

Cytogenetic Effects of Gestogens on Women with Threatened Miscarriage in Duhok Province, Kurdistan Region, Iraq

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Abstract

Background: Gestogens have been recommended to have a place in the prevention of threatened miscarriage and spontaneous pregnancy loss during the first 3 months of pregnancy. Certain studies consider that this steroid hormone has genotoxic effect because they directly involved in the change of DNA structure. **Objectives:** This study aims to investigate the cytogenetic effects of gestogens on peripheral blood lymphocytes of pregnant women, by damaged cells and chromosomal aberrations, as well as evaluate the models for hormonal therapy *in vivo*. **Materials and Methods:** Blood sample from 30 women with threatened miscarriage, who received gestogen therapy in the first 3 months, Were taken. Cytogenetic analyses and karyotyping were performed for Each patient Before and after therapy. **Results:** The numerical chromosomal aberrations which found in blood lymphocytes of women before taking gestogens were 44,XX and 48,XX. The same result was observed after taking gestogens. The percentage levels of structural chromosomal aberrations were ring chromosome (2.06%), chromatid breaks (12.47%), chromatid gaps (1.65%), dicentric (0.55%), acentric chromosome (4.02%), and interchange chromosome (12.5%). Whereas, the abnormalities after treatment with gestogens were ring chromosome (5.31%), chromatid breaks (42.19%), chromatid gaps (20.86%), dicentric (7.50%), acentric chromosome (17.75%), and interchange chromosome (43.97%). **Conclusion:** Gestogens as hormonal substitute therapy do not induce numerical chromosomal aberrations but have a significant increase of structural chromosomal aberrations. Gestogens have genotoxic effects on human lymphocyte chromosomes at wide range of concentrations. So the potential benefits and side effects of it must be weighed up against the deleterious effects.

Keywords: Cytogenetic effect, gestogens, threatened miscarriage

INTRODUCTION

Progestins include the naturally occurring hormone progesterone, which is rarely used therapeutically, as well as a number of frequently used synthetic compounds that have Progesterogenic activity. Gestogens are synthetic derivative of female sex hormone progesterone, and its chemical structure closely resembles to that of naturally produced progesterone and it therefore works in a similar way.^[1] These synthetic components are frequently used in the treatment of different conditions such as endometriosis, dysmenorrhoea, uterine bleeding and during the first trimester of pregnancy for habitual or threatened miscarriage. Threatened miscarriage is defined by National Library of Medicine, Medical Subject Headings (2012 MeSH), as bleeding during the first 20 weeks of pregnancy while the cervix is closed.^[2]

The naturally secreted progesterone, which is secreted during early pregnancy period from the ovary by corpus luteum, is

an essential hormone for the establishment and maintenance of pregnancy by inducing secretory changes in the lining of the uterus, and is important for implantation of the fertilized ovum. It also modulates the immune response of the mother to prevent rejection of the embryo, and it enhances uterine quiescence and suppresses uterine contractions.^[2]

The synthetic gestogens are steroid hormone which plays a crucial role in each step of human pregnancy because they can bind to the progesterone receptors and do the same actions that natural progesterone can do.^[3] Gestogens have been played a role in the prevention of threatened spontaneous pregnancy loss during the first trimester of pregnancy, because insufficient

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secretion of progesterone in early pregnancy has been supposed to be the main etiology of miscarriage.^[4]

Gestogens could be administered by three routes: orally, vaginally, or rectally and intramuscularly. Oral administration has the best optimal compliance for patients, but shows several disadvantages; this route also results in side effects such as nausea, headache, and sleepiness. The vaginal route results in higher concentrations in the uterus, but does not reach high and constant blood levels. The drug administered intramuscularly occasionally induces nonseptic abscesses, although it is the only route, which results in optimal blood levels.^[5,6]

Certain studies consider that synthetic gestogens have genotoxic effect since they directly involved in the change of DNA structure while others suppose that these hormones are acting indirectly by oxidation of reactive metabolites formed during their metabolism.^[7]

Several studies demonstrated the cytogenetic effects of gestogens on peripheral blood lymphocytes of pregnant women since they directly involved in the change of DNA structure while others suppose that these hormones are acting indirectly by oxidation of reactive metabolites formed during their metabolism.^[7]

