared to another fifty women with normal delivery. The forms included the following parameters : consanguinity degree, maternal age and reproductive health parameter as (spontaneous abortion, still birth, and infant mortality in age less than two years). Forty two women are selected randomly from aborted women. They were classified into two groups, the first group contained thirty three women who aborted during first trimester. While the second group included nine women aborted in the 2nd trimester. Comparison was made with ten women who had normal pregnancy and delivery. Blood samples were collected in period from December 2010 till June 2011 in hospitals of Baghdad .

Cytogenetic analysis of aborted women blood were made: they included chromosomal abnormalities and mitotic index, hence chromosomal abnormalities are important factors that are linked with recurrent spontaneous abortion in more than 60% of aborted women, where the studies found that at least one of parents (in 2-4% of couples suffering from recurrent spontaneous abortion) had special type of chromosomal abnormalities.

Genetic study has reached the following results:-

1. Absence of numerical and structural chromosomal abnormalities in blood samples of aborted women.
2. Decline in mitotic index in blood samples of aborted women during 1st and 2nd trimester of pregnancy in comparison with controlled group.
3. presence of positive correlation between the total percentage of spontaneous abortion with maternal age and consanguinity degree in aborted women and control group ($p < 0.05$).

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Introduction

The term "abortion" most commonly refers to the induced abortion of a human pregnancy, while spontaneous abortion ;are usually termed "miscarriages"(1).Spontaneous abortion in the general reproductive population is very common occurrence,recurrent pregnancy loss affects up to 5% of couples who are trying to establish family (2).After either two or three spontaneous pregnancy losses couples are labeled as recurrent aborters (3).There are many causes associated with pregnancy loss includes :Chromosomal abnormalities are the most frequent cause of spontaneous abortion (4).Chromosomal abnormalities are found in more than half of embryos miscarried in the first 13 weeks (5).

In 4-8% of couples with recurrent pregnancy loss, at least one of the partners has chromosomal abnormality that probably contains balance chromosomal abnormalities (6).Endocrine abnormalities such as deficiency of estrogen and progesterone in circulation to produce estrogen dominance is atheoreticalcause of abortion,hypothyroidism, poor diabetic control, and polycystic ovarian syndrome contribute to pregnancy loss (7).Hormonal causes potentially contribute to recurrent abortion in 10-20 of cases (7).

Congenital or acquired anatomic causes reportedly are present in 10-15% of women who have recurrent spontaneous abortion. Acquired lesions are intrauterine adhesions, Leiomyomas, and possibly adhesions due to endometriosis (7), up to 15% of pregnancy losses in the second trimester may be due to uterine malformation, cervical problems (8).Also infections etiology may be found in 5% of cases. Bacterial, viral, parasitic, and fungal infections are associated with recurrent spontaneous abortion (7). Herpes simplex virus has been linked to spontaneous abortion and chronic HSV is a possible of recurrent spontaneous abortion (5).Immunological causes may contribute in up to 60% of recurrent spontaneous abortions. Both the developing embryo and the trophoblast may be considered immunologically foreign to the maternal immune system (7).But still ,in nearly 50% of recurrent miscarriage patients the underlying causes remains unknown (9). Also sporadic pregnancy losses have been associated with cocaine (10), smoking,alcoholand caffeine(11). Inaddition,miscellaneous factors may account for as many as 3% of recurrent spontaneous abortion. Other contributing factors implicated in sporadic and recurrent spontaneous abortions include: environment,drugs,placental abnormalities and male-related causes(24). So this study was aimed to:Perform a cytogenetic study on blood to identify chromosomal abnormality in spontaneous aborted women, and determine the relationship between consanguinity degree, maternal age, and their effect on reproductive health especially spontaneous abortion.

Materials and Methods

Study Groups

This study was conducted on 42 blood and placentas samples from spontaneous aborted women during first trimester and seconde trimester. Also 10 blood and placenta from normal delivery women with an age (15-40) year, and the patient samples were collected from Baghdad city (Fatima- Al – Zahraa hospital and Al- Elwiya hospital) during the period from Decembar 2010 to June 2011.

Collection of blood samples

Peripheral blood samples were collected from each patient. The blood (5ml) was collected by venipuncture using a disposable syringe, and it was drawn into heparin tubes for the assessment of chromosomal abnormalities. The peripheral blood samples was cultured immediately and assessed for chromosomal abnormalities.

