ORIGINAL ARTICLE

CYTOMORPHOMETRIC CHANGES OF ORAL MUCOSA DURING NORMAL HORMONAL TURNOVERS IN HEALTHY YOUNG MENSTRUATING WOMEN

[1]**Usha Balan**, MDS; [1]**Master Luqman**, MDS; [2]**Abdelnasser Mohamed M. Soliman**, MDS, PHD; [1]**Hussain Almubarak**, PhD, FAAOMP

ABSTRACT

Oral mucous membrane is an excellent indicator of the constitutional state of a patient. The oral mucosa is under tropic influence of various hormones and thus reflects the systemic status of an individual. Very few cytomorphometric studies are done to evaluate the role of sex hormones on oral mucosa. This study was done to determine the morphometric changes in a cell and nuclear diameter of exfoliated cells from the oral mucosa during the course of normal menstrual cycle in healthy women. The purpose of this study was to determine whether oral smears reflect hormonal state of menstrual cycle. In this study consisting of 40 study subjects and 20 control subjects, smears were collected from buccal mucosa twice per week for a period of three months. Morphometric analysis of the cell and nuclear diameter was done and results were statistically analysed. Cell diameter showed significant changes during various phases of the menstrual cycle of healthy young women. In Comparison of both study and control groups, no significant changes were seen in cell and nuclear diameter of the exfoliated cells. The present study showed changes in cell diameter of the study group which could be related to the role of female sex hormones.

Key Words: Oral mucosa, Estrogen, Progesterone, Hormones, Cytomorphometry

INTRODUCTION

Oral mucous membrane is an excellent indicator of the constitution of a patient.[1] The oral mucosa possesses hormone receptors and is under tropic influence of various hormones,[2] thus it is a true reflector of the systemic status of an individual.[3] Menstrual cycle is the result of the sequence of hormonal influences that in a normal woman follow each other with great regularity from puberty to menopause except during pregnancy.

[1]Assistant Professor in Oral Pathology, Department of Diagnostic sciences and Oral Biology (DDS), King
[2]Associate Professor in Oral Biology, Department of Diagnostic sciences and Oral Biology (DDS), King Khalid University College of Dentistry, , Abha, Kingdom of Saudi Arabia

Correspondence should be addressed to:
Dr. Master Luqman
College of Dentistry, King Khalid University
Grainger, Abha, P.O Box 3263
E-mail: mluqman@kku.edu.sa
Contact No. : 0966557550023

Menstrual cycle commences with menstruation and has four phases –Bleeding, Proliferative, Ovulation and Secretory. Hormone levels fluctuate during various phases of the cycle. During first half of the menstrual cycle there is an increase in estrogen level followed by its decline. Second half of the cycle shows increased progesterone. During menstrual period both the hormone levels decline.[4]

Vaginal and buccal epithelia are microscopically similar[5] having stratified squamous epithelium and a desquamative growth pattern.[6] Both epithelia are predominantly non-keratinised.[5] Vaginal mucosa has been shown to respond to changes in hormonal levels and thus cytohormonal evaluation has been used for accurate assessment of hormonal effect on vaginal mucosa[7]. Whether oral mucosa exhibits properties similar to vaginal mucosa in relation to sensitivity to ovarian steroids and whether oral mucosa is an index of hormonal milieu is still a topic for debate.[5] Estrogen receptors have been identified in oral tissues including oral mucosal keratinocytes, thus variation in this hormone can affect oral mucosal cells[7]. With the advancement in the field of cytology, parameters like cytoplasmic and nuclear area have been shown significant in diagnosis of oral lesions. Oral cytomorphometric studies are mainly done for screening of oral malignant and premalignant lesions. The present study uses cytology to assess the effect of hormonal changes during various phases of the menstrual cycle by estimating the cell and nuclear diameter of normal exfoliated buccal mucosal cells.

MATERIALS AND METHODS

This study was initiated after obtaining an ethical clearance from the institution with an order number KMCTDC/IEC/2014/25. The experimental group was divided into study group and control group. Study group consisted of forty healthy young women volunteers with normal menstrual cycle of 28-30 days without any local or systemic diseases, abnormal endocrinal or immunological status. Control group consisted of twenty healthy young age matched men volunteers. With an informed written consent a thorough oral examination was done for both these groups of volunteers to rule out clinically evident and habit related lesions. All subjects were apparently healthy without any habits and were non anemic. None of them had any fixed or removable appliances.

Hemoglobin estimation was done to rule out anemia. A proper menstrual history was recorded from the study subjects and the entire cycle was divided into four phases consisting of bleeding phase, proliferative phase, ovulation phase and secretory phase Oral smears were collected from both the study and control groups at regular intervals twice a week for a period of three months. Smears were taken using cyto brush from the buccal mucosa anterior to the stenson's duct, which according to Timonen[8] is histologically similar to vaginal epithelium. Scrapings were smeared on a plain glass slide, fixed in absolute alcohol and stained using Rapid-pap^{TM}Papinicolau stain kit.

