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**Aim:** The objective of the study was to assess the effect of age related hormonal changes in the buccal mucosa of female subjects by estimating the cell diameter (CD) and nuclear diameter (ND) and nuclear cytoplasmic ratio

**Materials and methods:** A total of 60 apparently healthy female subjects with no known history of any systemic disease were chosen. The subjects were divided into three groups, group I: female subjects less than 15 years of age, Group-II: female subjects aged 15-45 years, Group-III female subjects aged more than 45 years. Buccal smears were taken and fixed in 95% alcohol, stained using the standard Papanicolaou laboratory procedure. Dimensions of 20 cells were measured from each smear and the mean was calculated. The Cytoplasmic Diameter and Nuclear Diameter were measured using image analysis software. Statistical analysis of the data was performed using ANOVA to evaluate the difference in the Nuclear Diameter, Cytoplasmic Diameter and Nuclear: Cytoplasmic ratio.

**Results:** The results showed an increase in Nuclear Diameter from the less than 15 age group to 15-45 age groups. In the 15-45 age groups, there was a decline in CD when comparing the first group. There was further decline in Cytoplasmic Diameter and an increase in nuclear diameter in the more than 45 age group. It was also observed that the nuclear cytoplasmic ratio increases as age advances.

**Conclusion:** The present study shows age related variations in the cells of buccal smears of healthy females.

**Key words:** Buccal smear, nuclear diameter, hormone, oestrogen.

## 1. INTRODUCTION

Oral mucosa reflects the systemic status of an individual. Hormones play a wide role in human tissues bringing about either a physiological or a pathological change. Oral Exfoliative cytology is the study of normally exfoliated superficial cells from mucous membrane. It has been used for the detection of oral potentially malignant or malignant lesions, is a technically simple, non invasive, less time consuming and cost effective procedure that has a sensitivity
of 89% and specificity of 89.5% [2]. Various parameters like nuclear size, nuclear pleomorphism, nuclear membrane discontinuity, degenerative changes of the nucleus and nuclear cytoplasmic ratio can be analyzed, from which Nuclear Cytoplasmic ratio is considered to be more significant in exfoliative cytology. Sex steroids play a very major and pivotal role in the life of a woman. It is a known fact that from the time of childhood, through menstrual age to menopausal stage there are cyclic fluctuations in a woman’s hormone levels [3]. There are numerous articles on cytomorphometric analysis of premalignant and malignant lesions and lesions due to tobacco chewing etc, but the articles available regarding that of the normal epithelial cells are relatively few in number.

The present study uses exfoliative cytology to assess the effect of age related hormonal changes in the buccal mucosa of female subjects by estimating the cell diameter (CD) and nuclear diameter (ND) and nuclear cytoplasmic ratio .

2. MATERIALS AND METHODS
A total of 60 apparently healthy female subjects with no known history of any systemic disease and those who are not under any medication were selected for the study. The patients who presented with a history of systemic illness, tobacco use or alcoholic consumption were excluded from the study. The subjects were divided into three groups , group -I: female subjects less than 15 years of age, Group-II : female subjects aged 15-45 years, Group-III female subjects aged more than 45 years.

Using a wet wooden spatula, exerting gentle pressure, cells were scraped from the buccal mucosa. Scrapings were smeared on to a microscopic slide. The Slides were fixed in 95% alcohol for thirty minutes. Air drying of the smears was not done to avoid potential alterations in the cellular morphology. The Smears were then stained using the Rapid-Pap kit. The smear was visualised in 40 X objective in a compound microscope and images of the cells were taken and nuclear diameter, cytoplasm diameter were measured using image analysis software. Dimensions of 20 cells were measured from each smear and the mean was calculated. Unfolded cells with a clear and definite outline were only selected for the study, excluding the clumped or folded cells. In order to avoid measuring the same cells again the sampling was done starting from left upper corner to the right and then down.

The values of nuclear diameter, cytoplasmic diameter and nuclear cytoplasmic ratio were tabulated and compared.

STATISTICS:
Statistical analysis was carried out using ANOVA.

