ORIGINAL RESEARCH

Oral Mucosal Cell Exfoliative Cytology – A Possible Tool for Age Estimation in South Tamilnadu Population

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Abstract:

Introduction: Forensic age estimation is an area of competence in forensic medicine that seeks to accurately determine the chronological age of an unknown individual in judicial or legal processes. Various morphological and radiological methods are available for age estimation. The age estimation from soft tissues remains an enigma and need to be studied

Aim and objectives: The aim of the study was to estimate the age of an individual by comparing the average cell size from exfoliative cells collected from the buccal smears using Image J Morphometric analysis software.

Materials and methods: Buccal smears were collected from 60 healthy individuals followed by fixation and standard PAP staining procedure. The average cell size was measured using Image J Morphometric analysis software. Statistical analysis was done using Analysis of Variance (ANOVA) test followed by post-hoc analysis using Bonferroni Test.

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

Conclusion: Age related morphometric variations are observed in buccal smears establishing that, exfoliative cytology can be used as an adjunct method along with standard age estimation methods.

Keywords: Age estimation, exfoliative cytology.

INTRODUCTION

Forensic age estimation is an area of competence in forensic medicine that seeks to accurately determine the chronological age of an unknown individual in judicial or legal processes.[1] Various morphological and radiological methods are available for age estimation. The age estimation from soft tissues remains an enigma.[2] Exfoliative cytology (EC) is a noninvasive procedure that collects intact cells from different levels of the oral epithelium for microscopic examination. The oral cavity is considered to be an ideal site for examining the manifestations of ageing. The oral epithelium is constantly replaced, hence objective signs of senescence should be visible in this area.[3] Thus, exfoliative cytology can aid in age estimation of an individual by examining the cellular morphology of the exfoliated cells.[4] This study was an attempt to establish the significance of exfoliative cytology as an age estimation tool.

The purpose of the study is to establish and understand the role of oral mucosal cell exfoliative cytology as an age estimation tool in South Tamil Nadu population.

MATERIALS AND METHODS:

The study was conducted in Department of Oral Pathology and Microbiology, Rajas Dental College and Hospital with the Institutional ethical Committee approval. The study was designed with a sample size of 60 healthy individuals between the age group of 18-60 years who were categorized under three groups :

Group I (18-30 years); Group II (30-45 years; Group III (45-60 years).

The patients with history of systemic illness and under medication and those with the habit of tobacco use and alcoholic consumption were excluded from the study, since these factors can have cause epigenetic modification in the cytomorphology of the exfoliated epithelial cells. The buccal mucosal smears were obtained after obtaining informed consent from the study participants. The patients were asked to rinse the oral cavity and the buccal mucosal smear were taken using moistened wooden spatula with a gentle scraping motion and collected cells were spread homogeneously in two slides labelled with sample number for future references.[1] After smear preparation, immediately fixed with 95% of ethanol for a period of 15 to 20 minutes. The slides were then stained using Papanicolaou staining technique and mounted with cover slip.[5] The stained smears were viewed in optical microscope under 40 X objective

magnification in a digital microscope attached with monitor having Image analysis software (Image J). The slides were observed and measured by two investigators. In all the smears, An average of 20 clearly defined cells were identified and manually marked using paint tool, projected on the monitor and images were captured using camera attached with digital microscope, the cell size were measured in both the horizontal and vertical axis in micrometre. The average cell size values were calculated and compared between the groups.

Statistical analysis was done using Analysis of Variance (ANOVA) test followed by post-hoc analysis using Bonferroni Test. p value less than 0.05 was considered to be statistically significant.