A Study in (2003) indicated that the highest doses of them induce the increase of average Micronuclei (MN) frequency which is a sign of genotoxic events and chromosomal instability in human peripheral blood lymphocytes of pregnant women.^[7]

In a review of the non-clinical literatures for embryo-fetal toxicity with one of progestin (17alpha-hydroxyprogesterone caproate), showed that there is a signal for embryo-fetal toxicity associated with 17-OHP-C in the two largest clinical trials.^[8,9]

Another study in 2016 revealed that Gestogens induced a significant increase of chromosomal aberrations in female patients with a diagnosis of threatened spontaneous abortions, who maintained their pregnancies by taking them.^[10]

The present study aims to investigate the cytogenetic effects of gestogens on peripheral blood lymphocytes of pregnant women, by damaged cells and chromosomal aberrations, as well as to evaluate the models of hormonal therapy. Therefore, the present study aims to investigate the cytogenetic effects of gestogens on peripheral blood lymphocytes of pregnant women, by damaged cells and chromosomal aberrations, as well as to evaluate the models for hormonal therapy.

MATERIALS AND METHODS

The current study was performed on 46 patients who attended an Obstetrical & Gynecological Private Clinic in Duhok city for antenatal care and follow-up of their pregnancies, during the period from April 2015 to October 2016. All the selected patients had threatened spontaneous miscarriage, or had bad obstetrics history with previous miscarriages, or

had pregnancy after a history of infertility, who needed to take Gestogens therapy in the first 3 months of gestation (1st trimester period - 13 weeks) to support their pregnancy, or/used Gestogens as treatment symptoms of threatened miscarriage and vagifor threatened miscarriage until the nal bleeding subsided.

The patients received one of the following models for hormonal substitution treatment: Oral: Duphaston tablets 1x3 (Dydrogesterone 10 mg). Injection Intramuscular: Primolute Depot 500 mg/week or 250 mg every 3 days + Duphaston tablets 1x2. And Vaginal suppositories: Cyclogest (micronized progesterone) suppositories of 200 mg, vaginally or rectally (2 tablets = 400 mg at night).

Lastly, only 30 patients continued with the study as 4 patients were not returned back, there were problems in the samples of 6 others, and the last 5 ended with a missed miscarriage.

Cytogenetic analyses and karyotyping for each individual before and after therapy were performed at the Scientific Research Centre in the College of Medicine, University of Duhok. All patients were subjected to full clinical and laboratory examinations. A questionnaire form was filled for each patient that included sociodemographic characteristics of the individual such as age, model of treatment, gestational age, history of infertility, and history of miscarriages and previous abortions.

All steps for the preparation of chromosome from the cultured lymphocytes of peripheral blood and all solutions needed were prepared according to genetic techniques.^[11-13]

For each patient, chromosomal culture was carried out by collection of 1ml of peripheral whole blood in sodium heparinized tube, then added to a flat culture tube that containing 10 ml of RPMI 1640/L-glutamine, 2ml of fetal bovine serum, 200µg of Phytohemagglutinin (10µg/ml), and 200µg penicillin-streptomycin solution (10µg/ml). For each patient 15-20 cells were counted and analyzed and finally designated the karyotype according to the International System for Human Cytogenetic Nomenclature (ISCN1995). /rpmi for 7 min. Then, 10 ml of 0.075 M KCl solution was added, mixed, and incubated at 37°C for 30 min. After centrifugation 1500/ rpmi for 7 min, hypotonic supernatant was added. Then, 10 ml of cold, fresh fixative solution (3:1 methanol:glacial acetic acid) was added drop by drop for the first 2 ml to the cell pellet. Centrifugation was done afterward, and the supernatant was removed, and the last step was repeated until a clear pellet was obtained. Finally, the cells obtained were dropped on clean distinct slides, slides dried, and stained with Giemsa stain.

The slides were examined and analyzed with a bright-field microscope using BX51 Olympus microscope, and karyotyping was performed with the aid of computer-based karyotyping system (CytoVision version 7.2 from Leica microsystem). At least 15-20 of cell at metaphase stage (which is a stage

of cell division when the duplicated chromosome become along the center of the cell, and end in meiosis, the pair of chromosomes separate from one another) spread and captured by using a satellite capture station then the images transferred to an image analyzer. For each patient, 15–20 cells were counted and analyzed and finally designated the karyotype according to the International System for Human Cytogenetic Nomenclature (ISCN 1995).

