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Cytomorphological Changes in Oral Mucosa among Female Users of Oral Contraceptive Pills in Shendi, Sudan

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Abstract

Background: *Oral contraceptives are widely used among women of reproductive age and may influence the cytomorphology of oral mucosal cells. However, limited data exist on their potential cytological effects.*

Objectives: *This study aimed to assess the cytomorphological changes in buccal mucosa among Sudanese females using oral contraceptives in Shendi Town.*

Methods: *A descriptive cross-sectional study was conducted from December 2022 to February 2024, including 100 women (60 oral contraceptive users and 40 non-users as controls). Buccal smears were collected and stained using the Papanicolaou method. Cytological changes including nuclear atypia, inflammation, keratosis, micronuclei, and maturation index were evaluated. Data were analyzed using SPSS version 28.*

Results: *The majority of users (83%) were taking combined estrogen-progestin pills, while 17% were using progestin-only pills. Most participants had been on contraceptives for 1–2 years. Cytological analysis revealed that keratosis was significantly more frequent in the study group compared to controls ($p = 0.001$). However, no significant differences were observed between users and non-users in nuclear atypia ($p = 0.743$), inflammation ($p = 0.743$), infection ($p = 0.368$), or micronuclei formation ($p = 0.583$). The maturation index also showed no significant association with age, duration of use, menstrual cycle regularity, or contraceptive type.*

Conclusion: *Oral contraceptive use was associated with an increased occurrence of keratosis in buccal mucosa, while no significant cytomorphological changes were observed in nuclear atypia, inflammation, infection, or maturation index. Further large-scale and longitudinal studies are recommended to clarify the long-term effects of hormonal contraceptives on oral epithelial health.*

Keywords: Cytomorphological, Contraceptive Pills, Female, Oral Cytology.

Introduction

Currently, interference with ovulation is the most common pharmacologic method for the prevention of pregnancy [1]. Major classes of contraceptives include combination oral contraceptives, transdermal patches, vaginal rings, progestin-only pills, and injectable progestins. Combination oral contraceptives (COCs) contain both an estrogen and a progestin and represent the most commonly used oral contraceptive type. The transder-

mal patch is an alternative to COCs and delivers ethinyl estradiol and the progestin norelgestromin. Another option is the vaginal ring, which contains ethinyl estradiol and etonogestrel, is inserted into the vagina, remains in place for three weeks, and is then removed. Progestin-only pills, commonly containing norethindrone ("mini-pill"), are taken daily on a continuous schedule, while injectable progestins, such as medroxyprogesterone acetate, are administered intramuscularly or subcutaneously every three months [1]. The relative influence of estrogen and progesterone on oral mucosal development has been previously investigated. Increased estrogen levels are associated with enhanced maturation and keratinization of the oral mucosa, whereas higher progesterone levels are linked to delayed maturation. These relationships were statistically significant at the 0.05 level [1]. Specifically, high estrogen levels were associated with an increased number of superficial and keratinized epithelial cells, while elevated progesterone levels correlated with a higher proportion of intermediate epithelial cells and a decrease in keratinized cells [2]. Oral contraceptive pills have been used as a method of contraception for several decades, with millions of women worldwide relying on them over the last sixty years. The combined form of oral contraceptives contains both a progestogen and an estrogen [3]. In the United States, most oral contraceptives contain a low-dose combination of ethinyl estradiol and a progestogen, which may be of the norethindrone type (first generation), norgestrel type (second generation), or desogestrel or norgestimate (third generation). Progestogen doses in these combination oral contraceptives vary across products, ranging from 0.1 to 1.5 mg [4]. Oral contraceptive agents are among the most commonly used medications globally, with approximately 50 million women using them worldwide. Due to such widespread use, multiple systemic and oral side effects have been reported. However, there is a lack of studies assessing cytomorphological changes in the oral mucosa associated with contraceptive use. Therefore, the present study aims to evaluate the cytomorphological effects of oral contraceptives on the oral mucosa.

Methodology

This descriptive cross-sectional study was conducted in Shendi Town, located in northern Sudan on the southeastern bank of the Nile River, approximately 150 km northeast of Khartoum. Geographically, the town lies at a latitude of 16°41′N and longitude 33°26′E, covering a total area of 14,596 km². A hot desert climate characterizes Shendi, located approximately 45 kilometers southwest of the ancient city of Meroe, which was historically recognized as a significant trading center. The study was conducted over 14 months, from December 2022 to February 2024. The study population consisted of women residing in Shendi Town who were using oral contraceptives. Samples were collected from the buccal mucosa of participants and transferred to the Histopathology and Cytology Laboratory at Shendi University, where they were processed and examined using standard cytological techniques. Inclusion criteria were restricted to women currently using oral contraceptives, while those on other medications, smokers, or alcohol users were excluded to minimize confounding factors.

