Effect of different contraceptive methods on cervico-vaginal cytology

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Effect of Different Contraceptive Methods on Cervico-vaginal Cytology

Aliaa Mohamed Abd Elatif Mosallam, Nahed Ezzat Mahmoud, Naglaa Mohamed Moharam, Doaa Elsaid Abd Elaziz Salama

Abstract

Background: The link between hormonal contraception and the growth of several cancers of the reproductive system in women has yet to be established. This study's goal was to assess the impact of different contraceptive methods on the cervico-vaginal smear at family planning outpatient clinic at Al-Zahraa university hospital and Zefta general hospital.

Subjects and method: We conducted a case control study with 276 women who met the inclusion criteria, all participants undergo Pap smear and evaluated according to the Bethesda system. The first group consisted of 128 women who did not use contraception, while the second group was subdivided into 3 groups of women who used an IUD, 42 people who used a COC, and 41 people who used progesterone. The study was done in outpatient family planning clinics.

Results: statistically significant increase in ASCUS among COC users, significant increase in infection (NILM, NILM with metaplastic changes and NILM with reactive changes or clue cells) among non-contraception users.

Conclusion: Hormonal contraceptive usage has been linked to an elevated risk of dysplasia, which raises the chance of developing cervical cancer. Incidence of ASCUS is higher in hormonal contraceptive pills user than non-hormonal contraceptive users. Incidence of inflammatory changes increased among IUCD users.

Keywords: Cervico-vaginal cytology, Contraception, Contraceptive methods

1. Introduction

Invasive squamous cell carcinoma of the cervix is still the most common malignant tumor of the female genital tract in many nations.1 If cervical dysplasia is not treated, it may develop into cervical cancer, which is a pre-malignant disease. Cervical invasive carcinoma has been seen as a preventable disease due to its lengthy pre-invasive stage, the effectiveness of pre-invasive lesions as a target for therapy, and cervical cytology screening programs.2 Cytologic screening of cervical smears is the most efficient cervical malignancy screening test ever created.3 Due to advancements in screening and treatment, cervical cancer incidence and fatality rates have significantly declined in industrialized nations over the last several years.4 Infection with the human papillomavirus, smoking, the use of oral contraceptives, and the male factor (multiple relationships) have all been associated to cervical cancer in various studies.5,6 The oral contraceptive pill

Abbreviations: ASCUS, Atypical squamous cells of undetermined significance; CIN, Cervical intraepithelial neoplasia; COC, Combined oral contraceptive pills; DMPA, Depo medroxyprogesterone acetate; E6, E7, oncoproteins; HC2, Hybrid Capture 2; HIV, human immunodeficiency virus; HPV, Human papilloma virus; IARC, The International Agency for Research on Cancer; IUCD, Intra uterine contraceptive device; LSIL, low-grade squamous intraepithelial lesions; NILM, Negative for intraepithelial lesion or malignancy; OCP, oral contraceptive pills; PCR _ISH, polymerase chain reaction insitu hybridization; pop, progesterone only pills; WHO, World health organization.

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(OCP), followed by female sterilization, is the most utilized modern form of contraception worldwide. In this research, women who were referred to the family planning outpatient clinic at Al-Zahraa university hospital and Zefta general hospital were assessed for the incidence of abnormal Pap smears while using various contraceptives.

2. Methods

It was a control study case. To assess how various forms of contraception affect cervicovaginal cytology, which undertaken on females attending Al-Zahraa university hospital and Zefta general hospital outpatient clinics, due to our conservative community, so all women that under investigation Are not smokers, drinkers, or not sexually promiscuous.

2.1. Inclusion criteria

Women who came to the Obstetrics and Gynecology outpatient clinic with gynaecological complaints or for follow-up without any complaints were utilizing various contraception techniques.

2.2. Exclusion criteria

Insufficient Pap smear, pt presented with cervical polyp, antibiotics treated patients, a patient with uterine tumour, using medications that administrated intravaginally.

2.3. Sample size

PASS software was used to compute the minimal sample size (PASS 11 citation: Hintze, PASS 11. NCSS, LLC. Kaysville, Utah, USA). According to Garg et al., contraceptive users had a negatively passive for Malignancy (NILM) or Intraepithelial Lesion with metaplastic cells of 25% compared to 11% in the control group, setting alpha error at 5 percent and power at 80%. The minimum demanded sample size for the study is determined to be 256 participants (128 per group) with accounting for dropout rate.