Pap stained smears were observed under a binocular light microscope LABOMED. Cell and nuclear diameter of the exfoliated cells were measured using a calibrated 5x eyepiece and 40x objective for both groups. Eyepiece graticule was calibrated for 40x objective by reference to a stage micrometer. The stage micrometer had gradations as small as 10 µm (0.01mm). By superimposition of eyepiece graticule on cytological smears, a direct measurement of individual epithelial cells was done.

Only clearly defined cells were measured avoiding clumped or folded cells. Fifty cells were measured for cell diameter (CD) and nuclear diameter (ND), from each slide which included the superficial, parabasal and intermediate cells. Diameters were obtained along the longest axis and shortest axis of cells and nuclei (Figure1).

For the study group, the mean value was obtained for each case during various phases of the menstrual cycle. Mean value of cell and nuclear diameter in all the three cycles of the study group were then compared with that of the control group.

Statistical analysis included estimation of value of significance using student's unpaired't' test and

analysis of variance was performed using Fischers test and Tukey's Honestly Significant difference test.

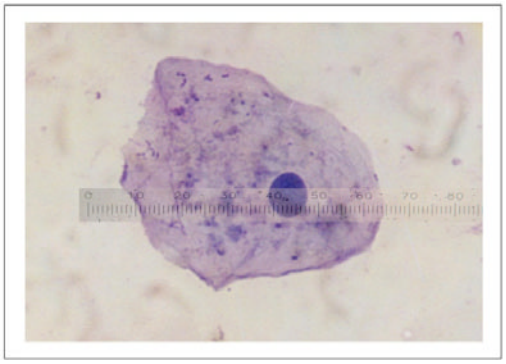

Fig. 1: PAP stained cytologic smear of an intermediate cell- Linear measurements of cell

RESULTS

In our study significant changes were observed in the cell diameter and nuclear diameter of the exfoliated buccal cells in the study group during various phases of the menstrual cycle (Table1).

Comparing cell diameter and nuclear diameter of the exfoliated cells of one phase with other phases of the menstrual cycle, cell diameter showed statistically significant changes in all the phases of menstrual cycle whereas nuclear diameter showed significant changes in a few phases of all the three cycles studied in study group (Table 2).

Comparison of morphometry of cells between the study and control groups during the three month study showed no significant changes in the cell diameter and nuclear diameter statistically (Table 3).

Table 1: Showing cell and nuclear diameter during various phases of a menstrual cycle in study Group

CYCLE	D	PHASE	N	MEAN mm	S.D.	F	p
1	CD	Bleeding	40	0.0540	0.00258		
		Proliferative	40	0.0547	0.00162		
		Ovulation	40	0.0580	0.00188		
		Secretory	40	0.0565	0.00150	12.935	0.001 vhs
	ND	Bleeding	40	0.00778	0.0005329		
		Proliferative	40	0.00768	0.0004894		
		Ovulation	40	0.00763	0.0003754		
		Secretory	40	0.00793	0.0005723	2.88	0.038 sig
2	CD	Bleeding	40	0.0548	0.003304		
		Proliferative	40	0.0544	0.002521		
		Ovulation	40	0.0552	0.002063		
		Secretory	40	0.0558	0.002104	2.384	0.071 ns
	ND	Bleeding	40	0.00767	0.0004763		
		Proliferative	40	0.00754	0.0004751		
		Ovulation	40	0.00762	0.0004262		
		Secretory	40	0.00792	0,0006201	4.229	0.007 hs
3	CD	Bleeding	40	0.0538	0.002263		
		Proliferative	40	0.0545	0.002135		
		Ovulation	40	0.0558	0.002621		
		Secretory	40	0.0562	0.002860	7.951	0.001 vhs
	ND	Bleeding	40	0.00765	0.000458		
		Proliferative	40	0.00757	0.000345		
		Ovulation	40	0.00782	0.000504		
		Secretory	40	0.00822	0.000812	10.618	0.001 vhs

CD- Cell Diameter ND- Nuclear Diameter D- Diameter
S.D = Standard Deviation

Table 2 -Showing comparison between the cell diameter and nuclear diameter of one phase with the other phases in the menstrual cycle

	DIAMETER	PHASES COMPARED		p
1	CD	Bleeding	Proliferative	0.096 ns
			Ovulation	0.001 vhs
			Secretory	0.001vhs
		Proliferative	Ovulation	0.023 sig
			Secretory	0.001 vhs
		Ovulation	Secretory	0.072 ns
	ND	Bleeding	Proliferative	0.411 ns
			Ovulation	0.178 ns
			Secretory	0.163 ns
		Proliferative	Ovulation	0.598 ns
			Secretory	0.028 sig
		Ovulation	Secretory	0.007 hs
2	CD	Bleeding	Proliferative	0.469 ns
			Ovulation	0.444 ns
			Secretory	0.70 ns
		Proliferative	Ovulation	0.137 ns
			Secretory	0.012 sig
		Ovulation	Secretory	0.292 ns
	ND	Bleeding	Proliferative	0.233 ns
			Ovulation	0.178 ns
			Secretory	0.03 sig
		Proliferative	Ovulation	0.448 ns
			Secretory	0.028 sig
		Ovulation	Secretory	0.009 hs
3	CD	Bleeding	Proliferative	0.195 ns
			Ovulation	0.001 vhs
			Secretory	0.001 vhs
		Proliferative	Ovulation	0.027 sig
			Secretory	0.003 hs
		Ovulation	Secretory	0.425 ns
	ND	Bleeding	Proliferative	0.523 ns
			Ovulation	0.185 ns
			Secretory	0.001 vhs
		Proliferative	Ovulation	0.05 sig
			Secretory	0.001 vhs
		Ovulation	Secretory	0.002 hs

vhs- Very highly significant hs-Highly significant
sig- Significant ns- Non significant