3. RESULTS

Nuclear diameter: The nuclear diameter in group I was found to be 7.44 µ, 7.85 µ in group II and increased to a maximum of 8.30 µ in group III. (Table-1) (p value 0.001)

| Age group           | Mean   |
|---------------------|--------|
| Group –I            | 7.44   |
| Less than 15 years  |        |
| Group –II           | 7.85   |
| Age 15-45 years     |        |
| Group –III          | 8.30   |
| Age more than 45    |        |

Cytoplasmic diameter:
The cytoplasmic diameter was found to be 49.50 µ in group I, 48.36 µ in group II and 47.16 µ in group III. (Table-2) (p value 0.000)

Nuclear cytoplasmic ratio
The nuclear cytoplasmic ratio was found to be 0.15 in group I, 0.162 in group II and 0.175 in group III. (Table-3) (p value 0.000)

There was significant increase in the mean nuclear diameter and significant decrease in the cytoplasmic diameter as age advances. It was also observed that the nuclear cytoplasmic ratio increases as age advances.

4. DISCUSSION

The results of this study showed a significant difference in the Nuclear Diameter, Cytoplasmic Diameter and Nuclear Cytoplasmic ratio in females of various age groups. There was an increase in the ND from the < 15 years age group to 15-40 years age group. This could be due to the rise in the levels of estrogen and progesterone in the blood which cause the acceleration of cell metabolism and growth which is reflected as an increase in the dimensions of the cytoplasm and nucleus. There was a decline in cytoplasmic diameter from < 15 age group to > 45 age group. This could be attributed to the decrease in the serum estrogen levels in the post-menopausal females. A study by Ziskin and Moulton [4] expressed rhythmic alterations in cells of the oral cavity that coincided with changes found in those of vaginal smears. In a study by Nayyar and Sivapatha sundharam, [6] the Nuclear diameter was reported to increase with age and Cytoplasmic Diameter was said to decrease with age and our results are in accordance with their study. Montgomery et al in their study stated that there was no relationship between menstrual cycle and the oral cells. [6] Rijssinghani, Peters in their study concluded that hormones played no role in the epithelial lining of the oral cavity. [7] These studies were not in conformity with the present study. Preethy et al stated that age related variations were present in the buccal mucosal cells in female subjects [1] which is in concordance with our study.

| Age group          | Mean   |
|--------------------|--------|
| Group – I          | 49.50  |
| Less than 15 years |        |
| Group – II         | 48.36  |
| Age 15-45 years    |        |
| Group – III        | 47.16  |
| Age more than 45   |        |

| Age group          | Mean   |
|--------------------|--------|
| Group –I           | 0.150  |
| Less than 15 years |        |
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Table 1

| Group   | Median Value |
|---------|--------------|
| Group II | 0.162        |
| Age 15-45 years |
| Group III | 0.175        |
| Age more than 45 years |

5. CONCLUSION
The present study shows age-related variations in Nuclear Diameter, Cytoplasmic Diameter and Nuclear: Cytoplasmic ratio in healthy female subjects and these changes could probably reflect fluctuations in the hormonal levels of oestrogen and progesterone.

6. REFERENCES
1. Preethy Mary Donald, Renjith George, G. Sriram, B. Kavitha, and B. Sivapathsundharam. Hormonal changes in exfoliated normal buccal mucosal cells. J Cytol. 2013; 30(4): 252–256.
2. T Ramaesh, B.R.R.N Mendis, N Ratnatunga. Diagnosis of oral premalignant and malignant lesions using cytomorphometry. Otontostomatologietropicale 1999; 22(85):23–8.
3. Bibbo M. 2nd ed. Philadelphia: W.B. Saunders Company; 1997. Comprehensive cytopathology.
4. Ziskin DE, Moulton R. A comparison of oral and vaginal epithelial smears. J Clin Endocrinol Metab.1948;8:146–65.
5. Nayar AK, Sundharam BS. Cytomorphometric analysis of exfoliated normal buccal mucosa cells. Indian J Dent Res. 2003;14:87–93.

6. Montgomery PW. A study of exfoliative cytology of normal human oral mucosa. J Dent Res. 1951;30:12–8.
7. Peters H, Rijsinghani K. The cytologic interpretation of the mouth smear; cell changes in cancer and other diseases. J Indian Med Assoc. 1956;27:231–6.

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