2.4. Ethical and legal consideration

Clearance from the Department of Obst & Gynae, Faculty of Medicine, Al-Azhar University for girls to review the records was obtained.

2.5. Methodology

All participants were exposed to:

Detailed history that involved: Personal history as name, age, residency, education qualification, and working status, menstrual history, Regular or not, obstetric history, number of deliveries, and mode of delivery, if any contraception used or not, and duration of contraception use, past history and family history of malignancies, or benign breast disease, sexual history if dyspareunia was present or no, superficial or deep.

2.6. Study procedure

All women were divided into three groups: those who used hormonal contraception, those who did not use hormonal contraception, and those who did not use contraception at all. Every woman who matched the inclusion criteria and presented with gynecological issues or came for follow-up without any concerns attended an outpatient clinic and used various methods of contraception had one pap smear taken using sterile vaginal cuscus speculum to explore and fix cervix, inspection of the cervix was done and cleaned with sterile dressing then smear was taken using Ayres spatula imbedded into cervical os with smaller end at ectocervix while longer end at cervical canal with rotation of spatula with 360° to maintain contact with ectocervix. Using a spatula with both sides spread, the sample was applied uniformly on the slide and fixed right away. Fixation of smear was done using 95% alcohols. Our pathologist, Dr. (Doaa Elsaid), used the Bethesda technique to do a cytological analysis of a pap smear.

2.7. Study outcome

We investigated whether contraception method is implicated in development of cervical dysplasia in our population.

2.8. Data statistical analysis

To look at data that was supplied into the computer, we used IBM SPSS software package (version 20.0). (Armonk, New York: IBM Corporation) To describe qualitative data, numbers and percentages were used. To verify that the distribution was regular, the Kolmogorov-Smirnov test was used. The minimum and maximum ranges together with mean, and standard deviation are utilized to describe quantitative data. The importance of the obtained results was determined at a 5% level.

Chi-square test was used: To compare several groups for categorical variables. ANOVA test: it is used as comparison tool between groups under study for normally quantitative variables, while Kruskal-Wallis H test: is used for abnormally quantitative variables.
3. Results

Our Study included 276 participants, 128 with no contraception used, 65 used IUD, 42 used COC and 41 used Progesterone Only. As shown in Table 1, Fig. 1 there was no substantial statistics variations between groups under investigation as regard to age, residence, and education level, and occupation, duration of marriage, parity and mode of labor.

As shown in Table 2 there was increased incidence of irregular menstruation among progesterone only users 78% in 32 women. There was substantial improve in duration of contraception usage among IUD users 92.3% in 60 women. The mean of duration of IUCD usage was SD $+3.052$.

Also, Table 3 showed that non-contraception users have high incidence of superficial dyspareunia 14.1% in 18 women with no statistically significant differences.

Table 4, Fig. 2 show statistically significant increase in ASCUS among COC users, 8 women (19%) using COC had abnormal pap smear (ASCUS) which shows atypical squamous cells with increased N/C ratio and hyper chromatic nuclei (Fig. 3a and b). The mean duration of use was SD $+3.47$, among non-contraceptive group 3 women (2.3%) with abnormal pap smear (ASCUS), In IUCD users 2 women (3.1) had ASCUS, and 4 women (9.8%) had ASCUS in progesterone only users. We reported significant increase in infection smear (NILM 50% in non-contraceptive users, in IUCD users NILM was 32.1%, 9.5% in COC users, 41.5% in progesterone only. Also NILM with metaplastic changes was increased in COC users which shows squamous cells with spidery cytoplasmic process in 31% (Fig. 3c and d). And NILM with reactive changes shows cytoplasmic vacuolization, polychromasia, neutrophilic infiltrate and others with giant cells.

4. Discussion

Cervix carcinoma is a dangerous kind of cancer that is the second most frequent form of cancer in developing nations and the fourth leading cause of death in women globally. Despite the low prevalence of cervical cancer in Egypt, it is nonetheless regarded as the primary cause of death (100%) due to late detection. Smoking and oral hormonal contraception could play a role in global cancer burden variations, Also, sexually transmitted diseases (HIV and Chlamydia trachomatis).

