Original Article

Exfoliative Cytology for Age Estimation: A Correlative Study in Different Age Groups

Vadivel Ilayaraja, Thambu Keerthi Priyadharshini, Nalliappan Ganapathy, Andamuthu Yamunadevi, Janardhanam Dineshshankar, Thangadurai Maheswaran

Department of Oral Pathology and Microbiology, Vivekanandha Dental College for Women, Tiruchengode, Tamil Nadu, India

Abstract

Background: Oral exfoliative cytology is a simple and relatively pain-free procedure which can be carried out repeatedly with minimum discomfort to the patients. Oral exfoliative cytology has a sensitivity of 89.5%; its accuracy could be enhanced by cytomorphometric analysis of the cells. **Aim and Objective:** The present study was undertaken to evaluate the cell diameter (CD), nuclear diameter (ND), and nuclear-cytoplasmic ratio (N:C) and their variation with age using buccal smears. **Materials and Methods:** Buccal smears were collected from 100 apparently normal healthy individuals. The patients were divided into five groups 10–20, 21–30, 31–40, 41–50, and more than 50 years. The smears were fixed and stained. The CD and ND were measured using MS paint tool. The CD, ND, and nuclear-cytoplasmic (N:C) ratio were calculated for each case. **Results:** The cytomorphometric analysis of the exfoliated normal buccal mucosal cells revealed that there is a decrease in CD and ND with increasing age. No variation is found in N:C ratio. **Conclusion:** Age-related changes are observed in buccal smears, which could be helpful in age estimation.

Keywords: *Cell diameter, cytomorphometric analysis, exfoliated buccal cells, nuclear-cytoplasmic ratio and nuclear diameter*

Introduction

In forensic medicine, estimating the chronological age of a person involved in judicial or legal proceedings is very important.[1] Chronological age is determined by the date of birth and the period of time or number of years elapsed after that to any point of time.[2] There is no medical test to find the accurate chronological age of a human being. Anyway, this type of expert report is needed in everyday practice in Justice Courts and other Public Institutions by forensic physicians and Legal Medicine Institutes.[1]

Exfoliative cytology is a noninvasive, simple, and less time-consuming procedure which allows pain-free collection of intact cells from various layers within the epithelium for microscopic examination. It has a sensitivity of 89% and specificity of 89.5%.[3,4] In exfoliative cytology, various parameters such as nuclear size, cell size, cell and nuclear pleomorphism, nuclear membrane discontinuity, degenerative changes of nucleus, and nuclear-cytoplasmic ratio were analyzed. Nuclear-cytoplasmic ratio was considered to be more significant among all the parameters.[3]

The oral cavity can be an ideal site to observe the manifestations of aging. However, few of the associated signs and symptoms provide themselves as a factor for quantifying age. Epithelial cells undergo continuous renewal as a part of normal physiological turnover, but as age progresses, the renewal capacity of tissues is declined showing age-related variation irrespective of gender.[5] Thus, cytomorphometric analysis of these exfoliated cells can aid in age estimation of an individual by visualizing the cellular morphology.

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Address for correspondence: Dr. Thambu Keerthi Priyadharshini, Department of Oral Pathology and Microbiology, Vivekanandha Dental College for Women, Elaiyampalayam, Tiruchengode, Tamil Nadu, India.
E-mail: drtkeerthi@gmail.com

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Since there are conflicting data regarding the age changes in human buccal cell dimensions, the present study was proposed to assess the age changes of exfoliated buccal cells which may be helpful in forensics for age estimation.

Aim and objective

To estimate the cell diameter and nuclear diameter and nuclear-cytoplasmic ratio of buccal cells of all the study participants.

To compare the cell diameter and Nuclear diameter and nuclear-cytoplasmic ratio between various age group.

Materials and Methods

A total of 100 apparently healthy subjects with 20 in each age group were selected randomly from Outpatient Department of Vivekanandha Dental hospital. The subjects were divided into five age groups:

- Group I – 10–20 years,
- Group II – 21–30 years,
- Group III – 31–40 years,
- Group IV – 41–50 years
- Group V – >50 years.

Samples were collected from the patients who visited our outpatient department. Clinically normal individuals without past history of systemic disease or therapeutic medications were included in the study. Patients with a history of systemic illness, oral-related habits such as smoking, tobacco usage, and alcohol consumption were excluded from the study.