Study Sample and Data Collection

Buccal smear samples were collected from each participant to assess oral cytomorphological patterns. A total of 100 participants were included, comprising 60 women using oral contraceptives and 40 women not using contraceptives. Primary data were collected through a structured questionnaire, while laboratory analyses were performed to detect cytomorphological changes. Cytological changes were identified using the Papanicolaou (Pap) staining method. Other analytical and sociodemographic data were analyzed using appropriate statistical methods.

Sample Collection and Fixation

Each participant was asked to rinse her mouth to reduce contamination, after which buccal mucosa cells were gently scraped using a sterile disposable spatula. The collected material was immediately smeared onto frosted-end labeled microscopic glass slides, air-dried, and fixed in 95% ethanol for at least 15 minutes to preserve cellular morphology.

Papanicolaou Staining Procedure

Fixed smears were rehydrated sequentially in 90% ethanol, 70% ethanol, and distilled water for two minutes each. The slides were then stained using the Papanicolaou method, beginning with Harris's hematoxylin for three minutes, followed by differentiation in 1% acid alcohol and bluing under running tap water. Smears were subsequently stained with Orange G6 for two minutes, rinsed in 95% ethanol, and then stained with Eosin Azure 50 for three minutes. The slides were dehydrated in absolute ethanol, cleared in xylene, and mounted using Disterene A Plasticizer and xylene. All smears were examined under a light microscope by the researchers and independently confirmed by experienced cytologists.

Interpretation of Results

Cytological changes were interpreted based on the presence of primary criteria of malignancy, including irregular chromatin patterns, unequal chromatin strands, and condensation of large chromatin clumps along the nuclear border with empty centers, indicating cancer cells. Dyskaryotic cells were identified by malignant chromatin with normal cytoplasmic volume. Secondary criteria of malignancy were used to detect cellular atypia, characterized by hyperchromasia, increased chromatin content, enlarged cells and nuclei, multinucleation, irregular nuclear borders, mitotic figures, and abnormal or multiple nucleoli. Other cellular alterations included metaplastic cells, keratosis such as parakeratosis and hyperkeratosis, acute inflammation indicated by neutrophilia, and chronic inflammation evidenced by lymphocytosis and macrophage infiltration. The maturation index was calculated as the percentage of parabasal, intermediate, and superficial cells among 100 observed cells, with superficial cells appearing polygonal with pyknotic nuclei regardless of cytoplasmic staining, intermediate cells being polygonal with open nuclei, and parabasal cells being round or oval with abundant basophilic cytoplasm and round or oval nuclei.

Quality Control

To ensure quality, sterile disposable spatulas were used for sample collection and smears were immediately fixed to prevent air-drying artifacts. All staining solutions were filtered prior to use, and dishes and Coplin jars were washed before and after use. The quality of staining solutions was checked before application, and all containers were tightly closed during procedures to avoid evaporation and contamination. Additional precautions were taken during mounting and coverslipping to maintain the

integrity of the samples.

Data Analysis

After examination of the sections, the results of the laboratory investigation, as well as the demographic data from the patient's records, were processed using the Statistical Packages for Social Sciences (SPSS) computer program. Frequency, mean, and chi-square test values were calculated at <0.05 and considered statistically significant.

Results

A total of 100 women participated in this study, including 60 oral contraceptive users and 40 non-users serving as controls (Table 1). The participants' ages ranged from 18 to 50 years, with most between 29 and 38 years. About 85% of participants reported having a regular menstrual cycle. Regarding contraceptive type, 83% (n = 50) of users were on combined estrogen–progestin pills, while 17% (n = 10) used progestin-only pills. The majority (58%) had been taking oral contraceptives for 1–2 years. Cytological examination revealed nuclear inflammation in 9 participants and nuclear atypia in 10, with no statistically significant differences between users and controls (p = 0.743 for both). Similarly, infection and micronuclei were not significantly associated with contraceptive use (p = 0.368 and p = 0.583, respectively). In contrast, keratosis was observed in 16 participants, mostly among contraceptive users, and this association was statistically significant (p = 0.001) (Table 6). Analysis of the maturation index by age, duration of contraceptive use, menstrual cycle regularity, and contraceptive type showed no significant differences between groups (Tables 2–5). For instance, the mean proportion of intermediate cells did not significantly vary with age group (p = 0.923) or length of use (p = 0.923). Likewise, the percentage of superficial cells showed no significant differences across age groups (p = 0.210), menstrual cycle regularity (p = 0.618), or contraceptive type (p = 0.553). Overall, while most cytomorphological parameters were similar between contraceptive users and non-users, keratosis was notably more common among oral contraceptive users (p = 0.001), indicating a possible localized epithelial effect.