Table 3: Showing comparison of cell diameter and nuclear diameter between the study group and control group

DEPENDENT	GROUP	MEAN	S.D	t	p
C.D	S.G	0.0555	0.0024	-0.58983	>0.05 ns
	C.G	0.0560	0.0033		
N.D	S.G	0.00775	0.00050	0.52028	>0.05 ns
	C.G	0.00768	0.00046		

S.G - Study Group C.G - Control Group
S.D - Standard Deviation ns - Non Significant

DISCUSSION

In our study significant changes were observed in the cell diameter and nuclear diameter during various phases of the menstrual cycle in all the three cycles studied. Comparing cell diameter and nuclear diameter of one phase with other phases of the menstrual cycle, a significant change in the cell diameter is seen from bleeding to secretory phase. Nuclear diameter shows a change during a few phases in the three cycles studied. Increasing cell diameter seen in the second half of the cycle could be attributed to the role of progesterone, which is seen in excess during this period. Age matched men were taken as controls to correlate the morphometric changes to be age- related. On comparing cytomorphometry of cells between study and control groups, no significant changes were seen in the cell diameter and nuclear diameter statistically. Thus in our study we could not establish a distinct difference in oral cytology with regard to morphometry between men and women.

Oral cellular changes associated with phases of menstrual cycle remains a controversial issue. Size of individual epithelial cells undergoes changes in the female reproductive tract as a result of cyclic changes in the menstrual cycle. Very few studies[9-11] have been done with regard to the cellular parameters like cell diameter and nuclear diameter of exfoliated oral cells in the course of normal menstrual cycle in women and most of them showed that buccal squames are unaffected by menstrual cycle.[10] Some authors[11,12] have proposed nucleus: cytoplasmic ratio as a

powerful parameter in reflecting the effect of hormones.

According to Driesch's Law[12] of volume invariance maximal cell size is genetically regulated and hormonal over stimulation has no effect on cell size. Wingrove FA (1979)[9] on cytomorphometric analysis of gingival epithelium during various phases of menstrual cycle found no change in cell size and nuclear surface area. Cowpe JG et al (1985)[10] found no change in the cell size and nuclear size in buccal squames during various phases of menstrual cycle and concluded that oral smears unlike vaginal smears do not clearly demonstrate stage of menstrual cycle or time of ovulation.

Gerasimov JG et al (1996)[11] found changes in buccal cell size, cell areas of cytoplasm, nucleus during menstrual cycle occurring throughout menstrual bleeding indicating a tensed state of female body during this period shown as smallest sizes in buccal epithelial cells. Chretion (1998)[12] on cytomorphometric analysis of superficial vaginal cells in women of various groups found increased nucleus: cytoplasmic ratio in women with oral contraceptives compared to women on in-vitro fertilization stimulation protocol.
Significant variations were seen in nuclear and cytoplasmic area between different sites and with the advancing age (Cowpe JG et al1985).[13,14]

Studies have shown that various factors like alcohol,[14] smoking,[15,16,17] age,[18,19,20] radiotherapy,[21] nutritionaldeficiencies,[22] anemia,[23] field change effect etc influence the cytomorphology of cells. Cytomorphometric analysis of exfoliated cells in premalignant and malignant lesions show variation from normal values.

In the present study in which cytomorphometric measurements were done with the help of an eyepiece graticule and stage micrometer, only linear measurements of cell and nuclear diameter were possible. A study of longer duration including more number of subjects would be more appropriate in determining the actual role of female sex hormones on oral mucosa. Cytomorphometric studies comparing cell and nuclear diameter changes in young females during various phases of menstruation with those of age-matched men are not much reported in the literature. Statistically no differences were found in the cell and nuclear diameter between the study and control groups. Thus in our study we could not establish a distinct difference in oral cytology with regard to morphometry between men and women.

CONCLUSION

The present study showed significant cytomorphometric changes in the exfoliated oral cells in the study group during various phases of the three cycles studied. An increase in cell diameter was seen during secretory phase of menstrual cycle which could be attributed to the role of progesterone. Comparison between study and control groups showed no changes in the cell and nuclear diameter statistically. This may be due to the influence of sex hormones on the oral mucosa both in men and women.

Clinical Significance

A study of longer duration including more number of subjects would be more appropriate in determining the actual role of female sex hormones on oral mucosa.

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Conflict of Interest: None declared

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