Scrapings were made from the buccal mucosa with moistened wooden spatula, then smeared on to a clear glass slide and immediately fixed with alcohol (Biofix spray fixative). Papanicolaou stain (Bio Lab Diagnostics) was used for staining the slides. The cells were examined using ×40 objective (Leica DMD108 microscope) and projected onto the monitor through camera attached to the microscope. A screenshot of each slide was captured and transferred to the computer for image analysis [Figure 1].

For each subject, 25 cells were chosen. Unfolded cells with clear outline were selected excluding the clumped or folded cells. Cell diameter (CD), Nuclear diameter (ND), and nuclear-cytoplasmic ratio (N:C) of 25 cells for each subject were recorded, and the average was found for all cells belonging to each age group. Sampling was done in a stepwise manner, moving the slide from left upper corner to right and then down to avoid measuring the same cells again. Photographs were exported to MS paint and viewed under gridlines command. A line is drawn along the maximum diameter of the cell and nucleus.

Number of grids from one end to the other end of the line is counted separately for each cell and nucleus diameter [Figure 2]. N:C ratio was then calculated. Mean of CD, ND, and N:C was calculated separately for all five groups. The obtained values were statistically analyzed using one-way analysis of variance, to find the difference in CD, ND, and N:C. Tukey's HSD (honest significance difference) *post hoc* test was done to identify the significance between various age groups. Ethical clearance was obtained from the Institutional Ethics Committee.

Results

Buccal smears were collected from 100 subjects with 20 subjects in each age group and CD, ND, and N:C ratio were measured. There was a statistically significant difference in ND and CD in different age groups. The average CD of each group is shown in Table 1. Mean CD was found to be 50.43 µm. CD appears to show a gradual decrease with increase in age. Table 2 shows ND measurements. Average ND is found to be 7.18 µm. ND displays a variation with age groups. N:C for all five groups was calculated manually and shown in Table 3. Mean N:C is found to be 0.146. The N:C ratio is found to fluctuate in different age groups with no specific pattern and is not statistically significant.(P = 0.490).

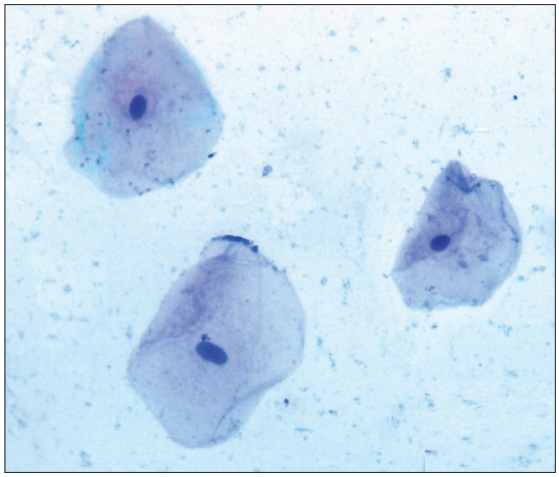

Figure 1: Cytological smear taken from buccal mucosa

Table 1: Comparison of cell diameter within age groups using one-way analysis of variance and Tukey - honest significance difference procedure

Groups	Age	Mean (µm)	SD	Significance
I	10-20	51.97	8.89	1 versus 2, 3, 4, 5*
II	21-30	54.77	8.95	2 versus 3, 4, 5*
III	31-40	53.82	10.54	3 versus 4, 5*
IV	41-50	50.21	11.52	4 versus 5*
V	>50	41.40	10.40	
Total		50.43	10.06	P<0.001 (HS)

*Statistically significant. SD: Standard deviation, HS: Highly significant

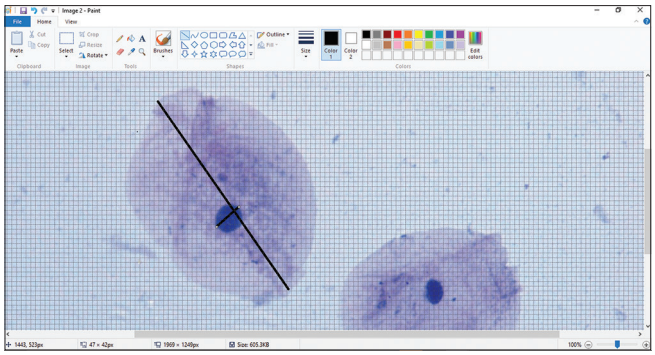

Figure 2: Screenshot demonstrates the measurement of cell diameter and nuclear diameter in MS paint tool with Grindline selection

