Alterations Caused by Hormonal Contraceptives on Cytomorphometric of Buccal Mucosa Detected by Computer-Assisted Method

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ABSTRACT

Background: Hormonal contraceptives (HCs) use has become the most important born control method. Several different previous studies have resulted in the effects of contraceptives on the mucosa, but quantitative parameters such as nuclear diameter (ND), cell diameter (CD), and the Nucleus-to-cytoplasm ratio (N/C) have become significant in oral diagnosing methods. **Aim:** This study was conducted to assess quantitative change in CD, ND, and N/C from the squamous obtained from the mucosa and also to evaluate the impact of contraceptives on cytomorphomeric measurement **Methods:** The study group consisted of twenty-five contraceptive cases and twenty-five apparently healthy controls. Abrasion of the mucosa and ND and CD were measured using the Optika camera microscope. **Results:** Age groups, headache feeling, and depression symptoms are significantly correlated with the type of contraceptives used ($P \geq 0.001$). Correlation between type of hormonal contraceptives used, hair loss, and weight change exhibited significant results ($P \geq 0.05$), however, a highly significant correlation was observed between the cytomorphometrics of superficial and intermediate cells, when the case and the control samples were compared ($P \geq 0.001$). **Conclusion:** The apparent rise in cell diameter, an increase in ND in contraceptives, and a rise in CD suggested that HCs cause significant changes in oral exfoliation cells and the quantitative measurement of smears is useful in detecting changes to contraceptives and certainly for many other health problems.

Key Words: *Cytomorphometrics, Buccal mucosa, Hormonal contraceptives.*

INTRODUCTION

Hormonal contraceptive (HC) is used to suppress ovulation. It includes birth control pills (oral contraceptives), and injections of Depo Provera and Norplant [1]. There are three types of oral contraceptive pills (OCPs): combination estrogen-progesterone, progesterone alone, and continuous or prolonged use of the pill. The birth control pill is the most widely used form of contraception in the United States. Approximately 25% of women aged 15-44 who are currently using contraception reported using a pill as their preferred option. The most widely prescribed drug is a combination of a hormonal drug with estrogen and progesterone. Progesterone is a hormone that prevents pregnancy, and the estrogen part regulates menstrual bleeding [2]. Birth control pills are used mainly to avoid pregnancy. The efficacy of this type of birth control is related to the standard and ideal usage. Typical usage, meaning that the procedure will not always be used regularly or correctly, results in an error rate of 9

out of 100 women who become pregnant within the first year of use. Due to negligence, the rate of failure reported for combined oral contraceptive pills is a normal 9% use. OCP can be used to manage other health conditions, especially menstrual-related disorders such as menstrual pain, irregular menstruation, fibroids, endometriosis-related pain, and menstrual-related migraines [2]. Depo-Provera CI is a birth control hormone progestin that is administered by injection to prevent pregnancy [3]. The possibility of getting pregnant depending on how well you follow the instructions to take your Depo-Provera CI [3]. Contraceptive implant is a thin, elastic rod implanted under the skin in the upper arm of a woman, which releases the form of a progesterone hormone. The hormone prevents the ovaries from releasing the egg and thickens the cervical mucus, making it impossible for sperm to reach the uterus. The implant involves a small operation using a local anesthetic to suit and remove the rod and must be replaced after three years [4]. Qualitative analysis and quantitative cytomorphometric evaluation of exfoliated buccal cells showed measurable improvements in cells [5]. Morphometry could be selectively applied to specimens that are difficult to measure reliably, such as cell and nuclear dimensions [6].

Cytomorphometry is a computer-assisted technique for cell analysis. Computer analysis of the digital microscopic image of the collected cells, using a neural network-based image processing method [7]. The aim of the current study was to evaluate alterations caused by HCs on cytomorphometric of the buccal mucosa.

MATERIALS AND METHODS

The current study is a retrospective analytical case-control, which was carried out to evaluate cytomprhological changes in buccal smears obtained from Sudanese contraceptive users who visited Khartoum state private clinic seeking medical advice and comparing their results with healthy individuals. Fifty Sudanese individuals of both contraceptive and non-contraceptive users were the main targets for this study. Participants were selected by stratified randomized sample size.

Inclusion criteria
All slides from the contraceptive users that showed good quality and clear stains without background stain were included in this study.

Exclusion criteria
Any individual with a history of any acute or chronic illness, visible oral lesions, smokers, alcoholics, and tobacco users were excluded from the study.
For obtaining good results, we counted three fields from which individual cells that showed a good nuclear

topographic area and outstanding delineation were selected; Moreover, separate cells apart from overlapped rejoin were used as a target for an optimal enumeration and measurement.