Figure 1: Materials used for smear preparation

Figure 2: Materials used for PAP Staining

Figure 3: PAP Staining procedure kit
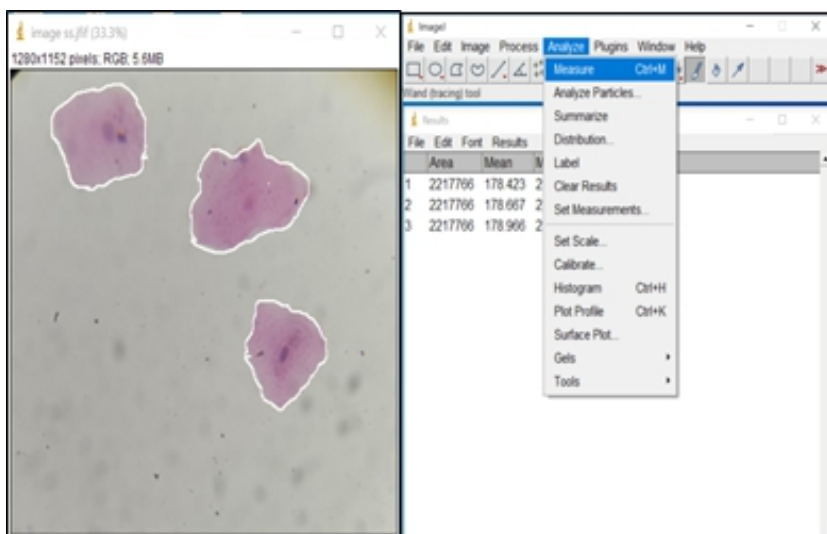

Figure 4:
Buccal mucosal smear collection

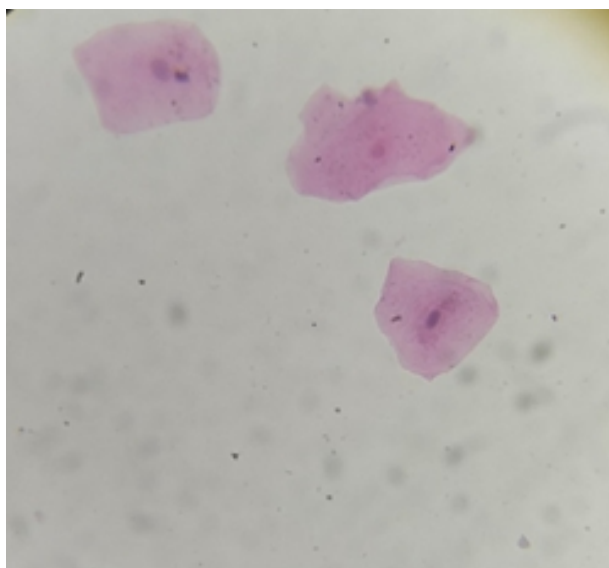Figure 5: Cytomorphological analysis using Image J Software

Figure 6:
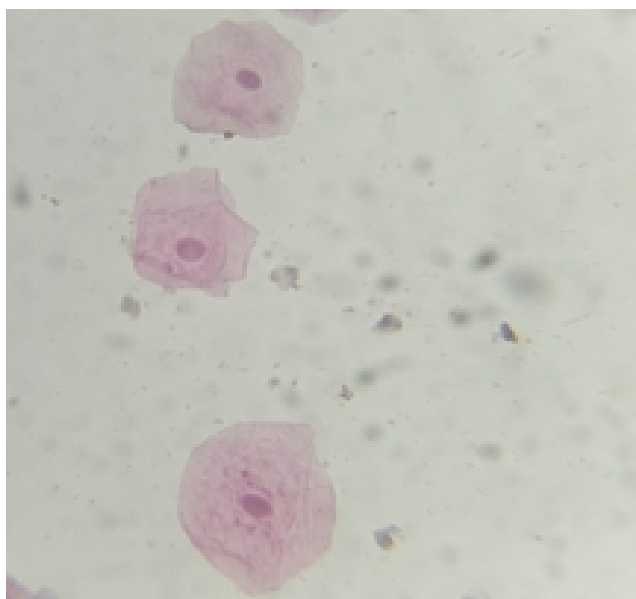PAP Stained cytological smear - Group I (18 to 30 years)

Figure 7 :
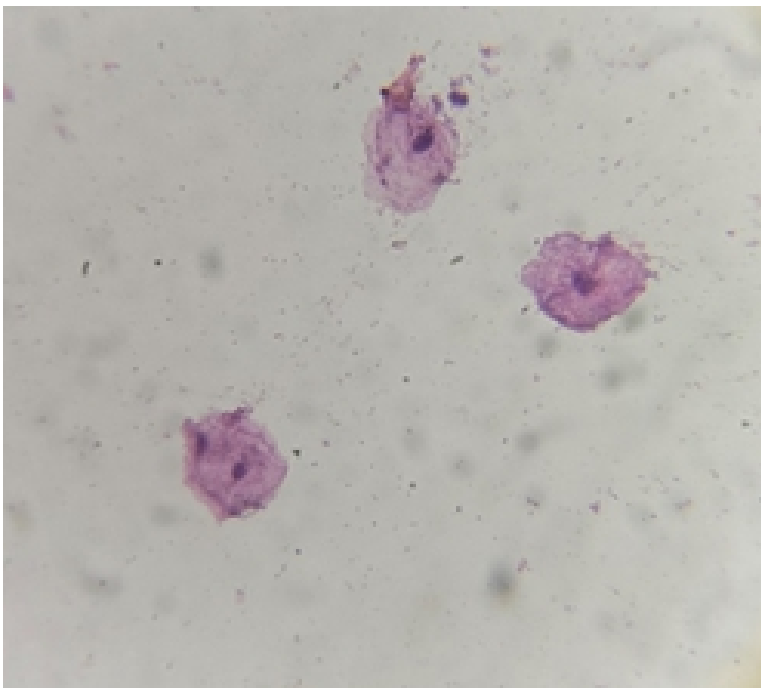PAP Stained cytological smear - Group II (30 to 45 years)

