



Assessment of Apoptosis in Women Using Oral Contraceptives

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Abstract

The aim of the study was the examination of the effect of birth control pills (Yasmin) on women who utilized the pills by measuring the percentage of apoptosis. The investigation was based mainly on the calculation of the apoptosis percentage of blood lymphocytes in women who used the pills for different durations. The results of the presented study was obtained from 25 women handling birth control pills and 15 women who had not used pills. According to the period of contraceptive pills treatment, the samples were classified into two categories: (2-5) years and (6-8) years. Results showed that the percentage of apoptosis in pills handling women (2-5) years were (7.08%) while the results were (12.2%) in women who used birth control pills (6-8) years. In addition, the results values were significantly different ($P \leq 0.05$) from those in control, which were (0.18%). Based on the results obtained in this study, it is concluded from that the percentage of apoptosis increase with the prolonged use of Yasmin.

Keywords: apoptosis, Yasmin.

تقييم الموت الخلوي المبرمج في النساء اللواتي يستخدمن حبوب منع الحمل

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الخلاصة

الكشف عن تأثير حبوب منع الحمل (ياسمين) على النساء اللواتي يستخدمن الحبوب من خلال قياس نسبة الموت الخلوي المبرمج هو الهدف من هذه الدراسة. وقد اعتمدت عملية المقارنة على حساب نسبة الموت الخلوي المبرمج لخلايا الدم للمفاوية عند النساء اللواتي يستخدمن الحبوب ولفترات مختلفة باستخدام فحص الموت الخلوي. جمعت 25 عينة دم من النساء اللاتي يستخدمن الحبوب و 15 عينة اخرى كعاملة سالبة. و اعتمادا على مدة الاستعمال، تم تقسيم عينات الدم لـ (2-5) سنوات و (6-8) سنوات. وقد بينت النتائج ان نسبة الموت الخلوي المبرمج للنساء اللواتي يستخدمن الحبوب للفترة من (2-5) سنوات كانت (7.08%) بينما عند النساء اللواتي يستخدمن الحبوب للفترة من (6-8) سنوات كانت (12.2%) والنتائج الملحوظة ادت الى زيادة معنوية ($P \leq 0.05$) بالمقارنة مع المعاملة السالبة والتي كانت (0.18%). من هذه النتائج نستدل على ان نسبة حصول الموت الخلوي المبرمج سوف تزيد مع طول فترة الاستخدام لعقار الياسمين في حياتنا اليومية.

Introduction

Prevents pregnancy by interfering with the normal process of ovulation, fertilization, and implantation is the process called contraception (birth control). There are various forms of contraception that act at various points in the process [1]. Contraception utilizes in developing countries has minimized the number of maternal deaths by 40% and if the full demand for birth control were met could prevent 70%. Birth control also can improve adult women's delivery outcomes and the survival of their children by prolonged the duration between pregnancies [2]. Oral contraceptives (OCs) are medicines taken by mouth to help avoid pregnancy. They are also called as the pills or birth control pills (BCPs) [3]. A combination of estrogen (ethinylestradiol or mestranol) and progestin is most oral contraceptive pills contain, but progestin-only preparations found as well [4]. Combined oral contraceptive pills were developed to prevent ovulation by suppressing the release of gonadotropins. A primary mechanism of action prevents ovulation and inhibit follicular development [5]. Apoptosis is the process of programmed cell death (PCD) that may take place in multicellular organisms [6]. Cell shrinkage, membrane blebbing, nuclear condensation, and formation of apoptotic bodies, it is a morphological characteristic of apoptosis [7]. PCD was already discovered by scientists more than 100 years ago and the German scientist Carl Vogt was first to explain the principle of apoptosis in 1842. In Greek, apoptosis means "dropping off" of petals or leaves of plants or trees [8]. The apoptotic process is started by "death" signals, which induce a complex series of events [9]. The activation of caspases, a group of enzymes belonging to the cysteine protease family is a central step of the apoptotic process so enzyme activation led to cleave many vital cellular proteins, breaking down the nuclear scaffold and cytoskeleton, and subsequently leads to nuclear DNA degradation [10]. Depend on the original source of "death" signals, caspase dependent apoptosis are categorized into two pathways: intrinsic pathway, which is activated by modulators within the cell itself, and extrinsic pathway, which responds mainly to extracellular stimuli [11]. In the receptor-mediated death pathway (also known as extrinsic pathway), death ligands induce apoptosis by activating the death receptors at the cell surface. The activated death receptors, which leads to the recruitment of the adaptor proteins such as FADD (Fas-associated protein with death domain) and further chain activation of caspase-8 and -3 [12]. While the mitochondria-mediated death pathway, which is also known as intrinsic pathway, is initiated by damage to the mitochondria, which led to the secretion of a series of proteins into cytoplasm including cytochrome C. [13]. For the intrinsic pathway, the initial enzyme activated is caspase-9 [14]. Finally, apoptosis is important in the sophisticated architecture of life, including normal cell turnover, organ metamorphosis, tissue homeostasis, and embryonic development [15]. Apoptosis is a programmed cell death process distinguished by morphological and biochemical characteristic take place at various stages. One of the plasma membranes differs during apoptosis was the translocation of phosphatidylserine (PS) from the inner to the outer layer of the plasma membrane, by which PS becomes exposed at the external surface of the cell. Annexin V has been suggested as a suitable assay of early apoptotic cell detection of such membrane changes [16]. The aim of this study is to evaluate the programmed cell death by examining the apoptosis percentage in the cells using flow cytometry.