Sampling
Smears were collected with a wooden spatula and instantly fixed in 95% ethyl alcohol for 30 minutes to maintain adequate fixation. The smears were then stained with the prepared Papanicolaou (PAP) stain.

Papanicolaou staining (Pap stain)
Using tongue depressors, doing steady pressure, the cells were scraped out of the clinically normal buccal mucosa. Scrapping was applied to clean labeled slides. Slides were fixed in 95% alcohol for 30 minutes. Air drying of the smears was prevented as it contributed to changes in cellular morphology, smear was stained using traditional pap staining. The technique is done through hydration of the smear in different concentrations of alcohol, 70% ethanol for 1 minute, 50% alcohol for 3 minutes, and distilled water for 1minute. Then the nuclear stain Harri's hematoxylin was applied for 5 minutes, bluing for 5 minutes, counter stain with OG6 (orange G6) for 2 minutes, differentiated with 95% alcohol, followed by cytoplasmic stain with EA50 (eosin azure 50) for 2 minutes, differentiated with 95% alcohol just rinse, finally dehydrate, clear and mounting. The Papanicolaou stained cells were examined under x40. The samples were taken stepwise, shifting the slide from the upper left corner to the right and then down to prevent measuring the same cell. Cells were classified on the basis of morphology and staining characteristics. All quality control measures were adopted during specimens' collection and processing. All stained smears showed fair quality.

Cytomorphometric Analysis
In each smear, six cells were selected by moving the microscope stage in a "Z" shape to avoid recounting of the same cell. Optika camera software program user interface, nuclear, cellular, and N/C diameters were obtained.

Ethical consideration
This research was approved by the ethics board of the Karary University – Faculty of medical laboratory science. Verbal consents have been obtained from the participants after a good discussion with them and have been told that the results of the study would be utilized for their benefits.

Data analysis
Data were analyzed using the statistical package of social science (SPSS) version 20. Nuclear, cellular, and nuclear-cytoplasmic ratio diameters (ND, CD, and N/C) were

measured on PAP stained smears using Optika digital microscope camera (Optika) digital innovation.

RESULTS

This study included 50 samples, 25 apparently healthy females were used as control and 25 were hormonal contraceptive users considered as cases. When age groups, headache feeling, and depression symptoms are correlated with the type of contraceptives used, there was a highly significant relationship (P≥0.001) as shown in Tables 1, 6, and 7 respectively.

Correlation between the type of hormonal contraceptives used, hair loss, and weight change exhibited a significant result (P≥0.05) as presented in Tables 2 and 4, while no significant correlation between types of contraceptives used and the duration of using (Table 3) and appearance of acne (Table 5).

Table 8 illustrated a highly significant correlation between cytomorphometrics of superficial and intermediate cells when the case and control samples were compared (P≥0.001).

OPTIKA Software was used to calculate the cells ND, CD, and N/D ratio and morphology of buccal mucosal cells presented in figures 1-4. The cells in the control group were primarily of a superficial and intermediate type. The cells were typically described and the nucleus was round to oval with a smooth peripheral skeleton. The nuclear manner was found to be homogeneous.

Table 1: The correlation between age categories and type of hormonal contraceptives

Type of contraceptives	Case n=25 (%)	Age groups/year			P-value
		15-25	26-35	36-45	
Oral	16 (64%)				
Injection	3(12%)	14(56%)	8(32%)	3(12%)	0.000**
Implant	6(24%)				

** P≥0.001 highly significant

Table 2: The relationship between hormonal contraceptives and hair loss

Type of contraceptives	Case n=25 (%)	Hair loss		P-value
		No effect	Hair loss	
Oral	16 (64%)			
Injection	3(12%)	5(20%)	20(80%)	0.003*
Implant	6(24%)			

* P≥0.05 is significant

Table 3: illustrates the correlation between of types and duration of hormonal contraceptives

Type of contraceptives	Case n=25 (%)	Duration of contraceptives/ month			P-value
		1-12	13-24	25-36	
Oral	16 (64%)				
Injection	3(12%)	7(28%)	11(44%)	7(28%)	0.130NS
Implant	6(24%)				

NS: Non-significant

Table 4: The relationship between changes in weight and type of contraceptives

Type of contraceptives	Case n=25 (%)	Weight			P-value
		No effect	Weight gain	Weight loss	
Oral	16 (64%)				
Injection	3(12%)	1(4%)	11(44%)	13(52%)	0.007*
Implant	6(24%)				

