Research Article

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Exfoliative Cytology: Implications in Forensic Odontology

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ABSTRACT

Introduction: Age determination of unknown human bodies is important in the setting of a crime investigation or a mass disaster because the age at death, birth date, and year of death as well as gender can guide investigators to the correct identity among a large number of possible matches.

Aim: The aim of the present study is to compare cell size parameters among different ages and to evaluate its role in age estimation.

Materials and Methods: Buccal smears were collected from a 100 apparently healthy individuals of different age groups. After fixation in 95% alcohol, the smears were stained using standard Papanicolaou laboratory procedure. The average cell size was measured with ProgRes C3 image software analysis system. Statistical analysis of the data was done using one-way ANOVA, Bonferroni procedures.

Results: The results showed significant decrease in average cell size of individual with increase in age.

Conclusion: Age-related alterations are observed in buccal smears.

Keywords: Cell size, exfoliative cytology, image analysis Techniques.

INTRODUCTION

Human identity is considered as the fortress of civilization, and the identification of unknown individuals always is of paramount importance to the society. It is important to identify the deceased to ensure appropriate obsequies, but also there are issues such as criminal investigations, insurance settlements, and military proceedings that can be resolved only with a positive identification.[1]

The age estimation is the important event in forensic odontology. As it is useful in the setting of a crime investigation or a mass disaster because the age at death, year of death and birth date as well as gender can guide investigators to the correct identity among a large number of possible matching groups.[2, 3] Many age estimation methods are available in forensic odontology. In children age can be determined by the analysis of tooth development and subsequent comparison to development charts, usually to an accuracy of approximately 1.6 years. The use of attrition, volume of pulp cavity and development of the third molar have been suggested as means of age estimation for those individuals over 18 years of age. Newer techniques like translucency of dentine and aspartic acid racemization have been proposed and proved to be highly accurate in adult age assessment.[1, 3.]

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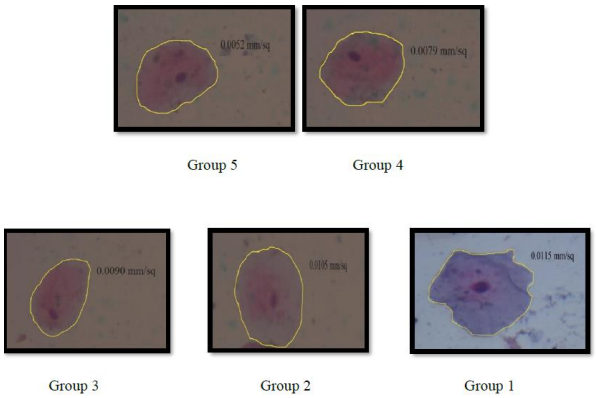

Fig 1: PAP stained buccal smear cells showing different sizes among age groups.

Exfoliative cytology is one of the unique methods that can be used for age estimation. Oral exfoliative cytology is a non-invasive technique, that allows simple and pain-free collection of intact cells from different layers within the epithelium for microscopic examination.[3, 4, 5, 6]

Oral epithelium, as a part of normal physiologic turnover undergoes continuous renewal. Epithelial cells move from basal layer towards the surface layers and are exfoliated. The use of exfoliative cytology as a diagnostic aid accentuates the need for establishment of an accurate baseline. However studies on oral epithelium are largely carried in pathological states. But secrets of pathology can be explored accurately, only when the fundamental observations in normal oral mucosal cells are established.[4] Since, initial studies of normal epithelial smears by Miller and Montgomery in 1951, there are only few instances where the normal buccal mucosal smears are studied in early days.[7] The use of oral exfoliative cytology in the past was limited, because of subjective nature of its interpretations and high false-negative results when it is used in diseased states like premalignant and malignant lesions. These limitations were overcome by the use of quantitative methods such as image analysis systems, especially in the assessment of cytomorphometric cellular alterations by Cowpe in the field of cytology.[5, 6]

In 1960, Prewitt and Mendelson first used the image analysis system for studying the leukocyte subsequently, it was extended by Weid and his coworkers to study the cells in the cervical smears.[3, 4] Hence, the quest for diagnosis with the simplest tool and with reasonable accuracy has led to various advancements in the field of oral exfoliative cytology.[4] It is a well known fact that, oral epithelium shows several changes in its morphology with the advancement of age i.e., from younger to older ages. Keeping this fact in mind, present study was intended to observe the changes in the morphometry of oral exfoliative cells in the different age groups of human life.

The aim of the present study is to compare cell size parameters among different ages and to evaluate its role in age estimation.

MATERIALS AND METHODS

50 apparently healthy subjects with no history of any systemic disease or therapeutic medication were taken for the study. All the subjects were free of deleterious habits like smoking, alcohol consumption or tobacco use and without any medical problems. A prior written consent was taken from all study subjects. The subjects were divided into five groups, with 10 patients per group, based on their age which included 5 male and five females per each group. (Table 1)

The subjects were asked to rinse their mouth thoroughly with water before sample collection. By using wet wooden spatula, buccal smears were collected from each subject in scrapping motion and smeared on to glass slide and immediately fixed with 95% ethanol for a minimum of 15 minutes. Finally, all the smears were stained using Papanicolau staining method.

The stained smears were observed under 40X objective in a stepwise manner for image analysis, moving from left to right and then down and across, in order to avoid measuring the same cells again and focused on the stage micrometer scale. In all the cases, cell sizes were measured with ProgRes C3 image software analysis system. Only clearly defined cells were measured, excluding clumped or folded cells. An average of 20 clearly defined cells were selected in each smear, markings were made manually using paint tool, projected on to the monitor and cell size parameters were determined on image analysis software.

Table 1: Different age groups and sample size included in the study.