Statistical analysis

After completing karyotyping, data analysis was performed using Statistical Package for the Social Sciences version 22 (SPSS, IBM Company, Chicago, USA). Descriptive data were presented for continuous variables as mean $\pm$ standard deviation, while qualitative data description was done by calculating number and percentage. *T*-test was used to compare between two means and Chi-square test was used to compare

between proportions; $P \leq 0.05$ was considered statistically significant.

RESULTS

Cytogenetic analysis was performed on peripheral blood lymphocyte of all 30 patients with threatened miscarriage before and after receiving different models of gestogen treatment. The age of the enrolled patients ranged from 20 years to 38 years with a median age of 28 years (mean 28.8 ± 5 years). Patients who received gestogens were classified into three groups according to the rout of administration of this hormone which are oral, injection, and vaginal boll.

Table 1 shows the different groups of patients who received gestogens with the duration of treatment and their gestational age. The A group included fifteen patients who received

Table 1: Distribution of the patients according to their groups about the types of gestogens are used, Age, the duration of treatment, the gestational age at the time of starting treatment and obstetrics history (G₀ P₀ A₀)

	Age	Mode of administration			Gestational age (weeks) starting treatment	Duration of treatment (weeks)	Obstetrics history G P A*
		Group C Vaginal tablet 200 mg Cyclogest 2 tablets	Group B Injection Primolut 500 mg (weekly) + Duphaston 10 mg 1×2	Group A Oral Duphaston 10 mg (1×3)			
1	28			Yes	8	10	G ₂ P ₁ A ₀
2	27			Yes	7	10	G ₁ P ₀ A ₀
3	22			Yes	5	9	G ₂ P ₀ A ₁
4	26			Yes	7	11	G ₁ P ₀ A ₀
5	31			Yes	6	10	G ₂ P ₀ A ₁
6	34			Yes	7	11	G ₂ P ₀ A ₁
7	32			Yes	6	12	G ₁ P ₀ A ₀
8	20			Yes	7	7	G ₂ P ₁ A ₀
9	25			Yes	6	8	G ₄ P ₃ A ₀
10	28			Yes	5	13	G ₄ P ₁ A ₂
11	26			Yes	6	9	G ₂ P ₁ A ₀
12	27			Yes	6	10	G ₄ P ₀ A ₃
13	35			Yes	5	12	G ₂ P ₀ A ₁
14	28			Yes	7	11	G ₁ P ₀ A ₀
15	30			Yes	6	12	G ₂ P ₀ A ₁
16	27		Yes		4	12	G ₇ P ₂ A ₄
17	34		Yes		6	10	G ₂ P ₁ A ₀
18	25		Yes		5	11	G ₁ P ₀ A ₀
19	32		Yes		7	8	G ₄ P ₂ A ₁
20			Yes		8	6	G ₂ P ₀ A ₂
21	28		Yes		6	8	G ₁ P ₀ A ₀
22	38		Yes		5	11	G ₆ P ₂ A ₃
23	28		Yes		5	12	G ₁ P ₀ A ₀
24	28	Yes			6	8	G ₁ P ₀ A ₀
25	29	Yes			6	8	G ₄ P ₃ A ₀
26	30	Yes			7	9	G ₂ P ₁ A ₀
27	31	Yes			5	11	G ₂ P ₀ A ₁
28	29	Yes			7	9	G ₅ P ₂ A ₂
29	35	Yes			5	12	G ₅ P ₁ A ₃
30	32	Yes			6	12	G ₁ P ₁ A ₂

*G (Gravida -- No. of all previous pregnancies). *P (Parity -No. of deliveries after 23 week of gestation). *A (Abortion --No. of previous abortions or Miscarriage, expulsion of fetus before 23 week of gestation).^[2]

Gestogens therapy orally in doses of (Dydrogesterone) 10 mg (1x3). The B group included eight patients who received Gestogens therapy in both Dydrogesterone orally in doses of 10mg (1x2) and Primolute Depot Injection in doses of 500mg (weekly). The C group included seven patients who received Gestogens therapy in form of vaginal or rectal boll of micronized progesterone in doses of 200 mg (2 vaginal tablets at night).

