



## Bacteriological and Cytokine Profiles for Women Using Hormonal Contraceptive Drugs

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### Authors' contributions

This work was carried out in collaboration between all authors. Author MAKAS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author AKG managed the literature searches, analyses of the study performed the spectroscopy analysis and author KAA managed the experimental process. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/BJMMR/2016/21391

#### Editor(s):

(1) Jingli Xu, College of Pharmacy, University of New Mexico, USA.

#### Reviewers:

(1) Gülsen Meral, Kagithane State Hospital, Istanbul, Turkey.

(2) Rodrigo Crespo Mosca, São Paulo University, Brazil.

(3) Rosliza Abdul Manaf, University Putra Malaysia, Malaysia.

Complete Peer review History: <http://sciencedomain.org/review-history/12025>

Original Research Article

Received 15<sup>th</sup> August 2015  
Accepted 3<sup>rd</sup> October 2015  
Published 30<sup>th</sup> October 2015

### ABSTRACT

**Background:** Hormonal contraceptives that contain estrogen and progesterone alter the genital tract epithelial configuration which has been suggested to be associated with shifts in the vaginal flora, changes that occurring as a result of contraceptive measures may disrupt the resident vaginal flora.

**Aim of Study:** To evaluate the effect of hormonal contraceptives on vaginal bacteriological profile and some of immunological markers.

**Study Design:** Case control study.

**Place and Duration of the Study:** This study was carried out in family planning clinic at Babylon hospital for maternity and children in Hilla, Iraq and Department of Medical Microbiology-College of Medicine -Babylon university, Hilla-Iraq. The period of study was from August 2014 to April 2015.

**Methodology:** This study involved 60 women, 40 of them were used oral combined contraceptive pills that contained progesterone and estrogen, and 20 women were used injectable contraception

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that contained only progesterone, while 30 women were healthy women never use contraception as a control. Samples taken from all women were high vaginal swab for Bacterial isolation and blood for measurement of serum interferon –gamma (IFN- $\gamma$ ), interleukin-2 (IL-2) and interleukin-8 (IL-8) by enzyme linked immunosorbent assay (ELISA) technique.

**Results:** *Lactobacillus* spp were the predominant among the control group (93.3%), while only (5%) in both contraceptive groups. Gram negative and Gram positive bacteria were isolated (68.39%, and 31.56%) respectively from women with contraceptive drugs. Immunological assay showed a significant decrease in serum concentration of both IFN-  $\gamma$  and IL- 8 in both contraceptive group compared to control group (p value less than 0.05). While, there is no significant difference in serum level of IL-2 among study groups.

**Conclusions:** Both types of hormonal contraceptives lead to inhibition of vaginal Lactobacilli and decrease serum level of both IFN- $\gamma$  and IL-8.

**Keywords:** Cytokines; bacteria; contraceptive drugs.

## 1. INTRODUCTION

Contraceptives or birth control, are devices or methods used to prevent pregnancy, provision and the use of birth control is also called family planning [1]. Birth control methods have been used since ancient times, but the effective and safe methods only became available in the 20<sup>th</sup> century [2]. Contraceptive methods include behavioral methods, hormonal contraceptive, intrauterine devices (IUDs), barrier methods and sterilization, each methods usually used during or before sex while emergency birth control are effective for up to a limited days after sex [3]. The most effective methods of birth control is a sterilization, implantable contraceptives, IUDs and hormonal contraceptives which including vaginal rings, patches, oral pills and injectable contraceptives [4]. About 60% of married fertile women use contraceptives worldwide [5], in Iraq the prevalence of contraceptives among women is 53.7% [6].

Hormonal contraceptive alter the genital tract epithelial configuration [7], that have been suggested to be associated with shifts in the vaginal flora, artificial changes that occurring as a result of hormonal contraceptives usage may disrupt the normal vaginal flora [8]. Normal vaginal inhabitants which maintain vaginal pH between 3.5-4.5 [9]. Lactobacilli play an important role in the maintenance of vaginal health, and prevention of vaginal infection [10]. Lactobacilli produce a variety of bacteriocins (proteins/peptides) that inhibit the growth of bacterial vaginosis-associated bacteria and other pathogens such as *N. gonorrhoea*, *Escherichia coli*, and *C. albicans* [11]. Vaginal infections primarily occur when normal vaginal flora is disrupted, since vagina is normally dominated by lactobacilli and its metabolites [12].