* P≥0.05 is significant

Table 5: represents the effect of hormonal contraceptives on skin pigments

Type of contraceptives	Case n=25 (%)	Acne		P-value
		No effect	Acne found	
Oral	16 (64%)			
Injection	3(12%)	9(36%)	16(64%)	0.162NS
Implant	6(24%)			

NS: Non-significant

Table 6: The relationship between pain and using hormonal contraceptives

Type of contraceptives	Case n=25 (%)	Headache		P-value
		No effect	**Pain feeling**	
Oral	16 (64%)			
Injection	3(12%)	2(8%)	23(92%)	0.000**
Implant	6(24%)			

** P≥0.001 highly significant

Table 7: The correlation between depression and using hormonal contraceptives

Type of contraceptives	Case n=25 (%)	Depression		P-value
		No effect	**Depressed**	
Oral	16 (64%)			
Injection	3(12%)	0(0%)	25(100%)	0.000**
Implant	6(24%)			

** P≥0.001 highly significant

Table 8: The correlation between cytomorphometrics of superficial and intermediate buccal mucosa cells in cases and controls

Cytomorphometrics	Population	Mean ± SD	95% confidence interval of the difference		P-value
			Lower	**Upper**	
NDs	Case	40.9 ± 159.6	-28.62	99.74	0.271[NS]
	Control	5.3 ± 0.73	-30.32	101.44	0.000**
CDs	Case	68.2 ± 13.0	17.06	28.07	0.000**
	Control	45.7 ± 4.14	16.96	28.16	0.000**
N/Cs	Case	19.1 ± 3.37	4.32	7.50	0.000**
	Control	13.2 ± 2.04	4.23	7.51	0.000**
NDi	Case	8.6 ± 1.11	2.77	3.84	0.000**
	Control	5.3 ± 0.72	2.77	3.84	0.000**
CDi	Case	73.2 ± 10.21	23.61	32.88	0.000**
	Control	44.9 ± 5.34	23.57	32.92	0.000**
N/Ci	Case	18.9 ± 3.56	5.04	8.12	0.000**
	Control	15.3 ± 1.39	5.02	8.14	0.000**

NS: Non-significant, ** P≥0.001: highly significant, ND: nuclear diameter, CD: cytoplasm diameter, N/C: nuclear cytoplasm ratio, (s): superficial cell, (i): intermediate cell.
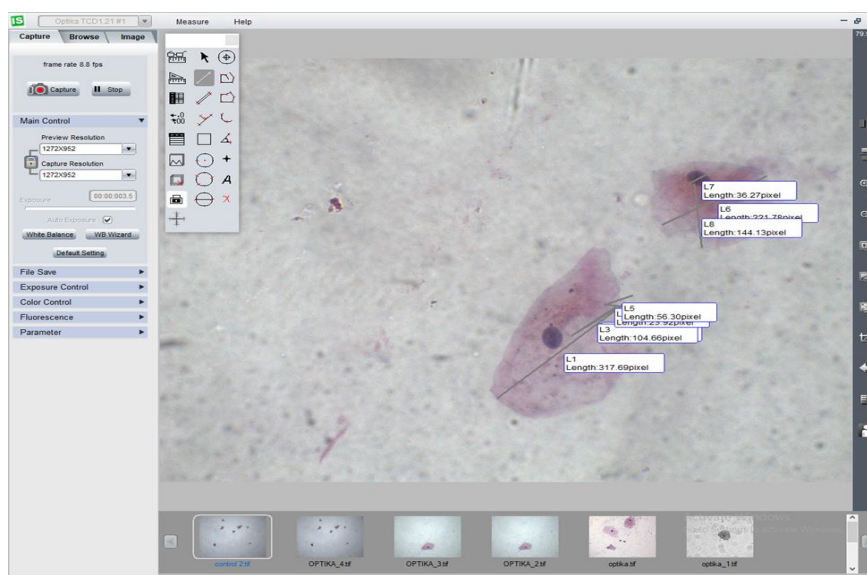
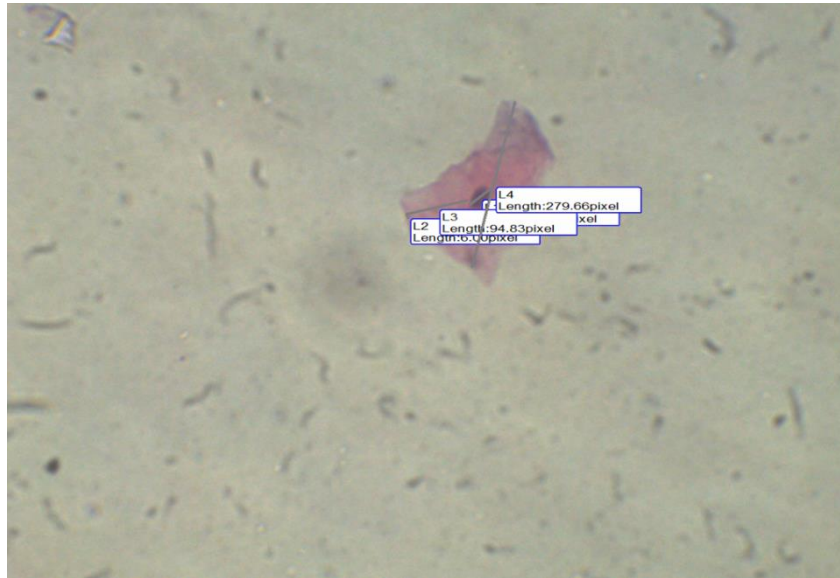**Figure 1: OPTIKA Software used to calculate the cells' ND, CD, and N/D ratio.**