Table 1: Distribution of Study Participants

Group	Number
Cases (users)	60
Controls (non-users)	40
Total	100

Table 2: Comparison Between Age and Maturation Index

Maturation Index	Age group (years)	No	Mean	Std. Deviation	P-value
Intermediate	18–28	13	7.92	3.013	0.923
	29–38	22	7.50	3.876	
	39–50	25	7.92	4.349	
Superficial	18–28	13	87.92	15.003	0.210
	29–38	22	92.50	3.876	
	39–50	25	92.08	4.349	

Table 3: Comparison Between Duration and Maturation Index

Maturation Index	Duration of use (years)	No	Mean	Std. Deviation	P-value
Intermediate	< 1–2	46	7.70	3.876	0.923
	3–4	10	7.92	4.349	
	> 4	4	7.92	4.349	
Superficial	< 1–2	46	91.50	8.376	0.210
	3–4	10	92.50	4.187	
	> 4	4	92.08	4.349	

Table 4: Comparison Between Menstrual Cycle Regularity and Maturation Index

Maturation Index	Menstrual cycle	No	Mean	Std. Deviation	P-value
Intermediate	Regular	51	7.82	3.846	0.789
	Irregular	9	7.44	4.187	
Superficial	Regular	51	91.12	8.378	0.618
	Irregular	9	92.56	4.187	

Table 5: Comparison Between Types of Contraceptives and Maturation Index

Maturation Index	Type of contraceptive	N	Mean	Std. Deviation	P-value
Intermediate	Combination	50	7.86	3.949	0.679
	Progesterone-only	10	7.30	3.561	
Superficial	Combination	50	91.06	8.486	0.553
	Progesterone-only	10	92.70	3.561	

Table 6: Association Between Cytological Findings and Contraceptive Use

Parameter	Present (cases)	Present (controls)	P-value
Nuclear atypia	1	4	0.738
Inflammation	1	3	0.555
Infection	0	3	0.368
Keratosis	12	1	0.001
Micronuclei	0	2	0.583

Discussion

Hormonal contraceptives exert systemic and local effects due to fluctuations in estrogen and progesterone, which influence the physiology and pathology of multiple tissues, including the oral mucosa. The oral cavity has long been recognized as a sensitive site for detecting systemic hormonal changes, as reflected in variations during puberty, pregnancy, menstruation, and menopause [5, 6]. In the present study, keratosis was found to be significantly more common among oral contraceptive users compared to non-users ($p = 0.001$). This suggests a possible chronic inflammatory response, which is consistent with the findings of Edgar et al. (2017), who reported that hormonal contraceptive use can be associated with inflammatory changes in oral tissues [7]. However, no significant differences were observed between contraceptive users and controls with respect to nuclear atypia, inflammation, infection, or micronuclei. These findings are in agreement with Osman et al. (2020), who concluded that hormonal contraceptives did not induce marked cytomorphological alterations in buccal mucosa [8]. On the other hand, our results contradict those of Chretien et al. (1998), who reported increased nuclear-to-cytoplasmic ratios in buccal epithelial cells of women on oral contraceptives, indicating cytomorphometric change [9]. Furthermore, the maturation index did not show significant differences when analyzed against variables such as age, duration of contraceptive use, menstrual cycle regularity, or contraceptive type. This observation supports previous work by Cowpe & Semmens (1985), who suggested that buccal squames are not markedly affected by hormonal fluctuations during the menstrual cycle [10]. Overall, the present findings highlight keratosis as the most notable cytological change in contraceptive users, while other parameters remained unaffected. The discrepancies between our results and those of previous studies may be attributed to differences in study design, sample size, population characteristics, and type or duration of contraceptive use.

Study Limitations

The main limitations of this study include its relatively small sample size, cross-sectional design that prevents establishing causality, and restriction to a single geographic area, which may limit generalizability. In addition, the use of only buccal smear cytology without molecular or immunological analyses may not fully capture subtle cellular alterations, and potential confounding factors such as nutrition, systemic diseases, and medication use were not fully controlled.

Conclusion

In conclusion, while most cytomorphological parameters were not significantly affected, the consistent finding of keratosis highlights a potential localized epithelial response to hormonal contraceptives. Routine oral cytology may help in early detection of epithelial changes in long-term users.

Recommendation

This study recommends increasing awareness among women regarding the potential oral effects of long-term hormonal contraceptive use and encouraging regular oral cytological screening for early detection of epithelial changes. Future research with larger and more diverse populations using standardized methods is needed to confirm these findings and to explore the biological mechanisms underlying keratinization in contraceptive users.

Consent

The patient's written consent has been collected.

Ethical Approval

The study was approved by the Department of Histopathology and Cytology in Medical Laboratory Sciences at Shendi University, and the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the area of study. The aims and the benefits of this study were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

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Conflict of Interest

The authors have declared that no competing interests exist.

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