

Exfoliative cytology: A possible tool in age estimation 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. **Objective:** The study was undertaken with an aim to estimate the age of an individual from their buccal smears by comparing the average cell size using image analysis morphometric software. **Materials and Methods:** Buccal smears were collected from 100 apparently healthy individuals. After fixation in 95% alcohol, the smears were stained using standard Papanicolaou laboratory procedure. The average cell size was measured using Dewinter's image analysis software version 4.3. 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. The difference was highly significant in age group of above 60 years. **Conclusion:** Age-related alterations are observed in buccal smears.

Key words: Cell size, exfoliative cytology, image analysis

Introduction

The identification of human bodies, where there are no clues as to the identity from circumstantial data, poses a difficult problem to the investigators. The determination of age and sex of the body can be crucial to the investigators to limit the search for individuals that could possibly match missing person lists and therefore, minimize efforts involving very unlikely alternatives. Although gender today can be determined with DNA methods, age determination is not as straightforward.^[1] Forensic Age Estimation (FAE) defines an expertise in forensic medicine which aims to define in the most accurate way the chronological age of person of an unknown age involved in judicial or legal proceedings.^[2]

Age estimation in children and adolescents often depends on morphological methods, such as radiological examination of skeletal and dental development.^[3] In adults, however, age estimation based on these methods is much less accurate. Current methods of age estimation include simple, yet less precise morphological methods (such as evaluation of dental or skeletal morphology), tooth wear, root dentine transparency, apposition of secondary dentine, OPG of the maxillary canines to study the pulp/tooth area ratio, tooth cementum annulations etc. More accurate laboratory methods such as racemization of aspartic acid in dentin or tooth enamel or radiocarbon dating of tooth enamel^[1] but are not cost-effective methods.

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

The mouth can be considered as an ideal site for observing the manifestations of aging. However, few of the associated signs and symptoms of age lend themselves to quantification.

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This is unusual, as there is continuous replacement of the oral epithelium, suggesting that this accessible tissue should provide objective indications of senescence. Thus, cytology can aid in age estimation of an individual by visualizing the cellular morphology.^[4]

Epithelial cells, as a part of normal physiological turnover, undergo continuous renewal. They move from the basal layer to the surface and are exfoliated. Oral exfoliative cytology is a simple, non-invasive, less time-consuming procedure with sensitivity of 89% and specificity of 89.5%.^[5]

The studies on oral epithelium are largely done in pathological state. Oral exfoliative cytological technique has been used for the detection of oral premalignant, potentially malignant or malignant lesions.^[6] But secrets of pathology can be explored only when the fundamental observations in normal oral mucosal cells are established. Since the initial studies of normal epithelial smears by Miller and Montgomery in 1951,^[1] there are only few instances where the normal buccal mucosal smears are studied. The use of oral exfoliative cytology in the past was limited due to the subjective nature of its interpretations and high false-negative results. These limitations were overcome by the introduction of quantitative methods such as image analysis systems, especially in the assessment of cytomorphometric cellular alterations.^[5]

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. Image analysis technology is programmed to analyze cells, replacing the human eyes. In 1960, Prewitt and Mendelson first conceived image analysis system for studying the leukocyte; subsequently, it was extended by Weid and his coworkers to study the cells in the cervical smears.^[6,7]

Morphometric analysis can be used in oral smears for diagnosis of malignant, premalignant conditions, diabetes mellitus (type 2) etc.

Old age is often accompanied by atrophy which refers to a decrease in the size and in the functional activity of a part which may be general or local. In local atrophy certain portions undergo changes which may be simple, degenerative, or numerical. In the simple variety the individual cells undergo a decrease in size.^[6]

The present study uses exfoliative cytology and image analysis for scrutinizing the exfoliated cells from buccal mucosa to estimate the cell size and to quantify the age related changes in the above variables.

Aims and Objective

To estimate the age of an individual from their buccal smears by comparing the average cell size using image analysis morphometric software.

Materials and Methods

The sample size consisted of 100 patients (47 females and 53 males), divided into 5 groups, 20 individuals from age group 5-15 years, 15-30 years, 30-45 years, 45-60 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 stepwise manner for image analysis, moving from left to right and then down and across, in order to avoid measuring the same cells again at 40X objective and focused on the stage micrometer scale. In all the cases, the cell sizes (Cs) were measured in both the horizontal and vertical axis in micrometer. Only clearly defined cells were measured, excluding the clumped or folded cells. An average of 20 clearly defined cells were selected in each smear markings were marked manually using paint tool, projected on to the monitor and images were captured using Olympus camera attached to Olympus BX-41 microscope. The average cell size (Cs) values were obtained for each case and statistically analysed using one-way ANOVA, Bonferroni comparison tests.