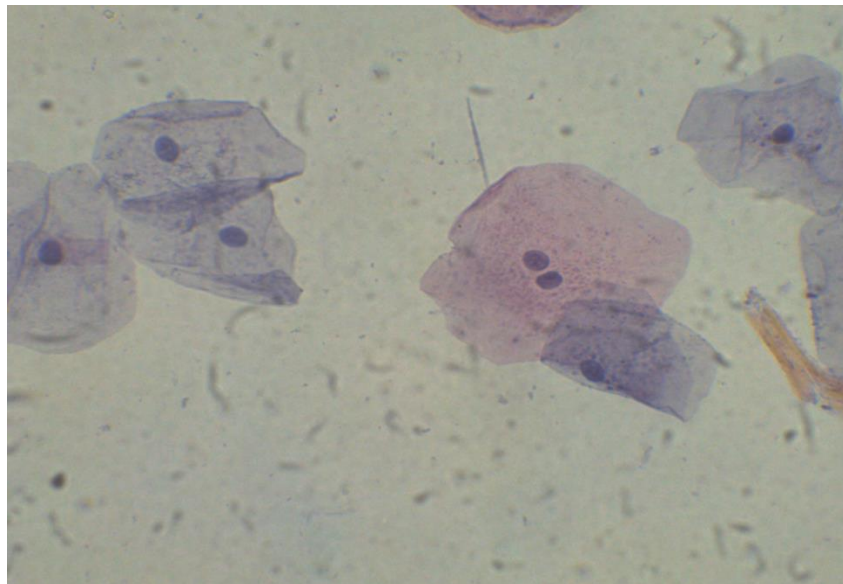**Figure 2: Morphometry of epithelial cell**

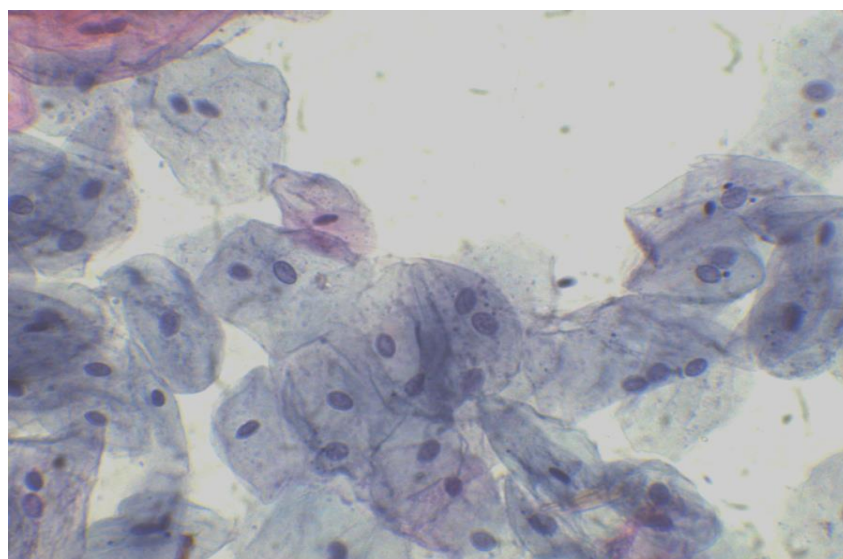**Figure 3: Bi-nucleated cell by using a 40× microscope photo by computer**

Figure 4: A large nucleus with vacuolation by using 40 × microscopic photos

DISCUSSION

The main goal of this research was to assess cellular alterations in buccal mucosa in HCs users comparing with those of healthy control subjects using OPTIKA Software, a technique that measures the cytomorphometrics of exfoliative cells.