Figure 8:
PAP stained cytological smear -
Group III (45 to 60 years)

RESULTS:

In the present study, the samples were selected with equal gender distribution. The mean age group in Group I is 23.90, Group II is 38.75 and Group III is 52.25. The study sample has a total average cell size of 0.009 mm/sq. The average cell size in Group I is 0.011 mm/sq, Group II, the cell size is 0.010 mm/sq and in Group III showed average cell size of value 0.008 mm/sq. When comparing the cell sizes between the groups there was a statistically significant difference between the groupa. Inter group comparison showed statistically significant decrease in the cell size in Group III compared to Group I and II (table 1) The results showed that cell size decreases with increase in age.

Groups	N	Mean	Standard Deviation	p-value
Group – I (18 to 30 years)	20	0.011	0.002	$< 0.001^*$
Group – II (30 to 45 years)	20	0.010	0.002	
Group – III (45 to 60 years)	20	0.008	0.001	
Groups			**Mean Difference**	**p-value**
Group – I	**Group – II**		0.001	0.292
	Group – III		0.002	$< 0.001^*$
Group – II	**Group – III**		0.001	0.039*
p-value based on Analysis of Variance (ANOVA) Test followed by post-hoc analysis using Bonferroni Test after adjusted for multiple comparisons * = Statistically Significant (p < 0.05)				

Table 1 : Inter-group cell size comparison

Age
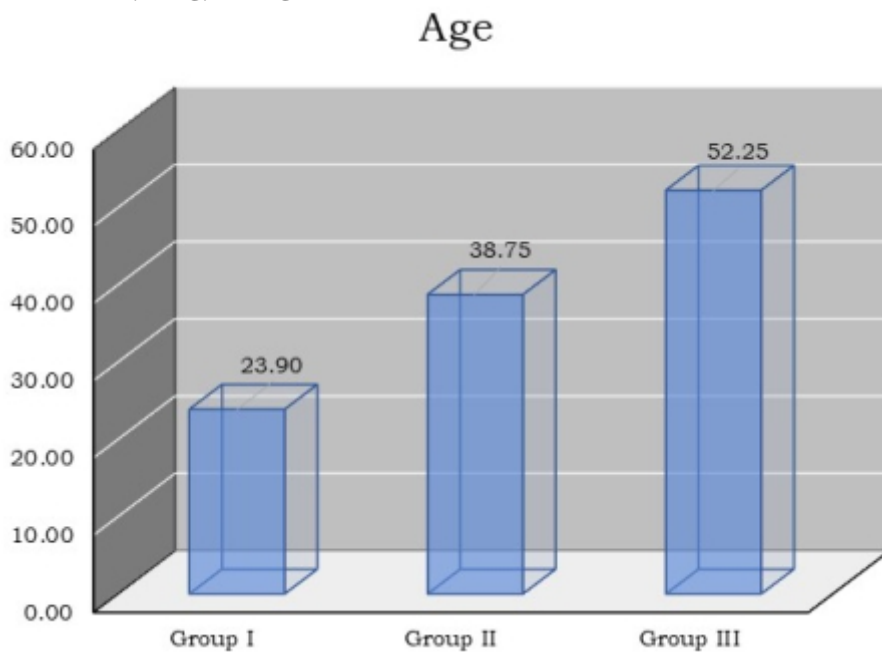
Graph 1: Age distribution
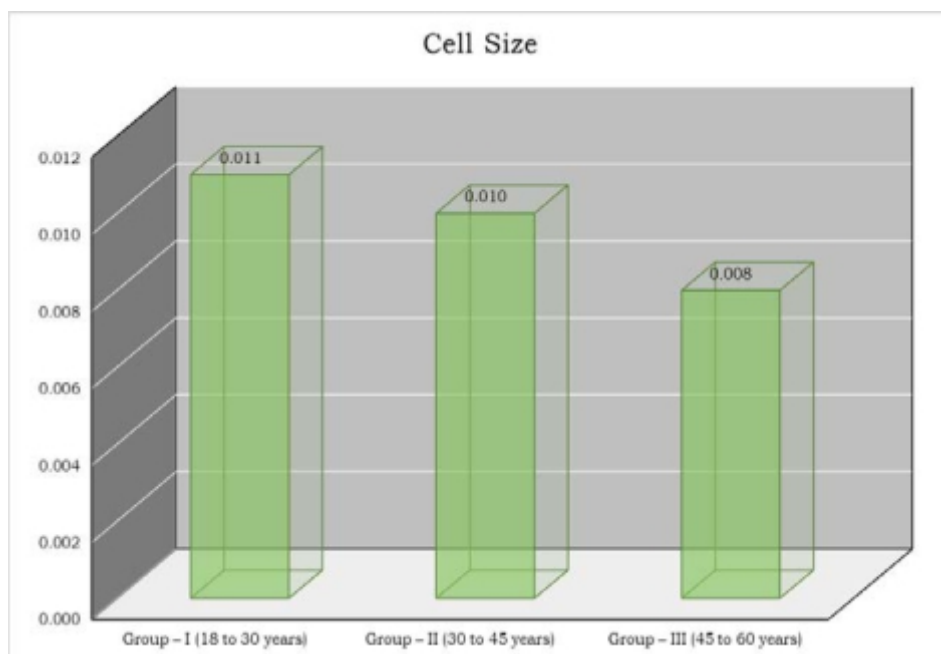
Graph 2: Variation in cell size