Results

The cell size in group 1 ranged from 0.008 mm/sq to 0.017 mm/sq with an overall average of 0.012 mm/sq. In group 2 the cell size varied from 0.009 mm/sq to 0.015 mm/sq with average of 0.011 mm/sq. Group 3 showed variation in cell size from a value of 0.008 mm/sq to 0.013 mm/sq with average cell size of 0.010 mm/sq [Figure 1]. In group 4 the cell size showed variation from a minimum value of 0.008 mm/sq to a maximum value of 0.014 mm/sq and a average value of 0.010 mm/sq, group 5 showed cell size ranging from 0.002 to 0.009 mm/sq and average cell size of 0.006 mm/sq.

The Pearson correlation coefficient for variable cell size was found out to be -0.692 ($P = 0.00$, $P < 0.005$) which was statistically significant showing that the cell size decreases with increase in age. The distribution of cell size with variable

group of different ages has significant difference, showing variation in cell size to be significant in different age groups.

Discussion

Role of exfoliative cytology can be regarded as a standard technique in screening of oral pathologies.^[7] Cytomorphometric analysis or image analysis of exfoliated cells has also been suggested as a key approach to define and identify the cellular and nuclear changes in cytological smears.^[11] The normal exfoliative cytology of the oral epithelium had been thoroughly studied by Miller and Montgomery. The histological structure of the normal oral epithelium is a stratified squamous type with an innermost basal layer (stratum germinativum), a prickle cell layer or acanthotic layer (stratum spinosum), and a surface cornified layer (stratum corneum). The direct scrapings of the surface epithelium may dislodge all the layers of epithelium including the basal cells. The basal cells are small oval cells approximately five times the diameter of the leukocytes, the nucleus is relatively larger with a diameter of about one-third to one-quarter of the cell. Cytoplasm of the basal cells is basophilic.^[7] Prickle cells are intermediate in their characteristic between the basal cells and cornified cells. The prickle cells are considerably larger than the basal cells, its outline is irregular and it tends to be more flat. The nucleus is usually round and smaller than the basal cell nucleus. The overall staining of the cytoplasm varies from pink to faint orange. The cornified cells are orangeophilic. As the epithelial cell matures, it is compressed and flattened until it assumes a wafer shape, the physiological activity of the nucleus decreases and the nuclear chromatin condenses to a dense structure less mass (pyknosis). As the cell continues to cornify, the nucleus disappears entirely leaving thin squamous epithelium.^[5] Morphometric analysis can be used in various conditions such as malignant, premalignant conditions,^[8] diabetes mellitus (type 2), etc. Previously, cytomorphometric analysis was done by using planimetric methods but with time, planimetric methods have been replaced by computer-assisted image analysis techniques, which are faster, more accurate, and more reproducible.^[9] The cytomorphometric result of the present study revealed an age-related significant variation in cell size i.e., area age-related variation, irrespective of gender can be ascribed 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.^[11] 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 cellular organelles decreases which could be the reason for the decrease in cell size.^[10] The results show that average cell size varied between different age groups. The average cell size cell

Table 1: Cell size range and average cell size in different age groups

Group no.	Age group (years)	Cell size range (mm/sq)	Average cell size (mm/sq)
1	5-15	0.008-0.017	0.012
2	15-30	0.009-0.015	0.011
3	30-45	0.008-0.013	0.010
4	45-60	0.008-0.014	0.010
5	60 yrs and above	0.002-0.009	0.006

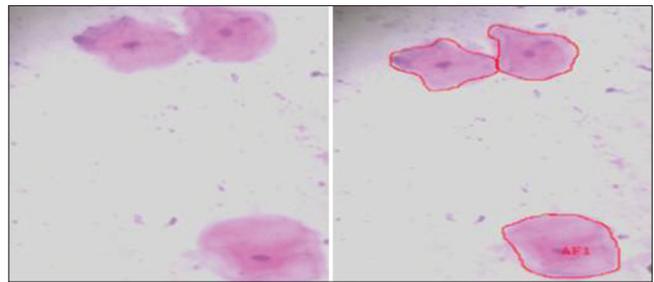


Figure 1: Cytological smear of 35-year-old male (x40)

varies from a minimum value of 0.002 to maximum value of 0.017 mm/sq [Table 1].

Conclusion

Cytomorphometric analysis of normal exfoliated buccal mucosa revealed that cell size varied from 0.002 to 0.017 mm/sq. The decrease in cell size can be attributed to aging process. Age determination can be used in personal identification of an individual. Comparison of cell size from buccal mucosa smears can be used as a marker for age determination. However, there were certain limitations in this study as the sample size was small comprising 100 samples thus representing small population with uneven distribution of female to male ratio. Various pathologies can also alter cell size for e.g., Diabetes (type 2). Progress in research in this area shows promise in contributing to investigation in a professional, unbiased, ethical, and truthful manner.

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