Estradiol and progesterone decrease the infiltration of macrophages, neutrophils and leukocytes to the upper and lower women genital tract and inhibits the expression of chemokine monocyte chemo- attractant protein-1 by human endometrial stromal cells [13]. Contraceptives that contain only progesterone also change the expression of cytokines that normally involve in T helper2 responses [14]. Usage of injectable (medroxy progesterone) contraceptives exert a number of effects on the T cell-mediated immune response including the inhibition of cytotoxic activity of T cells and blocking perforins expression in T cells [15]. A current study focused on evaluation of the effect of oral combined contraceptive pills (estrogen and progesterone) and injectable (medroxy progesterone) contraceptive on the vaginal bacteria and on the serum levels of cytokines, interferon-gamma, Interleukines- 2 and 8.

## 2. MATERIALS AND METHODS

### 2.1 Patients

The study involved 60 randomly selected women using hormonal contraceptives.

Forty of them used oral combined contraceptive pills that contained progesterone and estrogen, and twenty used injectable contraception that contained only progesterone. Thirty healthy women who never used contraception were involved as a control. Blood samples and high vaginal swab using vaginal speculum during late follicular phase of menstrual cycle were collected from all women. All samples were collected from family planning clinic in Babylon hospital for maternity and children. Women excluded from this study were pregnant, diabetics, those who were under steroid, anti-cancer and antibiotics

treatment and those with signs and symptoms of genital tract infection. Bacterial isolation and identification was carried out according to Forbes et al. [16]. Immune parameters: Serum IFN- $\gamma$ , IL-2 and IL-8 were estimated by ELISA technique according to the instruction company [Booster, China].

## 2.2 Ethical Approval

The necessary ethical approval from family planning clinic in Babylon hospital for maternity and children / Hilla -Iraq was obtained. Moreover, all subjects involved in this work were informed and the agreement required for doing the experiments and publication of this work was obtained from each one prior the collection of samples.

## 2.3 Statistical Analysis

The statistical analysis of this study was obtained by the SPSS and Excel programs, the statistical parameter that used in this study were include Z Test, Mean, Standard deviation, percentage and P value(<0.05) considered statistic significant.

## 3. RESULTS AND DISCUSSION

### 3.1 Gram Stain Distribution of Bacterial Isolates

The types, number and percentage of Gram negative and Gram positive bacteria isolated from women of both groups are shown in Table 1.

The percentage of Gram negative isolates in oral combined contraceptive pills and injectable (medroxy progesterone) administered women was (68.39%), *E-coli* isolates had the highest percentage (28.07%) between them, followed by *Enterobacter cloacae* (19.29%) and *Klebsiella pneumonia* (14.03%), the lowest percentage was represented in both *Pseudomonas aeruginosa* and *Neisseria gonorrhea*, which recorded (3.50%) for each one. While, there is no bacterial isolate in control group. These findings are similar to those obtained by Gupta et al. [17] who found that there was an inverse association between vaginal lactobacilli and *E. coli* colonization. Furthermore, after controlling for intercourse, antimicrobial use and birth control method in the preceding week. The risk of *E. coli* colonization at any visit was 2-fold greater

among women without *Lactobacilli* as compared with women with *Lactobacilli*. Other study done in Pakistan showed that *E. coli* was isolated from 24% of women with hormonal contraceptives [18]. The presence of *K. pneumoniae* in cases of contraceptive women can be attributed to absence or decrease in numbers of lactobacilli and subsequently their defense factors [19]. *P. aeruginosa* had also been isolated from both groups. It is potentially opportunistic bacteria within the vagina. Such microorganism may become increasingly prevalent upon minor alterations of the vaginal environment. Investigators had isolated this bacteria from cases of vaginitis and showed that this bacteria was prevalent among non- pregnant women using intra uterine device [20]. Only two isolate of *N. gonorrhoeae* had been isolated in this study from both groups. These organisms are gram negative intracellular diplococci organisms that are sexually-transmitted causing infection in the genital tract which may disseminate to other organs [21].

The percentage of Gram positive isolates in oral combined contraceptive pills and injectable progesterone administered women was (31.56%). *Staphylococcus epidermidis* isolates had the highest percentage (14.03%) followed by *Staphylococcus aureus* (10.52%) and *Lactobacillus* (5.26%). *Streptococcus feacalis* represents the lowest percentage (1.75%). While in control group the highest percentage was *Lactobacillus* spp (93.3%). Hiller et al. [22] were studied the vaginal flora of some women and they found that the organisms commonly isolated in women with normal smears were lactobacilli, coagulase negative Staphylococci, *S. aureus*, diphtheroids, *Candida* and group B *Streptococci*.

