Original Research Article

ORAL SQUAMOUS CELLS AND AGE ESTIMATION IN EXFOLIATIVE CYTOLOGY WITH HEMATOXYLIN AND EOSIN STAIN– A QUANTITATIVE STUDY

Running title: Age estimation and exfoliative cytology

ABSTRACT

Introduction

Exfoliative cytology in age estimation is a simple, painless, less invasive collection of exfoliative cells from epithelial layers, used as a diagnostic aid for age estimation. The oral cavity is an ideal site for exfoliative epithelial cells with a physiological turnover of cells, turnover decreases as the age increases show age variation with cellular morphological changes. Age estimation is one of the important factors to identify an individual and also helps to know the chronological age of a person.

Aim

To analyze and estimate the age from buccal smear and comparing the average cellular size under Image morphometric analysis.

Materials and methods

Buccal mucosal smears are taken using a wooden spatula in gentle motion of scraping and smeared on a clean glass slide and fixed in 95% ethanol immediately after smearing a minimum of around 15 minutes and stained with Haematoxylin and eosin stain. After staining, the cells were observed by microscope and measured by a paint tool. Pearson correlation analysis was done using SPSS software.

Results

The cell and nuclear size difference values observed using a Pearson correlation coefficient were statistically significant with pvalue<0.05 revealing that there is shrinkage in cells with increase in age.

Conclusion

Exfoliative cytology is a successful and vastly growing technology that is used for the detection of premalignant lesions.

Keywords:age, buccal smear, cell size, estimation, exfoliative cytology, innovative technique, nuclear size.

INTRODUCTION

Exfoliative cytology in age estimation is a simple, painless, less invasive collection of exfoliative cells from epithelial layers, used as a diagnostic aid for age estimation. (1) Both quantitative and qualitative analyses of exfoliated cells are obtained with cell size, shape, Nuclear cytoplasmic ratio, nuclear density, and texture for diagnosis. (2)

Age estimation is one of the important factors to identify an individual also helps to know the chronological age of persons in judicial proceedings, mass disasters, child marriage, elections, civil issues, refugees, dentistry, forensic, anthropology, and also in diseased conditions. The oral cavity is an ideal site for exfoliative epithelial cells with a physiological turnover of cells, turnover decreases as the age increases show age variation with cellular morphological changes. (3) Cytological smears are also used in Immunohistochemistry in tumor cell identification, RNA, DNA extract, and epigenetic alteration. (4) Identification of humans with sex and age helps in matching the missing humans through exfoliative cytology.(5) Variation in cytomorphology and nuclear morphology changes in oral epithelial cells are scraped and stained for cytological study and demonstration. (6)

In the last few years, exfoliative cytology is the most common diagnostic methodology. Few reasons like errors in findings, less number of samples, and interobserver bias give false-negative results. For precise results with fewer false-negative results parameters like nuclear and cytoplasmic areas, the N: C ratio has to be evaluated correctly. (7) Johnston (1952) measures nuclear-cytoplasmic (N: C) ratios of normal and malignant epithelial cells(8). Reagan (1957) underwent an elaborative study on the significance of normal and malignant cells. undertook a more extensive study.(9) Gold and stats(1963) analyzed factors like nuclear-cytoplasmic ratios from exfoliated cells of the oral cavity. (10) Cowpe(1985) oral smears collected from diseased mucosa and

contralateral areas of normal mucosa observed nuclear-cytoplasmic ratio are altered in the normal and diseased site. (11)

Other methods like Radiovisiography for morphometric analysis of pulp-tooth ratio in lower canine(3)Exfoliative cytology is based on microscopic examination of exfoliated epithelial cells after fixation and staining of the smears. Methods for collecting the samples are by Direct method by rubbing the mucosal surface, Indirect method is by aspiration with exfoliated cells and by Imprint method. The exfoliated cells are preserved in 10% methanol and stained.(12). Our team has extensive knowledge and research experience that has translate into high quality publications (13).(14–27) ,(28–32)

This study describes the quantitative analysis of age estimation with the oral smears where changes in nuclear size are seen with increasing age. Normal squamous cells can be collected from buccal mucosa, tongue, the floor of the mouth, gingiva, etc., This study was done quantitatively from the oral buccal smear and stained with Haematoxylin and eosin and examined for age-related changes in cells. The study aims to analyze and estimate the age from buccal smear and compare the average cellular size under Image morphometric analysis.