Cytogenetic analysis test

1- Chromosomal abnormalities assay:

Cytogenetic study for chromosomal aberrations was determined according to (12). As follow:

- a) Preparation of culture for cytogenetic study of peripheral blood lymphocytes.
- b) Blood sample collection and handling
- c) Culturing of samples
- d) Harvesting
- E) Dropping
- F) Staining
- G) Microscopic Examination

Microscopic examination under low magnification using (10X) objective lens was performed to determine the best metaphase, and then examination of numerical and structural chromosomal aberration was done under oil immersion (100X) objective lens.

2- Mitotic index (MI) assays:

The slides were examined under light microscope with (40X) power, and 1000 of the divided and non divided cells were counted and the percentage rate was calculated for only the divided ones according to this equation:

$$MI = \frac{\text{Number of dividing cells}}{\text{divided and non. divided cells}} \times 100 \text{ -----(13).}$$

Statistical analysis

Z test (test of proportion) to compare percentages (14).

Pvalue less than 0.05 is considered significant, SPSS (Statistical Package for Social Sciences) version 19 used for the analysis.

Results and Discussion

Cytogenetic studies

This study was carried out to assess the incidence of chromosomal aberrations in women who suffer from recurrent spontaneous abortion. And to detect the relationship between spontaneous abortion and the presence of chromosomal aberrations.

Because chromosomal aberrations are important causes of spontaneous abortion and recurrent miscarriage(15).

In this study we cultured 52 blood samples (42 were taken from aborted women and 10 were controlled) for cytogenetic studies at the cytogenetic Department in the medical city hospital . 33 blood samples were taken from aborted women in the first trimester of pregnancy and 9 blood samples were taken from aborted women in the second trimester of pregnancy , the result was 19 (57.5%) failed to respond and 4 (44.4%) failed to respond respectively.

This high percentage of failure is due to blood from women following abortion or still birth is often poor to respond in culture because it's influenced by stress and drugs, and it may be advisable to delay taken samples from these patients until some time after the event (12). From this 52 blood samples 29 (55.7%)

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succeeded to respond and all of them showed normal chromosomal pattern , and our results are in agreement with the study observed by Stirrat, (16); Egozcue et al., (17) who found that chromosomal content of couples with recurrent abortion usually are normal.

Mitotic index in the blood samples of different study groups

In this study, the percentage of mitotic index was (7.0%) , (2.2%) and (9.3%) in the first trimester, second trimester of aborted women and control group, respectively, referred to in the table (1).

Table 1 : Mitotic index in the blood samples of different study groups

Study groups	Total of divided cells	Total of non-divided cells	Total cell count	Mitotic index (MI)(%)
First trimester	91	1778	1869	*7.0
Second trimester	26	561	587	*2.2
Control	100	973	1074	9.3

Ztestpvalue (p<0.05),*(S)

(S)Significant

The statistic analysis show significant difference between first trimester and second trimester of aborted women group with pvalue (p<0.05) and it was significant difference had been shown between first trimester and control with pvalue (p<0.05), also there was significant difference between second trimester and control group with pvalue (p<0.05) (table,1).

This result agrees with the results of Bukvic et al., (18) who stated that the changes in factors as hormones cause decrease in the rates of mitotic index in lymphocyte.

The relation ship between marriage type and spontaneous abortion:

Table 2 : The relationship between marriage type and spontaneous abortion.

Abortion					Control			
Morriagetype	Number	No. of gravida	No. of abortions	The percentage (%)	Number	No. of gravida	No. of Abortion	The percentage (%)
First cousin	34	110	43	34.0%	30	90	20	22.2%
Second cousin	19	87	31	35.6%	10	25	5	20%
Non-cons an guincous	47	213	88	41.3%	10	30	5	16.6%
Total	100	410	162	34.5%	50	145	30	20.6%

Z test p value (P < 0.05) , *(S)

*(S) Significant

statistically there was significant relation ship between marriage type and spontaneous abortion in aborted women and control with P value (P < 0.05). the total percentage of spontaneous abortion with marriage type was (39.5%) in borted women , while the total percentage was (20.6%) in the control,

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as shown in table (2).

Bener and Hussain, (19);Assaf and khawaja(20) who found that a higher rate of prenatal losses among consanguineous couples.

This finding occurs in my result in control, the rates of spontaneous abortion in non – consanguineous marriage were higher than it in consanguineous marriage and this is related to the genetic compatability of the parents in consanguineous marriage and the differences ingenes between mother and her fetus which my be foreign to the maternal immunity system because the genetic difference in parents of non – consanguineous marriage (21,22).This result also occurs in our study in aborted group of women.

While Saad and Jauniaux , (23); Donbak ,(24) found that the rate of spontancous abortion in consanguineous and non - consanguineous mating is the same.

The relation ship between maternal age and spontaneous abortion

The relation ship between maternal age and spontaneous abortion were presented in table (3).