Discussion

Exfoliative cytology has been used as a standard screening aid for oral malignancy and premalignancy. The normal exfoliative cytology of the oral epithelium had been thoroughly studied by Miller and Montgomery.[5] Earlier cytomorphometric analysis was done using planimetric methods, but with time, planimetric methods have been replaced by computer-assisted image analysis techniques, which are faster, more accurate, and reproducible.[6] Hence, cytomorphometric analysis or image analysis of exfoliated cells has been suggested as a key approach to define and identify the cellular and nuclear changes.[5]

A number of studies have been carried out to evaluate the influence of systemic and local factors on normal cells. Factors such as smoking,[7] tobacco chewing,[8] anemia,[9] and diabetes mellitus[10] have shown cytomorphometric changes in exfoliated cells of oral cavity. It is also used in early detection of premalignant and malignant conditions of oral mucosa.[11] Fluctuations in hormonal levels also had an influence over the buccal cell morphology.[12] Other factors such as radiotherapy, chemotherapy, and medications are the possible factors that could contribute to the morphometric changes in the cells.[13] The cytomorphometric analysis result of the present study revealed that there was a decrease in CD and ND across different age groups.

The results show that average cell size varied between different age groups. When considering the cell diameter, in our study, there was a constant decrease in CD with age progression. Lee *et al.* could detect no significant variation in cell size in relation to age, between 6 and 80 years.[14] Cowpe stated that there is a significant variation in CD in his study from the smears taken from 75 patients obtained from various sites.[15] Nayar *et al.* found significant variation with a decrease in CD with age similar to our study.[16] In the study, among the 80 subjects of Karnataka population, Patel *et al.* indicated significant variation in cytoplasmic area with different age groups.[6]

Table 2: Comparision of nuclear diameter within age groups using one-way analysis of variance and Tukey - honest significance difference procedure

Groups	Age	Mean (µm)	SD	Significance
I	10-20	7.59	1.56	1 versus 2, 3, 4*, 5*
II	21-30	8.08	1.65	2 versus 3, 4*, 5*
III	31-40	8.01	1.99	3 versus 4*, 5*
IV	41-50	6.40	1.04	4 versus 5
V	>50	5.80	1.40	
Total		7.18	1.52	*P*<0.001 (HS)

*Statistically significant. SD: Standard deviation, HS: Highly significant

Table 3 : Nuclear-cytoplasmic ratio comparison within age groups using one-way analysis of variance

Groups	Age	*n*	Mean (µm)	SD	*P*
I	10-20	25	0.149	0.029	0.490
II	21-30	25	0.150	0.033	
III	31-40	25	0.155	0.053	
IV	41-50	25	0.135	0.043	
V	>50	25	0.145	0.037	
Total		125	0.146	0.039	

SD: Standard deviation

A study was done by Shetty *et al.,* using buccal smear exhibited a significant difference of CD similar to our study.[5] The reason attributed for this variation is 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. These cells which stay for a longer duration in oral cavity succumb to the effect of various local environmental factors.[5]

When considering the ND, our study shows a constant decrease in ND except for the first group and second group. Lee *et al.* reported no significant variation in ND with age.[14] Cowpe in his pilot study performed with clinically normal buccal squames displayed no statistically significant variation in ND.[15] Nayar *et al.* showed significant variation with an increase in ND with age.[16] Patel *et al.* presented significant variation in NA with age.[6]

When we consider the N:C ratio, our study shows no significant difference in the N:C ratio across different age groups and remains constant. The study done by Scott *et al.* reveals that there was a reduction in N:C ratio with advancing age from the cells of lingual mucosa.[17] Reddy *et al.* showed increase in N:C with age as there was a significant elevation in mean nuclear area and significant reduction in mean cytoplasmic area.[3] In our study as there was a proportionate decrease in the nuclear and cell diameter, the N:C ratio remains constant. Limitations of this study include small sample size with only 20 subjects

in each age group. In this study, measurements were not done in the age group of 1–10 years and subjects were not divided into different age groups beyond 50 years.

Although there are numerous methods available for age estimation such as visual examination, physical and chemical methods, and histological and radiographic methods, this semi-invasive cytomorphometric method can also be added to them. Thus, a combination of methods can reduce the biological variations and uncertainty associated with age estimation.

Conclusion

The present study shows that there is a correlation between CD, ND, and the age of the individual. As the age progression, there is a constant decrease in CD and ND. The cell size is influenced or altered in many other systemic conditions; hence, all those parameters have to be considered. One limitation of this study is that it cannot be used in deceased individuals. This study has to be carried out in a large number of samples to get a better objective baseline for estimating the age.

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Conflicts of interest

There are no conflicts of interest.

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