MATERIALS AND METHODS

The study was conducted in the department of pathology of Saveetha dental college with the ethical approval committee with ethical approval number IHEC/SDC/BDS/1955/01. The study was a simple, painless, and less invasive study and cost-efficient but included smaller populations. A sample size of 30 patients, divided into Group I as 10 individuals under the age group of 20-30 years of age, Group II 10 individuals above 60years of age, and GroupIII 10 individuals as control groups. Sample bias was stratified, validation of the procedure was done by a guide and expert pathologist. Smears are taken from the buccal mucosa from individuals of different age groups. Individuals with oral pigmentation, premalignant lesions, any other systemic illness, smoking, and alcohol habits are all excluded from the study.

Buccal mucosal smears are taken using a wooden spatula in gentle motion of scraping and smeared on a clean glass slide and fixed in 95% ethanol immediately after smearing a minimum of around 15 minutes and stained with Haematoxylin and eosin stain.

The stained smears image analysis was done by moving the slides in a zigzag manner from right to left so that to avoid imaging the same cells again at 40X. Cell size has to be measured in both vertical and horizontal manner in image analysis software in micrometer(mm). Cells without overlapping are taken. 10 clear cells are taken

from each slide and marked in a paint tool; manually projected images are captured in Olympus BX 41 Microscope. The cell and nuclear size difference values are observed using a Pearson correlation coefficient and were done using the software SPSS version 23. Dependent variables included age and independent variables included gender, height, weight.

RESULTS:

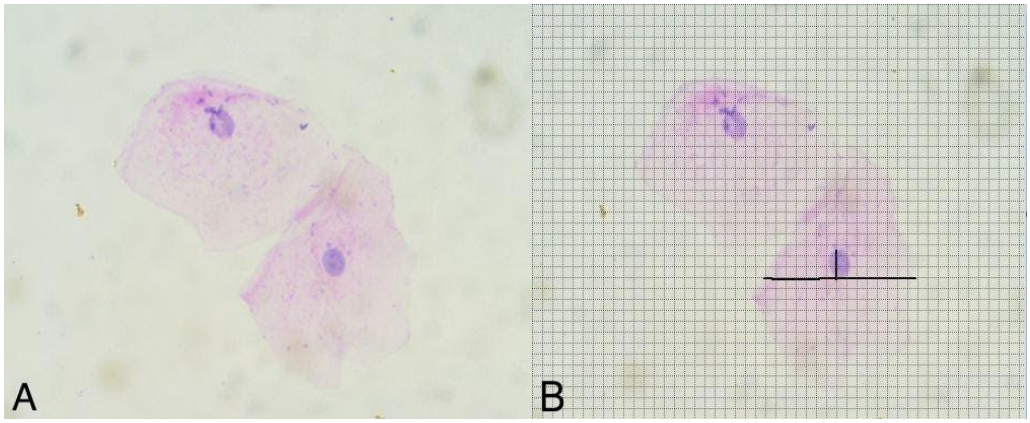

Figure 1: (A) Represents the Haematoxylin and eosin-stained image (40x) of 30years old. (B) represents the measurement of a cell and nuclear diameter vertically and horizontally.