The most bacterial agents causing vaginitis include *Staphylococcus aureus*, *Echerichia coli*, Group B *Streptococci*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Neisseria gonorrhoea* [23]. Other studies have proved that the rate of isolation of *Streptococcus agalactiae* from vaginal swabs ranged from 5-40% [24].

There is an inhibitory effect of *Lactobacillus reuteri* on some pathogenic bacteria that cause bacterial vaginosis, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Streptococcus agalactiae* [25]. No bacterial isolates obtained from control group.

**Table 1. Distribution of gram negative and gram positive isolates in both contraceptive groups**

Isolates	Groups	Combined oral contraceptive pills users		Injectable contraceptive users		Total number of isolates
		Total number of isolate	%	Total number of isolates	%	
<b>Gram negative isolates</b>						
<i>E. coli</i>		10	26.31%	6	31.57	16(28.07%)
<i>Enterobacter cloacae</i>		8	21.05%	3	15.78	11(19.29%)
<i>Klebsiella pneumonia</i>		7	18.42%	1	5.26	8(14.03%)
<i>Pseudomonasaeruginosa</i>		1	2.63%	1	5.26	2(3.50%)
<i>Neisseria gonorrhea</i>		1	2.63%	1	5.26	2(3.50%)
Total		27:38	71.04 %	12:19	63.13	39(68.39%)
<b>Gram positive isolates</b>						
<i>Staphylococcus aureus</i>		4	10.52%	2	10.52%	6(10.52%)
<i>Staphylococcus epidermidis</i>		4	10.52%	4	21.05%	8(14.03%)
<i>Streptococcus feacalis</i>		1	2.63%	0	0%	1(1.75%)
<i>Lactobacillus Spp.</i>		2	5.26%	1	5.26%	3(5.26%)
Total		11:38	28.93%	7:19	36.83%	8:57(31.56%)

**Table 2. Number and percentage of *lactobacillus* spp isolates in contraceptive and control groups**

Groups	<i>Lactobacillus</i> isolates		
	Total number	No. of isolates	Percentage
Combined oral contraceptive pills users	40	2	5%
Injectable contraceptives users	20	1	5%
Control	30	28	93.3 3%

### 3.2 Distribution of *Lactobacillus* Isolates among Study Groups

There is a significant difference in the number and percentage of *Lactobacillus spp* isolates between contraceptive and control groups. These results reflect the effect of contraceptives on vaginal *Lactobacilli*, since *Lactobacilli* were highly prevalent in control group (93.3%) while highly decreased in contraceptive groups (5%). These results are similar to other results obtained by other researchers who studied the effects of variety of factors on vaginal micro flora. Some researchers show that vaginal flora can be disrupted by a variety of factors including sexual intercourse, antimicrobial use and douching [26]. Long-term use of depot medroxy progesterone acetate results in endothelial dysfunction which may result from hypoestrogenism and serum estradiol was significantly reduced in users of depot medroxy progesterone acetate compared with controls [27]. Other study showed that hypoestrogenism state associated with decreased H<sub>2</sub>O<sub>2</sub>- positive *Lactobacillus* colonization and slight thinning of the glycogen vaginal epithelial layer in users of depot medroxy progesterone acetate. Such change may

compromise the vaginal barriers to infection [28]. *Lactobacilli* play important role in protection of the vagina from colonization by pathogens and this could be attributed to blocking the attachment of pathogens to the vaginal epithelium and production of substances that inhibit their multiplication [29].

### 3.3 Measurement of Immune Parameters

#### 3.3.1 Interferon gamma (IFN- $\gamma$ )

There is a significant difference between control and injectable contraceptive users (P value less than 0.05), also there is significant difference between control and oral combined contraceptive pills users (P value less than 0.05) Table 3.