The numerical chromosomal aberrations those found in blood lymphocytes of women before taking gestogens were 44,XX in patient number 12 and 48,XXYY in patient number 6. The same result was observed after taking gestogens [Figure 1].

The percentage values of structural chromosomal aberrations were ring chromosome (2.06%), chromatid breaks (12.47%), chromatid gaps (1.65%), dicentric (0.55%), acentric chromosome (4.02%), and interchange chromosome (12.5%). Whereas, those abnormalities after treatment with gestogens

Table 2: Mean $\pm$ standard error for the damage cells and percentage of chromosomal aberrations in patients before and after taking Gestogens

Variable	Before treatment	After treatment
Mean $\pm$ SE	13.60 $\pm$ 4.69	22.93 $\pm$ 6.81
Ring chromosome (%)	2.06	5.31
Chromatid break (%)	12.47	42.19
Gap chromosome (%)	1.65	20.86
Dicentric chromosome (%)	0.55	7.5
Acentric chromosome (%)	4.02	17.75
Interchange (%)	12.5	43.97
P	0.005353	

SE: Standard error

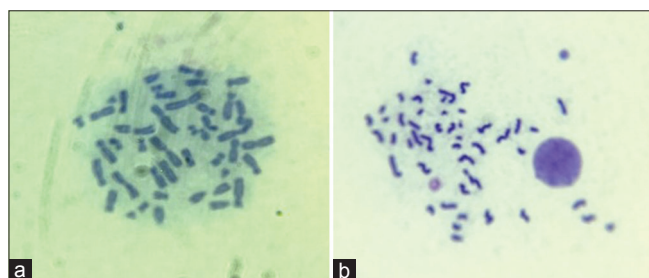


Figure 1: Metaphase of numerical chromosomal abnormality: (a) chromosomal abnormalities with 44, XX. (b) Chromosomal abnormalities with 48, XX

were ring chromosome (5.31%), chromatid breaks (42.19%), chromatid gaps (20.86%), dicentric (7.50%), acentric chromosome (17.75%), and interchange chromosome (43.97%) as in Table 2 and Figure 2.

Summarizing the results for all three analyzed groups, we observed that the average chromosomal aberrations after therapy with gestogens were statistically significantly higher ($P < 0.0053$), in comparison with average chromosomal aberrations in the same patients before taking therapy.

DISCUSSION

Progesterone is an essential hormone in the process of reproduction. It is involved in the menstrual cycle and implantation and is essential for pregnancy maintenance. Although the pharmacokinetics and pharmacodynamics of progesterone have been extensively studied since it was first synthesized in 1935, its use in the pathophysiology of pregnancy remains controversial.^[5]

Progesterone and its synthetic form 17 α -hydroxyprogesterone caproate offer an effective intervention when the continuation of pregnancy is at risk from immunological factors, luteinic and neuroendocrine deficiencies, and myometrial hypercontractility. Progesterone has been successfully used as prophylaxis in the prevention of spontaneous miscarriage, with treatment beginning from the first trimester of pregnancy.^[14]

Otherwise, several studies have used progesterone and other related steroids as a treatment to prevent spontaneous miscarriage and threatened and recurrent miscarriage. During the past years, several studies have used Progesterone and others related steroids as a treatment to prevent spontaneous miscarriage, threatened and recurrent miscarriage. Wahabi *et al.*^[4] selected the 15 trials published in databases and references, the authors included only 7 trials were where the administration of progestagens started before pregnancy and continued during pregnancy. The results demonstrated that there is no evidence to support the routine use of progestagens for the treatment of threatened miscarriage, and no statistically significant difference in the miscarriage rates between Progestogens and pregnancy loss in pregnant women who use Gestogens as treatment or those who do not use gestogens during pregnancy.^[6] Carp *et al.* reported in a recent meta-analysis the beneficial effects of progestogens which were associated with a higher rate of live births or ongoing