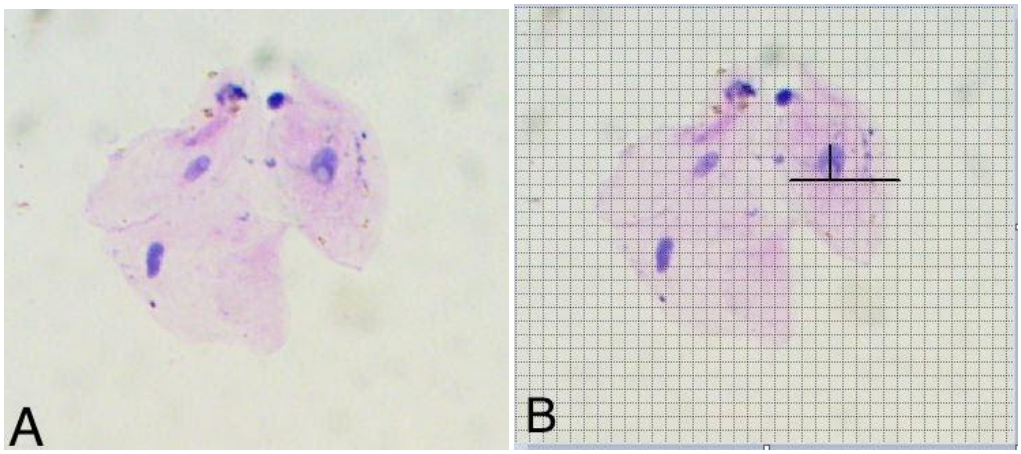

Figure 2: (A) Represents the Haematoxylin and eosin-stained image (40x) of 60 years old. (B) represents the measurement of a cell and nuclear diameter vertically and horizontally.

Table 1: Cell diameter comparison between age groups showed Significant differences using the Pearson correlation coefficient.

GROUP	AGE (years)	MEAN (µm)	Regression coefficient
Group I	>30	53.77	0.889
Group II	<60	41.40	0.876

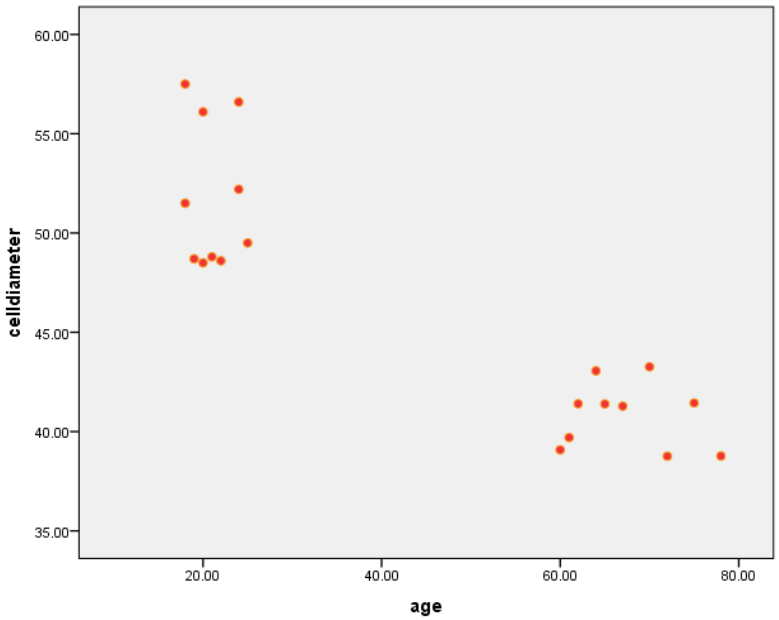

Figure 3: Dotted graph depicting the relationship between age and cell diameter (n=20) with the decrease in cell diameter with an increase in age. The X-axis represents the age in years and the Y-axis represents cell diameter (µm).

Table 2: Nuclear diameter comparison between age groups showed Significant differences using a Pearson correlation coefficient.

GROUP	AGE (years)	MEAN (µm)	Regression coefficient
Group I	>30	8.09	0.869
Group II	<60	5.79	0.899