This finding indicates that injectable and oral combined contraceptive pills uses may diminish the production of IFN-  $\gamma$ . These results are similar to Vicetti- Miguel et al. [30] who showed that contraceptive usage reduce the ability of cytotoxic T cell mainly CD<sub>8</sub> T cells for activation and secretion of cytokines especially IFN-  $\gamma$ . Gonadotropin releasing hormone and Hypothalamic-pituitary-gonadal activation is

involved in thymus maturation and exerts a potent immune-stimulatory effect leading to activation of T helper cells and increased levels of serum interferon-gamma(IFN- $\gamma$ ) [31]. Oral combined contraceptive administration lead to suppression of Hypothalamic-pituitary-gonadal axis [32] which leads to reduce activation of T helper cells and decrease serum level of IFN- $\gamma$ . Many studies have shown that bacterial endotoxin and exotoxin in vaginal infection of women without contraceptive are the cause of hyper production of pro- inflammatory cytokines [33]. Loncar [34] found that serum level of IFN- $\gamma$  was elevated in pelvic inflammatory disease and in vaginal bacterial infection of women without contraceptives. The results of this work exhibited a decreasing level in the concentration of IFN-  $\gamma$  in women with bacterial infection and using contraceptive drugs. This result may give an indicator for the inhibiting effects of these drugs on the production of TH1 cytokines which is supported by Koldehoff [35].

### 3.3.2 Interleukin -2 (IL-2)

There was non-significant increase in serum level of IL-2 in both oral and injectable contraceptive users groups compared to control group (p value less than 0.05) Table 4.

Antigens such as bacterial cells are internalized by endocytosis and processed by the antigen presenting cells then presented to immunocompetent lymphocytes to initiate the early steps of the immunological response. Processing by a macrophage for example results in attaching antigenic materials to the surface of the membrane in association with MHC II molecules on the surface of the cell. The antigen-class II MHC complex presented antigens to T-helper cells causing activation of these cells that begin to produce Interleukin 2 (IL-2) and to express a membrane receptor for IL-2. The secreted IL-2 autostimulates proliferation of the

TH2 cells. Stimulated TH2 cells produce a variety of lymphokines including IL-2, IL-4, IL-6 and gamma Interferon which mediate various aspects of the immune response [36]. Other study showed that serum progesterone levels did not correlate with the activation of cytokine-secreting cells [37]. The effects of synthetic hormones on IL-2 level were studied and the results were conflicting: Oral combined contraceptive use did not affects IL-2 level [38]. Injectable contraceptive of Depot Medroxy progesterone acetate levels did not correlate with the production of IL-2 [37].

### 3.3.3 Interleukin -8 (IL -8)

Serum level of IL-8 has a significantly decrease in injectable contraceptive users group compared to control group (P value less than 0.05) also IL-8 was significantly decrease in oral combined contraceptive pill users group compared to control group (P value less than 0.05) Table 5.

IL-8 produces by monocytes, neutrophils, endothelial cells, epithelial cells and fibroblasts [39]. IL-8 exerts several effects on neutrophils [40]. These effects include chemo-attraction, increase expression of adhesion molecules, modulation of trans endothelial cell migration and respiratory burst [41]. Progesterin inhibits the expression of IL-8 by human endometrium stromal cells which leads to virtual reduction of neutrophils after progesterone-dominated mid-luteal phase human endometrium [42].

Other study showed that medroxy-progesterone acetate and estradiol cause reduction in the secretion of IL-8 by human endometrium stromal cells monolayers [43]. Moreover, IL-8 production by endometrial stromal cells markedly decreases during progesterone-induced decidualization [44] also other study concluded that progestogens inhibit IL-8 and other chemokines [45].

**Table 3. Level of interferon -gamma in contraceptive and control groups**

Groups	Mean of (concentration) Pg/ml	(p) value
Oral combined contraceptive Pills users	112.2 pg/ml	P < 0.05
Injectable contraceptives users	116.8 pg/ml	P < 0.05
Control	215.6 pg/ml	

**Table 4. Level of interleukin -2 (pg/ml) in contraceptive and control groups**

Groups	Mea concentration Pg/ml	(p) value
Oral combined contraceptive Pills users	25.8 pg/ml	P = 0.05
Injectable contraceptives users	25.5 pg/ml	P > 0.05
Control	20.62 pg/ml	

**Table 5. Level of interleukin -8(pg/ml) in contraceptive and control groups**

Groups	Mean concentration Pg/ml	P value
Oral combined contraceptive Pills users	13.10 pg/ml	P < 0.05
Injectable contraceptive users	10.77 pg/ml	P < 0.05
Control	21.30 pg/ml	

#### 4. CONCLUSIONS

Hormonal contraceptives lead to inhibition of vaginal lactobacilli and decrease serum level of both IFN- $\gamma$  and IL-8 while IL-2 level not affected by hormonal contraceptives.

#### CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this research article.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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