In an analytical study that conducted in Egypt, researchers make a comparison of HPV detection by PCR-ISH tissue and other conventional approaches such as available histopathology, colposcopy and cytology. They decided to know which of these approaches are efficient in a developing country such as Egypt for HPV detection. They found that

![Table 1. Demographic data of studied groups.](image)

<table>
<thead>
<tr>
<th>No Contraception</th>
<th>Using contraception</th>
<th>Progesterone Only</th>
</tr>
</thead>
<tbody>
<tr>
<td>IUD (n = 65)</td>
<td>COC (n = 42)</td>
<td>Progesterone Only (n = 41)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>22–48</td>
<td>18–47</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>33.64 ± 6.919</td>
<td>32.97 ± 6.080</td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>94 (73.4)</td>
<td>48 (73.8)</td>
</tr>
<tr>
<td>Urban</td>
<td>34 (26.6)</td>
<td>17 (26.2)</td>
</tr>
<tr>
<td>Marital Status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>94 (73.4)</td>
<td>63 (96.9)</td>
</tr>
<tr>
<td>Not married</td>
<td>34 (26.6)</td>
<td>2 (3.1)</td>
</tr>
<tr>
<td>Duration of Marriage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>1–28</td>
<td>2–25</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>10.52 ± 6.535</td>
<td>10.34 ± 5.751</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min.-Max.</td>
<td>1–6</td>
<td>1–5</td>
</tr>
<tr>
<td>Mean ± S.D</td>
<td>2.46 ± 1.304</td>
<td>2.65 ± 1.007</td>
</tr>
<tr>
<td>Mode of labor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NVD</td>
<td>85 (66.4)</td>
<td>37 (56.9)</td>
</tr>
<tr>
<td>CS</td>
<td>43 (33.6)</td>
<td>28 (43.1)</td>
</tr>
</tbody>
</table>

$X^2$: Chi-square test.
H: Kruskal-Wallis test.
P: P value for comparing between the studied groups.

a Statistically significant at $P < 0.05$. 
conventional cytology and histopathological investigation were more sensitive than PCR-ISH tissue with positive predictive value 100% and 66.7% as negative predictive value together with reported sensitivity 87.5%, the specificity 78.6%.

We conducted a cross section study in our hospital to assess the possible association between various contraceptive methods and cervicovaginal smear abnormalities.

In the current study Ascus was believed to be significantly in combined contraceptive pills users (19%) followed by progesterone only pills users (9.8%) and the duration of use of combined pills was 4 years. Cytological examinations revealed that ASCUS (A typical squamous cells of undetermined significance) and LSIL (low-grade squamous intraepithelial lesions) are minor lesions at cervical epithelium detectable. Although tis abnormality considered non precancerous but it carries the risk. Arbyn et al., supported our finding that one third of CIN was discovered via follow-up with ASCUS of a previous smear, So that they recommend for Hybrid Capture 2 (HC2) to triage women with ASCUS.

Duggan et al., reported that the use of hormonal contraception has little influence on the occurrence and persistence of HPV infection, and thus cervical dysplasia.

The relationship between abnormal cervix cancer and hormonal contraception is mainly related to type of hormones used or to duration of exposure or both. IARC (International Agency for Research on Cancer)

![Fig. 1. Comparison between groups as regard to patient's marital status.](image)

Table 2. Menstrual regularity and duration of contraception among studied groups.

<table>
<thead>
<tr>
<th>Rhythm</th>
<th>No Contraception (n = 128) Number (%)</th>
<th>Using Contraception</th>
<th>Test of Sig.</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IUD (n = 65)</td>
<td>COC (n = 42)</td>
<td>Progesterone Only (n = 41)</td>
</tr>
<tr>
<td>Irregular</td>
<td>31 (24.2)</td>
<td>5 (7.7)</td>
<td>1 (2.4)</td>
<td>32 (78.0)</td>
</tr>
<tr>
<td>Regular</td>
<td>97 (75.8)</td>
<td>60 (92.3)</td>
<td>41 (97.6)</td>
<td>9 (22.0)</td>
</tr>
</tbody>
</table>

Duration of Contraception Use

<table>
<thead>
<tr>
<th>Min.-Max.</th>
<th>Mean ± S.D</th>
<th>P₁</th>
<th>P₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months-15 years</td>
<td>4.22 ± 3.052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 months-13 years</td>
<td>3.98 ± 3.247</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 months-8 years</td>
<td>1.82 ± 1.643</td>
<td>&lt;0.001*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

X²: Chi-square test.
H: Kruskal-Wallis test.
P: P value for comparing between the studied groups.
P₁: P value for comparing between IUD group and each of COC group and Progesterone Only group.
P₂: P value for comparing between COC group and Progesterone Only group.