DISCUSSION:

There are many invasive and non-invasive methods available for age estimation in forensic odontology. The relevance of the technique lies in the fact that exfoliative cytology is used as a standard technique of screening many pathologies associated with oral cavity.[6] In 1951, Paul W Montgomery conducted extensive research on the exfoliative cytology findings in normal oral epithelium. Since then, there has been limited research on normal buccal mucosal smears.[3] Numerous support-

ing literatures are there explain the significance of exfoliative cytology used for the detection of oral premalignant, potentially malignant and malignant lesions.[7] But its role in age estimation needed further exploration. The typical oral epithelium has a stratified squamous structure, consisting of an innermost basal layer (stratum germinativum), a prickle cell or acanthotic layer (stratum spinosum), and a surface cornified layer. Scraping directly on the surface epithelium can dislodge all layers, including basal cells.[1] As a part of normal physiological turn over, the epithelial cells undergo

continuous renewal by migration from the basal layer to the superficial layer and undergoes exfoliation due to cellular senescence.[8] The parameters such as nuclear and cellular size, ratio, pleomorphism be analysed in exfoliative cytology technique.[4] As we age, our cellular activity, organelles, and epithelium turnover rate diminish, leading to a decrease in cell size.[8] Our cyto-morphometric analysis showed a significant age-related variation in cell size or area. This can be attributed to cellular senescence regardless of gender. The study showed that average cell size had variations between different age groups. Cyto-morphometric analysis of buccal cells had a range of cell size of 0.002 to 0.018 micrometer/sq.

Similar to our study, Nallamala et al conducted a research on age estimation using buccal mucosal smears and compared it with measurement of pulp tooth ratio and stated that, age estimation using buccal mucosal smears were more accurate as compared to that of the pulp tooth ratio, their investigation found a considerable decline in cell size with age, which is consistent with our findings, and supporting our study.[9]

Shetty et al, In their investigation of normal buccal mucosal smears, found a substantial decrease in average cell size with age, which is consistent with our findings.[1]

Sankari et al conducted a study on age estimation using exfoliative cytology and stated that age estimated by cell size is comparable to that of chronological age and that the cell size decreases as the age of the individual increases which is in accordance with our study.[10] The decrease in cell size can be attributed to aging process. Cell size from buccal mucosa smears can be used as a marker for age determination.

CONCLUSION:

Age estimation using exfoliative cytology is a valuable and non-invasive technique with various potential applications in the fields of medicine and forensic science. Our study demonstrates that due to ageing, there is a noticeable reduction in cell size. This suggests that age estimated by cell size is comparable to that of chronological age and that the cell size decreases as the age of the individual increases.[10] Therefore, the use of exfoliative cytology for age estimation can be considered a valuable adjuant when combined with other established methods.

Conflict of interest: None

Source of support: Nil

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