Materials and methods

Collection of human samples

Subjects and patient group

Twenty- five patients using contraceptive pills of Yasmin type from Al-Karama Hospital was taken. Their age ranged between 25-35 years old with treatment duration between 3-8 years.

Healthy control group

Fifteen apparently healthy women who had not used the contraceptive pills were used. Their age ranged between 25-35 years old.

Blood sampling

Blood samples (5 ml) were collected by venipuncture from all patients and control groups. Blood was transmitted within few hours for apoptotic analysis using cooling container and stored in the refrigerator at 4°C. Heparinized tubes for apoptosis were used.

Principle

Region A: necrotic cells if it positive PI-/ negative annexin V-dye; region B: secondary necrosis if it positive PI and annexin V dye ;region C: vital cells if it negative PI- and annexin V- dye; while region D: apoptotic cells if it negative PI- and positive annexin V- dye.

Kit contains

Annexin V-FITC was employed in flow cytometry and performed according to the human Annexin V-FITC Kit-EXBio Praha. Reagents provided with the kit are the binding buffer (10X concentrated), Annexin V-FITC, and Propidium iodide [17].

Assay procedure

Preparation of reagent

The Annexin V binding buffer was a (10x) concentrated and diluted with deionized water prior to use in order to prepare 1x Annexin V binding buffer.

Procedure

Cells were harvested by centrifugation at 2,000 rpm for 5 min, the supernatant was discarded. Pellets were resuspended in cold PBS and cells were washed by gentle shaking or pipetting up and down. Re-centrifuged washed cells again and the supernatant was discarded. Then, the pellet was resuspended with 1X binding buffer and cell density was justed to $2-5 \times 10^5$ cells/ml. preparing a sufficient volume of cell suspension (100 μ l/ assay). Cells were stained with 5 μ l of Annexin V-FITC and propidium iodide (PI) and incubated for 15 minutes in dark at room temperature. Next, cells were harvested by centrifugation at 2,000 rpm for 5 min and pellet was resuspended in 100 μ l of binding buffer. Finally the mixture was analyzed by Flow cytometry of [18].

Statistical analysis

Analysis of variance was performed to test whether group variance is significant or not, the comparison between groups performed by the statistical package for the social sciences program (SPSS).

Results and discussion

Annexin V-FITC kit used to distinguish between cells of either apoptosis or necrosis or vital. In this assay the different labeling patterns, identify the various cell populations.

Concerning the use of oral contraceptives, the women were asked if they had used combined oral contraceptives for at least one month. Pictures of all pills types that were on the market in Iraq were used to obtain required information about oral contraceptive use history. During the interview, the woman was asked about the period and for how long she had used a specific type. If she could not remember the type of pills, pictures of the brands marketed in the relevant period were seen to the woman and women who used different kinds of combined oral contraceptives, and women who did not remember the type they had used were excluded. The total sample numbers were 40 (25 for contraceptive users and 15 for control). Contraceptive pills users were categorized into two groups depending on the duration of treatment to 2-5 years and 6-8 years.

The percentage of apoptosis in women who used Yasmin contraceptive pills for 2-5 years was 7.08%. On the other hand, there was observed an increase in the percentage of apoptotic cells to 12.2% in women used Yasmin contraceptive pills for 6-8 years as compared with the negative control 0.18%. The apoptosis percentage in contraceptive users were significantly different ($P \leq 0.05$) in comparison with those of the control group. The conclusion from these results shows that the prolonged use of these drugs led to increasing in apoptosis percentage as compared with the negative control.