* Statistically significant at P < 0.05.
pooled data from invasive cancer (8 cases) and HPV positive cases (2 of carcinoma in situ) that demonstrated positive association between parity, duration of exposure to hormonal contraception and cervical dysplasia. The cases revealed that no raised risk at women with using hormonal contraception for less than five years however, obvious risk is appeared after ten years.16 While the results from Steroid Contraceptives and WHO collaborative Study of Neoplasia revealed there was a slight risk even for ever-users with duration of less than four years.17 In agreement with our reported results, Mahgoub et al.,18 use of hormonal contraception has been related to an increased risk of developing dysplastic alterations in the cervical exfoliated cells leading to cervical cancer. Given that HPV infection is now well acknowledged as a key factor in cervical cancer development. Because estrogen has trophic effects, continuous exposure to higher-than-normal levels may result in this growth. Studies have shown that estrogen binds to particular DNA sequences within transcriptional regulating domains on HPV DNA and that women with greater estrogen receptor transcript values are much more susceptible to cervical HPV infection.19 The results showed that expression of E7 proteins and HPV-18 E6 directly with the estrogen receptor increased and HPV uses to bind to and deactivates the p53 tumor suppressor gene, resulting in apoptotic abnormalities and carcinogenesis.20 Cervical ectopy, which is common in women using hormonal contraception, may also make them more susceptible to HPV infection. These findings point to type-specificity and provide a biological explanation for HPV-16, 18’s higher detection and its greater oncogenic potential.21 Other biological factors, in addition to increased viral replication, may be significant. For example, sex steroid hormones substantially alter immune function to HPV-16 virus-like particles, raising the possibility that hormonal contraception may impact the host’s susceptibility to infection with HPV-16.19 The fact that there is a link between combined pills and DMPA hormonal contraception, which is frequently associated with poorer usage of barrier protection during sexual contact (exposure to other pathogens), as well as direct impacts of the combined pills, could explain the associations, and could be another contributing factor.

In the current research, pap smear results revealed that there were substantially more non-contraceptive users who had NILMs (Negative for Intraepithelial Lesion or Malignancy), which is different from NILM with Metaplastic Changes or Presence of Reactive or Clue Cells. These results, which are unrelated to cervical cancer, are thought to be symptoms of infection with yeast, herpes, or Trichomonas vaginalis. Among the current investigation, the cytological alterations caused by infection were more frequent in IUD users. The present study results demonstrate that combined pill users and progesterone only contraception users showed least rate of infection cytology this was attributable to the progesterone action, more susceptible to HPV infection. These findings point to type-specificity and provide a biological explanation for HPV-16, 18’s higher detection and its greater oncogenic potential.21

Table 3. Patients complain among studied groups.

<table>
<thead>
<tr>
<th>Dyspareunia</th>
<th>No Contraception (n = 128) Number (%)</th>
<th>Using Contraception</th>
<th>Progesterone Only (n = 41) Number (%)</th>
<th>X²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IUD (n = 65) Number (%)</td>
<td>COC (n = 42) Number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>110 (85.9) 52 (80.0) 33 (78.6) 32 (78.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (14.1) 13 (20.0) 9 (21.4) 9 (22.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep</td>
<td>5 (3.9) 4 (6.2) 2 (4.8) 0 (0)</td>
<td></td>
<td></td>
<td>2.302</td>
<td>0.512</td>
</tr>
<tr>
<td>Superficial</td>
<td>13 (10.2) 9 (13.8) 7 (16.7) 9 (22.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>128 (100) 65 (100) 42 (100) 41 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. PAP smear results among studied groups.