Cytomorphometric measures of oral epithelial mucosal cells can be affected by the characteristics of patients including age and gender, systemic diseases such as anemia and diabetes, or local irritants such as tobacco, alcohol, illicit drugs, and infectious diseases [8]. It is believed that the quantitative cytomorphometric alterations revealed in the present study could primarily be due to HCs themselves as confounding factors for buccal cell morphometric changes, also due to excluding all abnormalities as mentioned in exclusion criteria.

A strong association was found in the current study between HCs used and weight change. Clinical findings in this field are paradoxical: some women reported weight gain, while others reported weight loss. This is why both weight gain and weight loss are known as potential side effects of hormonal contraceptives. When people gain weight, it is typically due to one of the following changes: fluid retention, growing in muscle tissue, and an increase in body fat. Theoretically, HCs may lead to weight gain if they contributed to increase fluid retention and body fat content. Also, combining contraceptives are often suggested to enhance appetite; however, women who don't use hormonal contraceptives still gain weight as they age [9, 10].

In our study, there was an obvious correlation between the types of HCs and hair loss, headache feeling, and appearance of depression symptoms, which was consistent with prior studies that explored the favorable and negative impacts of injectable and oral contraceptives. In particular, complications reported by women using hormonal contraception include irregular bleeding, dysmenorrhoea, hair loss, hirsutism, nausea, acne, headaches, mood changes, reduced libido, weakness, breast pain, and bloating [11]. Users of HCs were more likely to show depressive symptoms than women in the control group. These results are consistent with the majority of published research on the psychological impact of these methods [12, 13]. While other recent studies have shown that injectable contraception and HCs may not adversely affect mood, many physicians still believe depression and anxiety to be side effects of hormonal contraception [11].

We observed the presence of significantly larger ND, NC, and N/C in contraceptives utilizers in comparison with the control group. In both types of cells superficial and intermediate cytomorphometrics have been increased. Since hormonal contraceptives contain estrogen and progesterone, estrogen promotes the spread and cornification of the oral mucosa. Previous researches on the impact of estrogen therapy and oral mucosa have not always shown similar results. A positive association has been established between the estrogen level and the maturation and keratinization of the oral mucosa. A number of studies have documented that oral mucosa is responsive to estrogen effect and that decreased estrogen levels may cause epithelial thinning and decreased keratinization of the oral mucosa. However, other studies found no effect of sex hormones on the oral mucosa. Estrogen also enhances the production of antibodies via a humoral reaction and as a general immunoinhibitor [14]. Recurrent aphthous stomatitis (RAS) occurring before menstruation is thought to be due to reduced estrogen levels prior to menstruation. Such a hormonal disorder induces inflammation in the epithelium of the oral mucosa and may contribute to the incidence of RAS. Decreased levels of estrogen prior to menstruation can also lead to a reduction in antibody synthesis, particularly in the mouth, resulting in inflammation [15].

Cowpe et al. have shown that exfoliative cytology is able to detect deformation by estimating the ratio of nuclear size to cytoplasmic size using the Papanicolaou-stained test planimeter [16]. Since then, a variety of studies have been performed using quantitative cytomorphometric techniques. Ramaesh et al. utilized cytomorphometric techniques to determine the nuclear diameter and cell diameter of the healthy oral mucosa, dysplastic lesions, and oral squamous cell carcinoma. They observed that cell diameter was higher in normal mucosa, lower in dysplastic lesions, and lower in oral squamous cell carcinoma. On the contrary, ND was lower in normal mucosa, higher in dysplastic lesions, and higher in oral squamous cell carcinoma. They stated that exfoliative cytology is of use for the evaluation of clinically suspected lesions and early diagnosis of malignancy [17].

According to the outcome of this study, the usage of hormonal contraceptives increasing the nuclear diameter of mucosal cells may be considered as dysplastic criteria. Further studies with large data samples are recommended for future studies for good evaluation and statistically pertaining results. Presenting quantitative measurement of cells in cytology applications would be very useful as a confirmatory diagnostic and research tool.

CONCLUSION

We concluded that HCs have a direct effect on cytomorphmetric measurements of buccal mucosa exfoliive cells; the significant rise in ND, CD, and N/C ratio indicated that the quantitative measurement of smears is useful in detecting changes to contraceptives and certainly for many other health problems.

Disclosure of potential conflicts of interest

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