Group	Age group in years	Males	Females
1	5- 15	5	5
2	16-30	5	5
3	31-45	5	5
4	45-60	5	5
5	Above 60	5	5
	Total (50)	25	25

Table 2: Cell size parameters within different age groups.

Group	**Age group in years**	**Cell size range (mm/sq)**	**Average cell size and S.D**
1	5-15	0.005-0.016	0.0111 ± 0.000516
2	16-30	0.004-0.016	0.0105 ± 0.000689
3	31-45	0.003-0.015	0.0092 ± 0.000413
4	46-60	0.003-0.014	0.0081 ± 0.000545
5	> 60	0.001-0.0104	0.0052 ± 0.000790

Table 3: Comparison among groups done by statistical Bonferroni multiple comparison test.

Group (I)	**Group (J)**	**Mean Difference (I-J)**	**Std. Error**	**(p-value)**	**Significance**
1	2	.00053500	.00027093	.545	NS
	3	.00188000*	.00027093	.000	S
	4	.00302000*	00027093	.000	S
	5	.00584000*	.00027093	.000	S
2	1	-.00053500	.00027093	.545	NS
	3	.00134500*	.00027093	.000	S
	4	.00248500*	.00027093	.000	S
	5	.00530500*	.00027093	.000	S
3	1	-.00188000*	.00027093	.000	S
	2	-.00134500*	.00027093	.000	S
	4	.00114000*	.00027093	.001	S
	5	.00396000*	.00027093	.000	S
4	1	-.00302000*	.00027093	.000	S
	2	-.00248500*	.00027093	.000	S
	3	-.00114000*	.00027093	.001	S
	5	.00282000*	.00027093	.000	S
5	1	-.00584000*	.00027093	.000	S
	2	-.00530500*	.00027093	.000	S
	3	-.00396000*	.00027093	.000	S
	4	-.00282000*	.00027093	.000	S

The average cell size values were obtained for each case and statistically analyzed using one-way ANOVA and Bonferroni multiple comparison tests in SPSS version 22 and p value less than 0.05 was considered as statistically significant.

RESULTS

The cell size changes as the age advances and the values obtained from 5 age groups in the present study are as follows, group 1 showed cell size range of 0.005-0.016 mm/sq (average cell size 0.0111 ± 0.000516 mm/sq), group 2 showed cell size range between 0.004-0.016 mm/sq (average cell size 0.0105 ± 0.000689 mm/sq), for group 3 cell size range was 0.003-0.015mm/sq (average cell size 0.0092 ± 0.000413 mm/sq), group 4 showed cell size range of 0.003-0.014 mm/sq (average cell size 0.0081 ± 0.000545 mm/sq) and finally group 5 showed cell size range of 0.001-0.0104 mm/sq (average cell size 0.0052 ± 0.000790 mm/sq). (Table 2). Finally, statistical significance between all the 5 groups was performed. Where, except between group1 and 2 ($p>0.05$), all other groups showed statistically significance difference ($p<0.05$). (Table 3)

DISCUSSION

Exfoliative cytology utilizes the microscopic examination of shed or desquamated cells from the epithelial surface most commonly the mucous membrane. It also includes the study of those cells that have been collected by scraping the tissue surfaces or fluids collected from body such as sputum, saliva, etc. Continuous exfoliation of epithelial cells is a part of physiological turnover. Deeper cells which are strongly adhered in normal conditions become loose in the case of malignancy and tend to exfoliate or shed along with superficial cells.[9, 10]

From the past, exfoliative cytological studies were done in various fields like normal cytomorphometry and in pathological conditions like in diabetes mellitus, oral potentially malignant conditions and oral cancers. The present study was intended to apply exfoliative cytomorphometric methods in forensic context i.e., comparison of cell sizes for age estimation. Because, oral epithelium show age-wise changes in their morphology.

The results of present study revealed an age related variation in cell size parameters, irrespective of gender and these changes can be attributed to cellular senescence. A basal cell can only divide for a set of number; then the renewal capacity of tissues declines with age, resulting in the accumulation of senescent cells.[7] The cells which stay for a longer duration in the oral cavity succumb to the effect of various local environmental factors. It is a well known fact that the cellular activity and the epithelial turnover rate decreases as age advances and also cellular organelles decreases which could be the reason for the decrease in cell size.[3] Present study has revealed, as the age advances the cell size decreases and these results are in accordance with study done by Shetty et al., Donald et al., Anuradha et al., and Nair et al.

Shetty et al., (2015) have done a study on buccal smears from healthy individuals of various ages for comparing the average cell size using image analysis morphometric software. Their results showed significant decrease in average cell size of individual with increase in age.[3] Donald et al., (2013) have correlated hormonal influence on exfoliated cells of normal buccal mucosa in healthy female individuals with age. Their results showed that nuclear and cell diameter increased from 5-10 to 15-35 years because of maximal hormonal activity and after, as the age advances the parameters gradually decreased where hormonal activity decreases.[11]

Anuradha et al., (2007) have done a study to correlate cell and nuclear diameter with age and sex by including gingival smears from healthy individuals of different age groups. The cell diameter in males and females was low in the 1-20 years then it gradually increased. After 60, there was a decrease in cell diameter.[4] Study by Nayar et al., (2003) also showed significant variation in the cell diameter with increasing age.[12]

The present study recommend to perform similar kind of studies in the different ethnicity, geographic locations by considering large sample size to achieve more accurate results and also advises to compare the ages estimated by this method with other methods for knowing efficiency of present method.

CONCLUSION

Exfoliative cytology has several implications in the field of biology and its role in forensic odontology is a promising field of research. The present study establishes significant age-related variations in cell size. The decrease in cell size can be attributed to aging process. Hence, present study stresses the use of exfoliative cytology in the age estimation processs.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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