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Cytomorphometric Analysis of Oral Exfoliative Cells for Age Estimation

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Abstract

INTRODUCTION:

The estimation of the age and sex of the human body is important to the investigators as it can reduce the time taken to compare the body with the missing person's reports and find a possible match. This could tremendously improve the identification process which of high interest in forensic odontology.

AIM:

The aim of this study is to estimate the age of an individual by comparing the average cell size of their oral smears, GIMP 2.10 morphometric software.

MATERIALS AND METHODS:

The study sample included oral smears collected from 30 patients; 15 male and 15 female patients. Oral smears for each patient was taken from 6 sites; Right buccal mucosa, left buccal mucosa, junction of the

hard and soft palate, dorsum surface of the tongue, floor of the mouth and lower labial region. The smears were subjected to papanicolaou stain. The stained smears were observed under a microscope for image analysis. The average cell size of the exfoliative cells was calculated using GIMP 2.10 image analysis software.

RESULTS:

The cytomorphometric analysis of oral smears from various age groups showed a decrease in cell size from 0.11mm/sq to 0.0677mm/sq.

CONLUSION:

Cytomorphometric analysis of exfoliated cells of oral mucosa using GIMP 2.10 image analysis software can serve as a potential alternative non-invasive procedure in evaluation of age of an individual compared to the other screening modalities, which are usually either expensive or invasive.

Keywords: Age estimation, cytomorphometric analysis, oral exfoliative cytology, papanicolaou stain, image analysis

INTRODUCTION

Identification of person/people is the prime priority when it comes to forensic odontology as it is the first step in the investigation process. The identification of people, with sparse clues to their identity, poses a difficult problem to the investigators. The estimation of the age and sex of the human body is important to the investigators since it can reduce the time taken to compare the body with the missing person's reports and find a possible match(Shetty *et al.*, 2015). Determination of age is not as simple as it sounds, unlike gender determination, which can be done using various methods including DNA analysis(Sakuma *et al.*, 2012). Age estimation of children and young adults are done mostly using radiological examination of skeletal development and dental eruption status. In adults, estimation of age can be quite difficult and less accurate(Willems, 2001). Age estimation methods for adults that are currently used include skeletal and dental morphology, teeth wear faucets, root dentin transparency, secondary dentin apposition, pulp tooth area ratio of maxillary canines, tooth cementum annulations etc.. Other methods include laboratory techniques such as radiocarbon dating of the tooth, but these are comparatively expensive(Sakuma *et al.*, 2012).

One other technique that can be used for age estimation is "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(Reddy *et al.*, 2012; Shetty *et al.*, 2015).

Oral epithelial cells undergo physiological turnover as a part of their routine. The cells proliferate and move from the basal layer to the superficial layer where they may or may not mature to form keratin, depending on the site. Oral exfoliative cytology is a simple, non-invasive, less time-consuming procedure with sensitivity of 89% and specificity of 89.5%(Anuradha and Sivapathasundharam, 2007).

Donne (1945) was the first to propose that the size of microscopic objects could be detected. Since then, measuring cells and their components has been a cognitive challenge. Image analysis technologies are programmed to analyze cells, substituting for the direct visual inspection. Prewitt and Mendelson (1960) first invented an image analysis system for studying leukocytes. It was later extended by Weid et al to study the cells in the cervical smears('Harrow, Stone. Borek, Wagreich, And Mazur. Laboratory manual of biochemistry. Philadelphia: W. B. Saunders Company, 1940.

119 p. $1.50', 1941),(Hande and Chaudhary, 2010).

A large portion of the studies done on the oral epithelium are done in pathological conditions. The oral exfoliative cytology technique has been used predominantly to study premalignant lesions, potentially malignant lesions and malignant lesions('Harrow, Stone. Borek, Wagreich, And Mazur. Laboratory manual of biochemistry. Philadelphia: W. B. Saunders Company, 1940. 119 p. $1.50', 1941; Hande and Chaudhary, 2010). Only a handful of studies have been done on normal oral epithelium. In the past, the use of oral exfoliative cytology was restricted due to the subjective nature of its interpretations and high false-negative results(Sakuma *et al.,* 2012). These limitations could be later overcome by the introduction of quantitative methods such as image analysis systems.

The epithelium of the old people is usually atrophied which is seen as a decrease in the size of the cells and reduced functional activity. Local atrophy of the oral epithelium undergoes various changes which can be simple, degenerative or numerical. There is a decrease in size in the simple variety('Harrow, Stone. Borek, Wagreich, And Mazur. Laboratory manual of biochemistry. Philadelphia: W. B. Saunders Company, 1940. 119 p. $1.50', 1941; Sakuma *et al.,* 2012).

