Original Article

Age Estimation by Exfoliative Cytology: New Era of Noninvasive Forensic Science

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Abstract

Background: Age determination in mass disaster is an important information that helps to identify the individual. Exfoliative cytology is one of the noninvasive techniques with minimal expenditure, which allows simple and pain-free collection of intact cells from different layers within the epithelium for examination. The present study uses exfoliative cytology smears from buccal mucosa, to estimate the age-related changes to guide the investigators for correct identification of unknown human bodies.

Aim and Objective: The aim of the study is to estimate the age of an individual from buccal smears.

Materials and Methods: Buccal smears were taken from 50 healthy individuals and analyzed for cellular and nuclear perimeter, using Dewinter's image analysis software (Version 4.3) using one-way ANOVA and Bonferroni method.

Results: There was a statistically significant reduction in the size of the cell with the age of the individual (P = 0.000). Nuclear size reduces with increasing age but was not consistent. NP:CP ratio increased with advancing age.

Conclusion: The cell size is a more reliable parameter to assess the age of the individual.

Key Words: *Age estimation, buccal smears, exfoliative cytology, morphometric analysis*

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Introduction

From time immemorial, ever since the modern man has evolved, his intelligence made him to conquer the world by discovering newer scientific technologies in various fields relating to survival of mankind.[1]

Forensic science is an advanced scientific arena where accurate identification of an individual either living or deceased is established using various techniques.

All human beings have their own identity in the form of fingerprints and DNA which is safeguarded even after death. Fingerprints are safe only if the body is not disfigured, but DNA is stored in every part of human body which is helpful in human identification.[1] However, DNA analysis is cumbersome and not straightforward.

Individual identification is an important aspect of forensic medicine and dentistry. Chronological age, as recorded by registration of birth date, is referred throughout an individual's life. Age is an important factor in clinical practice, research, and court of law. Age is estimated on the basis of chronological age, bone age, dental age, mental age and others.[2] The age estimation in children and adolescents depends on radiographic analysis of developing dentition and bones, especially when there is no clinical evidence.[3] In adults, however, various methods have been undertaken such as root dentin transparency, tooth wear, pulp/tooth ratio, and tooth cementum annulations. Most accurate laboratory method is racemization of aspartic acid in dentin or enamel. However, these methods are not cost-effective and have a tedious procedure.

Exfoliative cytology is a noninvasive, simple and pain-free technique for collection of intact cells from different layers within the epithelium for microscopic examination. This technique can be used for age estimation.

The use of exfoliative cytology as a diagnostic aid accentuates the need for establishing an accurate baseline. However, studies on oral epithelium are largely done in the pathological state. However, secrets of pathology can be explored only when the fundamental observations in normal oral mucosal cells are established. Donne, in 1945, first proposed that the size of microscopic objects could be detected. From then on, measuring cells and their components has been an intellectual challenge.[4]

The smear obtained by exfoliative cytology can be analyzed quantitatively and qualitatively. With advancements in the field of quantitative oral exfoliative cytology, various parameters

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such as nuclear size, cell size, nuclear-to-cytoplasmic ratio, nuclear shape, nuclear discontinuity, optical density, and nuclear texture can be evaluated collectively to confirm the diagnosis.[5]

The present study uses exfoliative cytology and image analysis for scrutinizing the buccal cells to estimate the cell and nuclear diameter and to quantify the age-related change in different age groups.

Materials and Methods

The sample size consisted of 50 patients, divided into five groups, 10 individuals from the age group of 20–30 years, 31–40 years, 41–50 years, 51–60 years, and above 60 years. Buccal smears were prepared from individuals of each age group. The patients, who presented with the history of systemic illness, tobacco use, or alcoholic consumption, were excluded from the study.

All smears were made using moistened wooden spatula. With a gentle scraping motion, scraped from the clinically normal appearing buccal mucosa and smeared on to a clear glass slide, immediately fixed with 95% ethanol for a minimum of 15 minutes, these smears were stained using Papanicolaou staining technique.