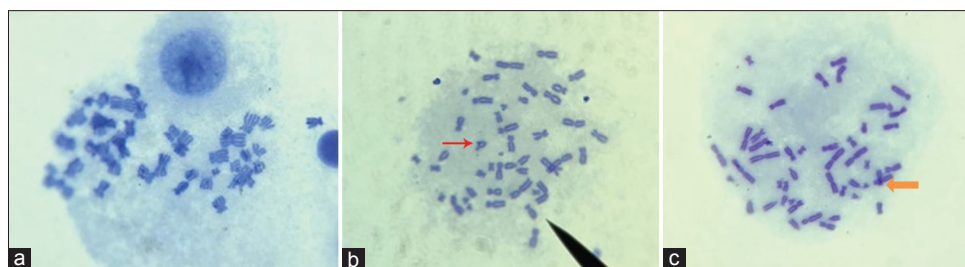


Figure 2: Metaphase of structural chromosomal abnormality: (a) Chromatid interchanges. (b) Ring Chromosome. (c) Chromatid gap

pregnancy in the progesterone group by 28%. Progestogens have therefore been used at all stages of pregnancy including luteal-phase support prior to pregnancy, threatened miscarriage, recurrent miscarriage, and to prevent preterm labor. In recurrent miscarriage, progestogens also seem to have a beneficial effect. For the last 30 years, progestogens have been used to prevent preterm labor.^[3]

Many studies conducted about the cytogenetic effects of Gestogens on chromosomal abnormalities in peripheral blood lymphocytes of pregnant women who take gestogens during first trimester of their pregnancy, which showed higher frequencies of MN and aneuploidy, because they directly involved in the change of DNA structure.^[7,10,15]

In this study, the results showed that numerical chromosomal aberrations which detected in blood lymphocytes of women before taking gestogens were 44,XX and 48,XX. The same result was observed after taking gestogens. This means that gestogen therapy dose not result in numerical chromosomal abnormalities in women with threatened miscarriage who used it as therapy or prophylaxis in the pregnancy. The same results were obtained by others who revealed the genotoxic effects of fludrocortisone steroid hormones in human lymphocyte by applying a wide range of concentrations (25 µg, 50 µg and 100 µg) at a different exposure duration (24, 48, and 72 h).^[15]

These results that obtained by may be affected by the low doses of Gestogens which are not adequate to affect peripheral blood lymphocytes to bind their steroid receptors. Also, may be due to the inability of Gestogens to generate free oxygen radicals and to form hydrogen peroxide as well as superoxide and hydroxyl radicals, as has been observed in the case of certain synthetic steroids or may be due to the new design and formulation of Gestogens that we used.^[16]

In another hand, the results of this study observed that the percentages of structural chromosomal aberrations in women with recurrent miscarriage before taking gestogens was high (mean = 22.93 ± 6.81), but this percentages significantly increased ($P < 0.005$) after taking therapy (mean = 13.60 ± 4.69) by (~2-fold) in comparison with the same patients before therapy.

These results are similar to results of another study which revealed that the average of MN frequency after therapy was significantly higher ($P < 0.001$), in comparison with average MN frequencies in the same patients before therapy (~1.5-fold) and with those of control group of healthy pregnant women (~3-fold).^[7]

A series of published studies also reported that synthetic steroid hormones "including gestogens" have genotoxic effect on pregnant woman who take gestogens during first trimester of their pregnancy by inducing higher frequencies of MN and aneuploidy, because they directly involved in the change of DNA structure.^[7,10,17]

There is no evidence to support the routine use of progestogen in order to prevent miscarriage in the first and second trimester

of pregnancy. However, there is an evidence about its benefit in women with a history of recurrent miscarriage.^[16] A finding of a significantly reduced miscarriage rate in women with a history of recurrent miscarriage was found only in studies that included women with at least two or three miscarriages. This finding was obtained with different types of progestagens.^[6] Treatment of these women who warranted had given reduced rates of miscarriage in the treatment group, and there was no statistically significant difference between treatment and control groups about the adverse effects. Regarding the three routes of administration (oral tablets, injections, and vaginal or rectal suppositories) also showed no statistically significant difference between these three groups.^[6]

CONCLUSION

Gestogens as hormonal substitute therapy do not induce numerical chromosomal aberrations but have a significant increase of structural chromosomal aberrations Gestogens have genotoxic effects on human lymphocyte chromosomes at wide range of concentrations. So the potential benefits and side effects of it must be weighed up against the deleterious effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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