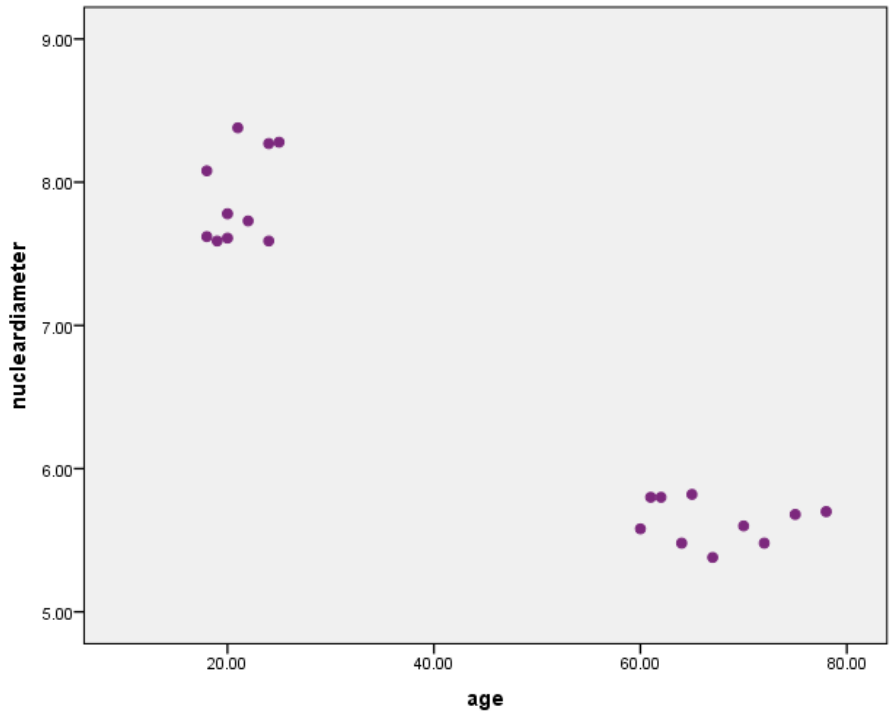

Figure 4: Dotted graph depicting the relationship between age and nuclear diameter (n=20) with the decrease in nuclear diameter with an increase in age. The X-axis represents the age in years and the Y-axis represents the nuclear diameter (µm).

DISCUSSION:

The age estimation of Pearson's correlation where the (Figure 1) depicts Haematoxylin and eosin-stained image (40x) of 30 years old and (Figure 2) depicts Haematoxylin and eosin-stained image (40x) of 60 years old. (Table 1) represents the cell diameter comparison between age groups showing a significant difference using the Pearson correlation coefficient. The average cell size varies from a minimum value of 40 µm/sq to a maximum value of 50 µm/sq**. (**Table 2) represents the nuclear diameter comparison between age groups showing a significant difference using the Pearson correlation coefficient. The cell size in group 1 ranged an overall average of 53.77 µm/sq. In group 2 the cell size varied ranging from an average cell size of 41.50 µm/sq. (Figure 3) depicts a dotted bar graph showing the relationship between age and cell diameter (n=20) with the decrease in cell diameter with an increase in age. (Figure 4) depicts a dotted bar graph showing the relationship between age and nuclear diameter (n=20) with the decrease in nuclear diameter with an increase in age. The results show that average cell size is diverse between different age groups. The Pearson correlation coefficient for variable cell size was found to be $r = 1$ which was statistically significant showing that the cell size

decreases with an increase in age. The distribution of cell size with various groups of different ages has significant differences, showing variation in cell size to be significant in different age groups.

Oral exfoliative cytology is a common technique for age estimation and screening oral pathological condition is a standard technique in medical cases and legal issues. (33) Exfoliative cytology involves calculating cytomorphological changes in the cell with the nuclear-cytoplasmic ratio which comprises all the layers of the keratinized and non-keratinized layers of the epithelium. The staining is from pink to orange. (2) Stratum corneum is the compressed cell with condensed nuclear chromatin called Pyknosis followed by the disappearance of the nucleus with thin cornified cells.(34) Exfoliative cytology along with computer-based image analysis of cell size is an accurate, faster, accurate, and easier method. With increasing age variation of cell size irrespective of gender, determination reveals the repeated division of basal cells with the decreased renewal of cells followed by senescence of cells with increasing age also influenced by environmental factors with decreased epithelial turnover and cell organelles(35) (36). The morphology of normal basal cells is normal cell size with a larger nucleus around one-fourth of the cell size with cytoplasm which is basophilic. Prickle cell layer with cell size larger than stratum basale but smaller and intermediate nuclear size with flat and irregular cell size.

The limitations of the study included sample size, keratinized lesions show negative cytology, larger sampling size, and development in the technology of exfoliative cytology may overcome the limitation of the study. The future scope can be that exfoliative cytology is a promising diagnostic technology for premalignant or malignant lesions. It can be used as a diagnostic tool in the medical field.

CONCLUSION

Even though exfoliative cytology cannot take the place of biopsy for detecting the nature of lesions, it is vastly concerned in the estimation of oral lesions. Early detection of a premalignant oral lesion can help to increase the survival rate of patients suffering from pernicious conditions. Further studies with a larger study population will promise the role of oral exfoliative cytology.

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