The stained smears were observed in a step-wise manner for image analysis, moving from left to right and then down and across, to avoid measuring the same cells again at 40X objective, and focused on the stage micrometer scale.

In all the cases, the cell perimeter (CP) and nucleus perimeter (NP) were measured in both the horizontal and vertical axes. Only clearly defined cells were measured, excluding the clumped or folded cells [Figures 1 and 2].

An average of 20 clearly defined cells were selected in each smear markings, marked manually using paint tool, projected on to the monitor and images were captured using a camera attached to Olympus BX-53 microscope. The average CP and NP values were obtained for each case by image analysis software and statistically analyzed using one-way ANOVA and Bonferroni comparison tests.

Results

The result showed statistically significant reduction in the size of the cell $P = 0.000$ as age increases. The nuclear size also showed reduction as age increases. However, Group 4 and 5 showed increases in the nuclear size. The NP to CP ratio was also calculated and was observed to be statistically significant. There was a statistically significant difference between various age groups in relation to cell size. Hence, the present study showed that cell size is more reliable to assess the age of the deceased individuals [Table 1].

The results showed that average cell size varied between different age groups. The average cell size varies from a minimum value of 13.7 to a maximum value of 21.03 μ, and average nucleus size varies from a minimum value of 2.3 to a maximum value of 3.2 μ. The average NP:CP ratio is increased with increasing age group [Graph 1].

Discussion

Forensic odontology is the branch of dentistry which, in the interest of justice, deals with proper handling of dental evidence, and with proper evaluation and presentation of dental findings.[6] Oral cytology plays a major role in preventing misdiagnosis of the lesions which are interpreted clinically. It is a simple chairside test which provides a predictive diagnosis. Exfoliative cytology is based on monitoring the exfoliated cells or cells that flake off the mucosa whether through natural or artificial means. Cytological specimens are recently analyzed for nuclear DNA content, immunohistochemical and molecular analysis.[7]

The present study was undertaken for age estimation in various age groups using exfoliative cytology. The results of this study showed a significant difference in the CP, NP and NP:CP in various age groups. This may be because, as the

Table 1: Showing the mean, standard deviation and ratio of perimeter of the exfoliated cells and their nucleus

Age groups	Mean cell perimeter	SD	Mean nuclear perimeter	SD	NP:CP ratio	SD
1	21.034	2.12	3.266	0.3	0.15	0.01
2	19.375	1.67	2.766	0.22	0.14	0.01
3	17.861	2.5	2.37	0.38	0.13	0.02
4	16.713	1.12	2.882	0.21	0.17	0.009
5	13.703	0.61	3.186	0.3	0.23	0.02
F	467.275		472.090		3.933	
Significant	1.09×10^{-63}**		8×10^{-144}**		3.83×10^{-25}**	

**Statistically highly significant $P<0.001$ cell size and nucleus size range and average cell size and nucleus size in different age groups. NP: Nucleus perimeter, CP: Cell perimeter, SD: Standard deviation

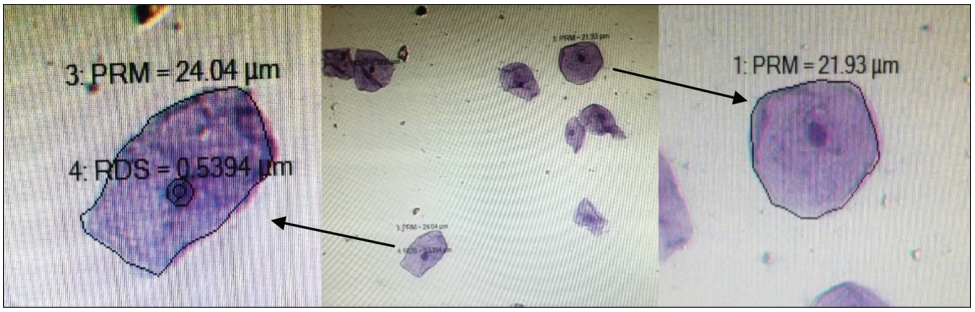


Figure 1: Photomicrograph showing cell perimeter and nuclear perimeter, (PAP, ×40)