Higher ($P \leq 0.05$) viable cells percentage was noticed in the negative control (99.3%) as compared contraceptive pill groups either for 2-5 years (92.18%) or 6-8 years (86.84%). The differences among groups in necrotic or late apoptotic cells lacked significance Table-1, Figure-(1, 2).

Table 1-poptosis percentage in women blood lymphocyte used contraceptive pills for different periods (Mean+SD).

Groups	Necrotic cells-A (%) (mean \pm SD)	Late apoptosis cells-B (%) (mean \pm SD)	Viable cell-C (%) (mean \pm SD)	Apoptotic cells-D (%) (mean \pm SD)
Negative control	A 0.22 \pm 0.09	A 0.24 \pm 0.06	A 99.36 \pm 0.44	C 0.18 \pm 0.09

Contraceptive pills (2-5 years)	A 0.36±0.03	A 0.38±0.003	B 92.18±1.75	B 7.08±0.55
Contraceptive pills (6-8 years)	A 0.54±0.021	A 0.42±0.035	C 86.84±2.47	A 12.2±0.93

Means with different superscripts within each column differ significantly ($P \leq 0.05$).

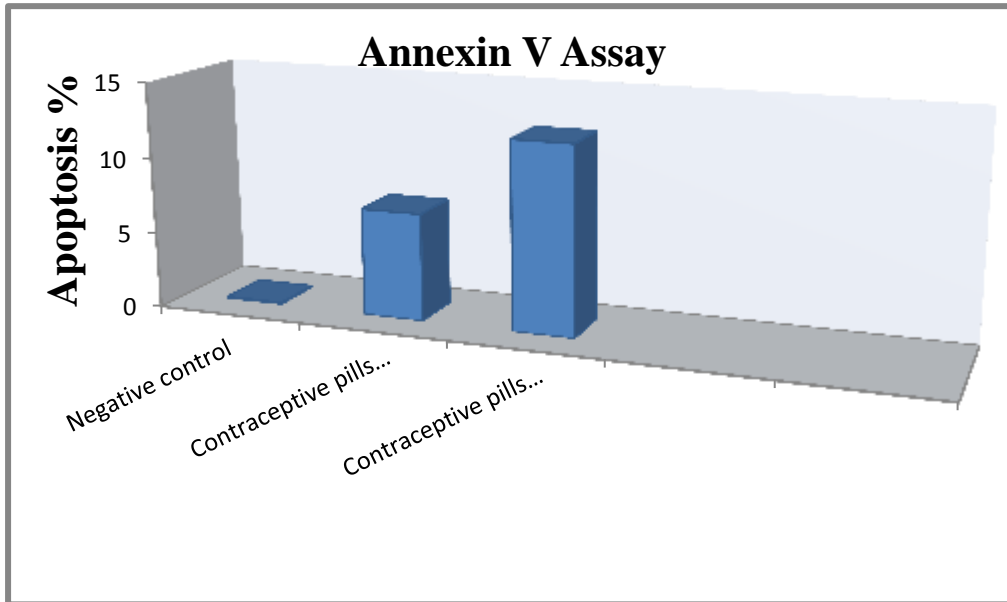
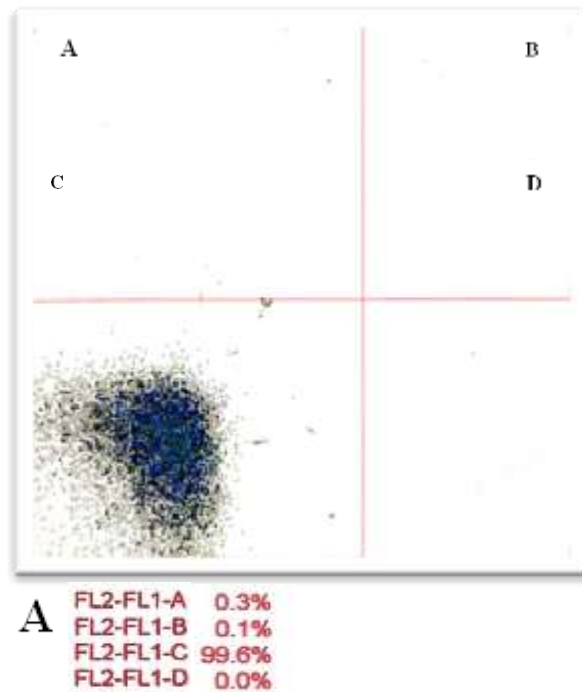


Figure 1-the apoptosis percentage in women blood lymphocyte by Annexin V assay.