Table 3 : The relationship between maternal age and spontaneous abortion.

Abortion					Control			
maternal age	Number	No. of gravida	No. of abortions	The percentage (%)	Number	No. of gravida	No. of Abortion	The percentage (%)
< 20 years	12	17	14	82.3%	15	55	15	27.2%
20-24 years	21	55	25	45.4%	5	40	5	12.5%
5-29 years	27	97	37	38.1%	5	25	5	20%
10-34 years	11	52	14	26.9%	5	25	5	20%
≥ 35 years	29	189	72	38.0%	5	25	5	20%
Total	100	410	162	39.5%	30	145	30	20.6%

Z test P value ($P < 0.05$) , * (S)

* (S) Significant

The total percentage of spontaneous abortion with maternal age was (39.5%) in aborted women, while the total percentage was (20.6%) in the control. The difference between spontaneous abortion and maternal age in aborted women and control was significant ($P < 0.05$).

In our study the highest percentage of spontaneous abortion (82.3%) was in age under 20 years. This findingresult agrees with Bullettietal.,(25) who found that abortion is also more common in women with curly menarche, i. e. occurred before the age of 12 years.

- Also our result was (38.05) in age ≥ 35 years which agrees with Rochebrochard and thonneau , (26); Quenbe et al .,(27) who found that women aged 35 years or more are increased risk of spontaneous abortion.

The increase in the rate of spontaneous abortion occurs in age ≥ 35 years , while in this study the rate was the highest in age under 20 years, this is

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expected to be due to lack of parental experience.

Another reports conducted by Christiansen , (28) and Anderson et al., (29) found that 15% of spontaneous abortion occurs in women in age group (30-34) years, while our result was (26.9%) , this is because pregnant women in Iraq are exposed to different conditions due to the situation of the country.

Also most spontaneous abortion in women (sporadic and recurrent) occurs during first three months of pregnancy in different ages (30).

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دراسة التغيرات النسيجية المرضية والوراثية الخلوية للنساء التي تعاني من الاسقاط

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الخلاصة

اجريت الدراسة الحالية بهدف القاء الضوء على بعض مسببات الاجهاض. اذ تم ملئ 100 استمارة استبيان لنساء مجهضات غير معروفة السبب وتم مقارنتها مع 50 من النساء ذوات الولادة الطبيعية. وكانت الاستمارات الاستبائية محتوية على المقاييس الاتية: درجة القرابة بين الزوجين، عمر الزوجة وبعض مؤشرات صحة التكاثر (الاجهاض التلقائي، موت الاجنة، موت الاطفال الذين هم دون سن الثانية من العمر). تم اختيار (٤٢) امرأة من النساء المجهضة عشوائيا، قسمت النساء الى مجموعتين، الاولى هي مجموعة النساء اللاتي تعرضن للاجهاض خلال المرحلة الاولى من الحمل وعددهن (٣٣) والثانية مجموعة النساء اللاتي تعرضن للاجهاض خلال المرحلة الثانية من الحمل وعددهن (٩). اجريت المقارنة مع (١٠) من النساء ذوات الحمل الطبيعي انتهى حملهن بولادة طبيعية. جمعت نماذج الدم خلال الفترتين كانونالاول ٢٠١٠ ولغاية حزيران ٢٠١١ في مستشفيات بغداد. اجريت التحليلات الوراثة الخلوية على عينات دم النساء المجهضات وقد شملت التحليلات: الانحرافات الكروموسومية ومعامل الانقسام. تعد الانحرافات الكروموسومية من العوامل المهمة والمرتبطة ارتباطا وثيقا مع الاجهاض التلقائي المتكرر عند اكثر من ٦٠% من النساء الحوامل. اذ اظهرت الدراسات الحديثة ان ٢-٤% من الأزواج الذين يعانون من الاسقاط التلقائي المتكرر على الاقل احد الأزواج يحمل نوعا معيناً من الانحرافات الكروموسومية.

وقد خلصت الدراسة الوراثة الى النتائج الاتية:-

- 1- عدم وجود انحرافات كروموسومية تركيبية وعددية في عينات دم النساء المجهضات.
- 2- انخفاض معامل الانقسام الخلوي في عينات دم النساء المجهضات خلال المرحلة الاولى والثانية من الحمل مقارنة مع مجموعة السيطرة.
- 3- وجود علاقة ترابطية بفرق معنوي ($P < 0.05$) ما بين نسبة الاجهاض التلقائي مع عمر الزوجة ودرجة القرابة بين الزوجين في النساء المجهضات والنساء ذوات الولادة الطبيعية.