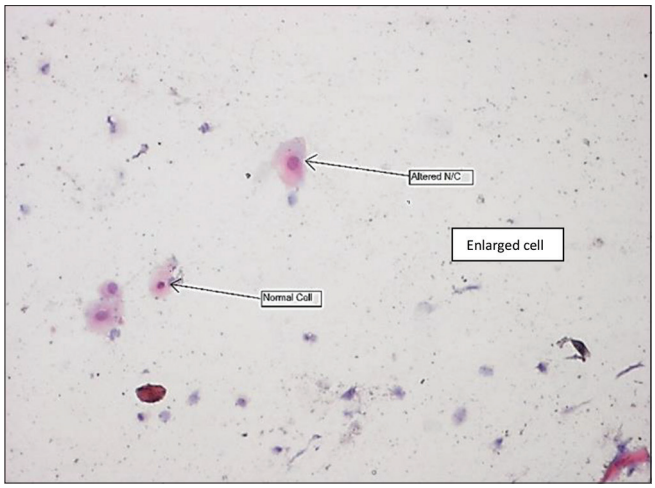


Figure 2: Photomicrograph showing normal cell and altered cell with increase in nuclear perimeter, (PAP, ×40)

age advances, the cellular activity and epithelial turnover rate decreases. Decrease in cellular organelles can be a possible reason for decrease in cell size.

Our study samples involved clinically normal individuals, with no systemic illness or habits. The present study was in accordance with the study of Shetty *et al*.[8] who reported the distribution of cell size with variable group of different ages has a significant difference, showing variation in cell size to be significant in different age groups.

Montgomery observed exfoliative cytology in the normal human mucosa. He observed differential counts of the three types of epithelial cells with definite cytologic patterns in various regions of the mouth which were proven statistically significant.[9]

Cowpe *et al*. described the development of quantitative cytological techniques and their application to oral smears. They observed significant variation in nuclear and cytoplasmic areas among different sites. Nuclear size varied significantly with advancing age; however, this was not the case for cytoplasmic area.[10]

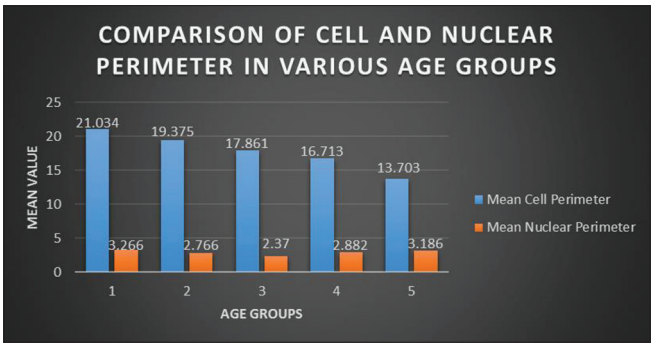
Anuradha and Sivapathasundharam showed age- and sex-related variations with advancing age in normal exfoliated gingival cells.[4]

There are studies on the comparison of cytomorphometric changes of oral mucosal cells in normal as well as premalignant and malignant cases and diabetes mellitus Type-2. Nivia *et al*.,[11] Acharya *et al*.,[12] Hande and Chaudhary[5] have done studies on tobacco and gutkha chewers, in premalignant and malignant individuals.

However, the present study is unique in its way to identify the age of various groups of individuals using exfoliative cytology and cytometry.

Conclusion

Cytomorphometric analysis of normal exfoliated buccal cells revealed that decrease in cell size and nucleus size can be attributed to the aging process. The comparison of cell size from buccal mucosa smears can be used as a marker for age determination.



Graph 1: The mean values of cellular and nuclear perimeters in various age groups. Cell size is an important marker of age estimation

However, there were certain limitations in this study. The sample size was small, the nucleus size increased in Group 4 and 5 and the NP:CP ratio showed variability in different age groups. More studies are required to be conducted with larger sample size to make exfoliative cytology and cytomorphometry an effective tool in forensic science.

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Conflicts of interest

There are no conflicts of interest.

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