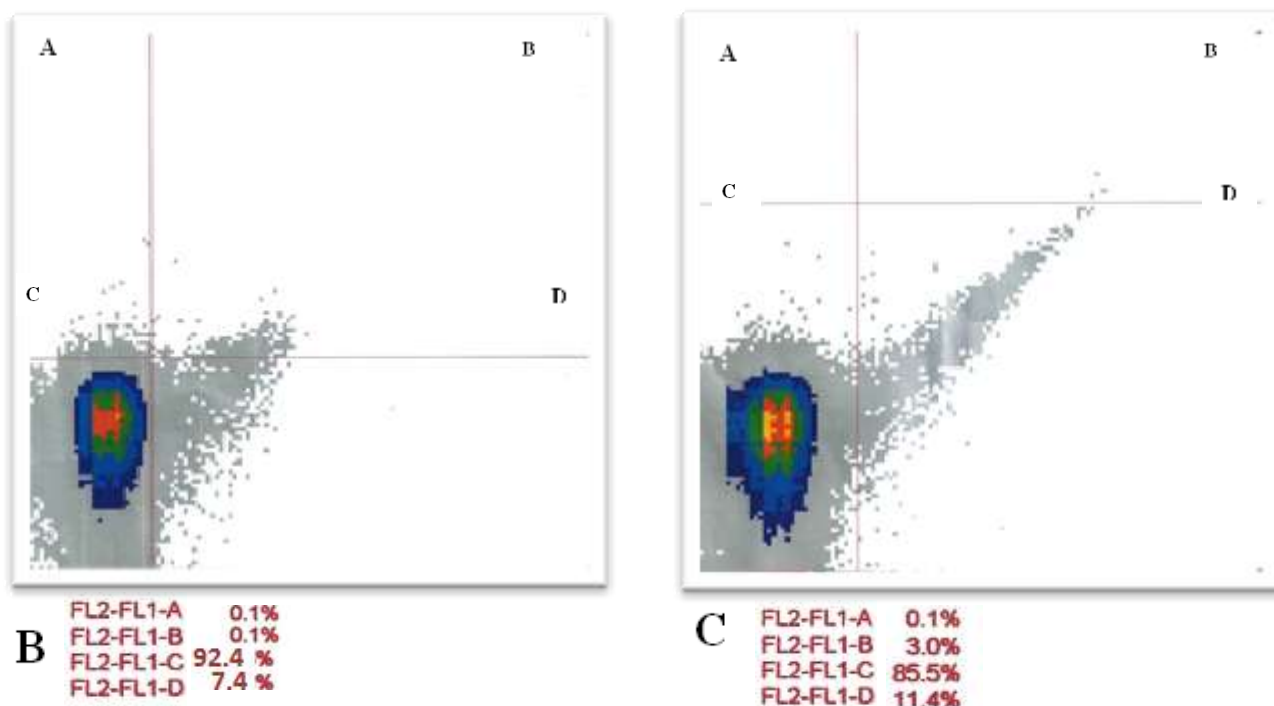


Figure 2-Annexin V expression for mice induced apoptosis analysis. The labeling patterns inside quadrants indicate the percentage of A: necrotic cells(PI- positive/ Annexin V- negative); B: late apoptosis(PI-positive/ Annexin V-positive); C:Vital cells(PI-negative/ Annexine V- negative); D: apoptotic cells (PI-negative/ Annexine V- positive).The labeling patterns outside quadrants indicate the percentage of apoptotic cells control (a), contraceptive pills treatment duration (2-5) years (b), contraceptive pills treatment duration (6-8) years (c).

Compatible with our findings (19) found that the combination estrogen–progestin OC has a potent apoptotic activity on the ovarian epithelium accomplished by the progestin component and the stimulating ability on apoptosis percentage increased more than six-fold by levonorgestrel alone and fourfold by the contraceptive combination of ethinylestradiol and levonorgestrel almost.

It was shown that just one month of pills was enough to significantly reduce observes of cell proliferation and increase the apoptotic index in the eutopic endometrium of patients with endometriosis (20).

In ovarian surface epithelium the synthetic progestin, levonorgestrel, may induce apoptosis (19), ethinylestradiol and levonorgestrel induce ovarian epithelial cell apoptosis in intact monkeys (21), while drospirenone was found to stimulate chromosome aberrations in human peripheral lymphocytes (22).

The estrogen component inhibits the release of FSH and led to suppresses the development of the ovarian follicle, but the progestin component inhibits the release of LH thereby preventing ovulation. They also reduce the amount of the cervical mucus, increase its viscosity and cell content, and change its molecular structure, making it less suitable for sperm penetration (23).

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