<table>
<thead>
<tr>
<th>Histopathological Changes</th>
<th>No Contraception (n = 128) Number (%)</th>
<th>Using Contraception</th>
<th>Progesterone Only (n = 41) Number (%)</th>
<th>X²</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IUD (n = 65) Number (%)</td>
<td>COC (n = 42) Number (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASCUS</td>
<td>3 (2.3) 2 (3.1) 8 (19.0) 4 (9.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLM</td>
<td>64 (50.0) 15 (23.1) 4 (9.5) 17 (41.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NLM with metaplastic</td>
<td>19 (14.8) 15 (23.1) 13 (31.0) 7 (17.1)</td>
<td></td>
<td></td>
<td>43.563</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>changes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NILM with reactive</td>
<td>42 (32.8) 33 (50.8) 17 (40.5) 13 (31.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or clue cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>128 (100) 65 (100) 42 (100) 41 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X²: Chi Square Test of significant.
P: P value for comparing between the studied groups.

Statistically significant at P < 0.05.
which thickens cervical mucus and inhibits bacteria penetration, preventing bacteria colonization by shortening menstrual periods.

In line with our result, Tekin et al.²² claimed that IUDs were linked to infections due to changes in the components of the cervical mucus’ glycosaminoglycans, which protect the female genital system from infection. To colonise the IUD, bacteria produce biofilms, which are made up of layers of host cells and bacteria coated in a matrix made up of an

![Fig. 2. Comparison between groups as regard to patient's Histopathological Changes.](image)

![Fig. 3. (a): A case of ASCUS shows atypical squamous cells with increased N/C ratio and hyperchromatic nuclei (arrow) (x400). (b): A Case of NILM with metaplastic changes shows squamous cells with spidery cytoplasmic process (arrow) (x400). (c): A case of NILM with Reactive changes shows cytoplasmic vacuolization, polychromasia, neutrophilic infiltrate (arrow) (x400). (d): A case of NILM with Clue cells shows squamous cells covered with rod shaped micro-organisms (arrow) indicative for bacterial vaginosis (x400).](image)
exopoly-saccharide layer that helps to characterise the infection. Antimicrobial resistance is common among biofilm bacteria, making treatment of infected women more challenging.

In agreement with the present study da Silva et al., Findik et al. found that IUD users had considerably more benign epithelial alterations than non-users, which is consistent with the current study. In addition, as compared to women who took oral contraceptives, they had a considerably greater frequency of bacterial vaginosis in cytological investigations and reported a higher frequency of bacterial vaginosis, Candida spp., and Actinomyces spp.

However, Discacciati et al. found no significant association in identifying inflammatory and cytopathological abnormalities in cervical smears in IUD users versus women who used another type of contraceptive method.

Our study strengths, included homogeneity of our study sample as it recruited from one hospital all patient of nearly same socioeconomic and educational levels. In addition, the pathologist in our study was only one to minimize inter-variability and human error.

The limitations confronted in this study originated from the Pap smear procedure itself as it has false-negative rate 20–30% according to June et al. When cells are not distributed consistently or evenly on the slide, or when contaminants like bacteria or yeast inhibit the identification of aberrant cells in the specimens, false-negative findings may ensue. Having insufficient resources is still another problem.

Given the widespread use of hormonal contraception, which is actually encouraged in our population, it is important that such women be advised and encouraged to have frequent Pap tests to improve early detection.

4.1. Conclusion

The use of hormonal contraception has been related to an increased risk of developing dysplasia, which in turn increases the risk of cervical cancer. Incidence of Ascus is higher in hormonal contraceptive pills user than non-hormonal contraceptive users. Incidence of inflammatory changes increased among IUCD users.

4.2. Recommendation

Following a thorough medical history and clear instructions on when to stop using it, a competent
doctor or midwife should prescribe hormonal contraception. Women on birth control pills are advised to get regular Pap tests.

**Ethics approval**

Al-Azhar University’s department of obstetrics and gynecology gave its consent for the examination of the records (Ethical approval number:……………). All of the women who signed up for the research provided their informed permission. Women were recognized by a serial number to maintain secrecy, and the details at the individual level were kept completely private.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The relevant Author will provide the datasets utilized and/or analyzed during the present work upon reasonable request.

**Authors and contributions**

AMM was responsible for data collection, methodology, project administration, NMA was responsible for supervision, visualization, and review & editing. NMM helped supervision, reviewing, and editing. DEA in was responsible for, formal analysis, and investigation, methodology.

**Disclosure**

The authors have no financial interest to declare in relation to the content of this article.

**Authorship**

All authors have a substantial contribution to the article.

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**Conflicts of interest**

None. No authors have disclosed any competing interests. ‘No direct or indirect participant involved in the topic of this document has received or will receive any money or non-financial advantages’.

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