In this study, we use oral exfoliative cytology and image analysis to examine the exfoliative cells from oral mucosa and estimate the cell size and therefore quantify the age related changes. The aim of this study is to estimate the age of an individual by comparing the average cell size of their oral smears, using image analysis morphometric software. The null hypothesis states that there is no difference in the cell size obtained from oral smears as the age increases.

MATERIALS AND METHODS

The sample population was randomly selected from the outpatient department of Saveetha Dental College and Hospitals. The sample collection was done from the department of oral and maxillofacial pathology and department of pedodontics and preventive dentistry of the institution. The study sample included oral smears collected from 30 patients; 15 male and 15 female patients. Oral smears for each patient was taken from 6 sites; Right buccal mucosa, left buccal mucosa, junction of the hard and soft palate, dorsum surface of the tongue, floor of the mouth and lower labial region. This concluded a total of 180 smears.

S.No	SITE	ANATOMICAL LOCATION
1.	Site 1	Right buccal mucosa
2.	Site 2	Left buccal mucosa
3.	Site 3	Junction of hard and soft palate
4.	Site 4	Dorsum of the tongue
5.	Site 5	Floor of the mouth

6.	Site 6	Lower labial vestibule

Table 1: Table represents the various sites from which oral smear where collected

The samples were divided into 6 age groups. Group 1 – 10-19.9 years, Group 2 – 20-29.9 years, Group 3 – 30-39.9 years, Group 4 – 40-49.9 years, Group 5 – 50-59.9 years and Group 6 – 60-69.9 years.

S.No	**GROUP**	**AGE RANGE**
1.	Group 1	10-19.9 years
2.	Group 2	20-29.9 years
3.	Group 3	30-39.9 years
4.	Group 4	40-49.9 years
5.	Group 5	50-59.9 years
6.	Group 6	60-69.9 years

Table 2: Table represents the distribution of age among various groups ranging from 10 to 70 years.

Oral smears were collected from individuals of all the age groups mentioned. For each patient, oral smears of 3 sites; right buccal mucosa, junction of hard and soft palate and floor of the mouth, were taken using a wooden spatula. Oral smears of other 3 sites i.e., left buccal mucosa, dorsum of the tongue and lower labial region, were taken using cytobrush.

The smears were collected in a gentle scraping motion from normal healthy-looking oral mucosa and were immediately smeared onto a glass slide. It was then fixed with 95% ethyl alcohol for 30 minutes before they were subjected to Papanicolaou staining technique.

The stained smears were observed under a microscope for image analysis. Images were taken at 10x magnification. An average of 10 clearly defined cells was examined and was measured in both horizontal and vertical axis. Folded and clumped cells were not included in the measurement. The cells were marked manually using GIMP 2.10 image analysis software.

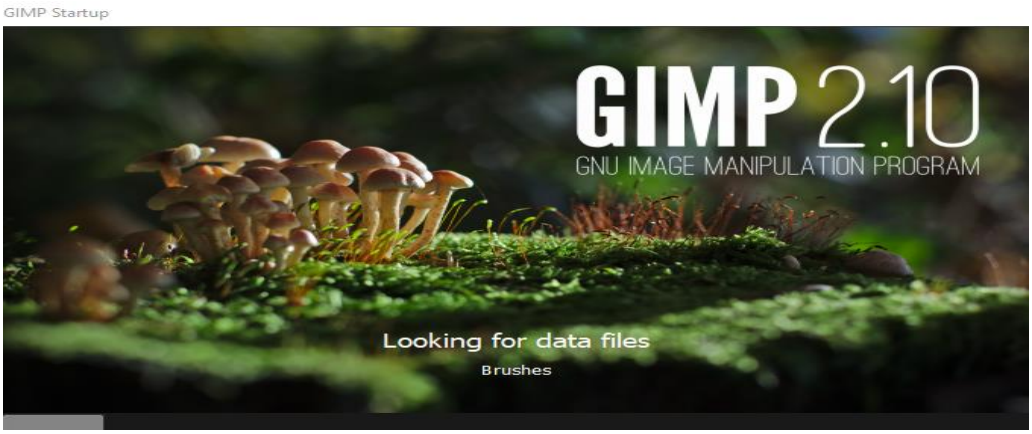

Figure 1: The GIMP 2.10 image analysis software which was used for the morphometric analysis of exfoliative cells

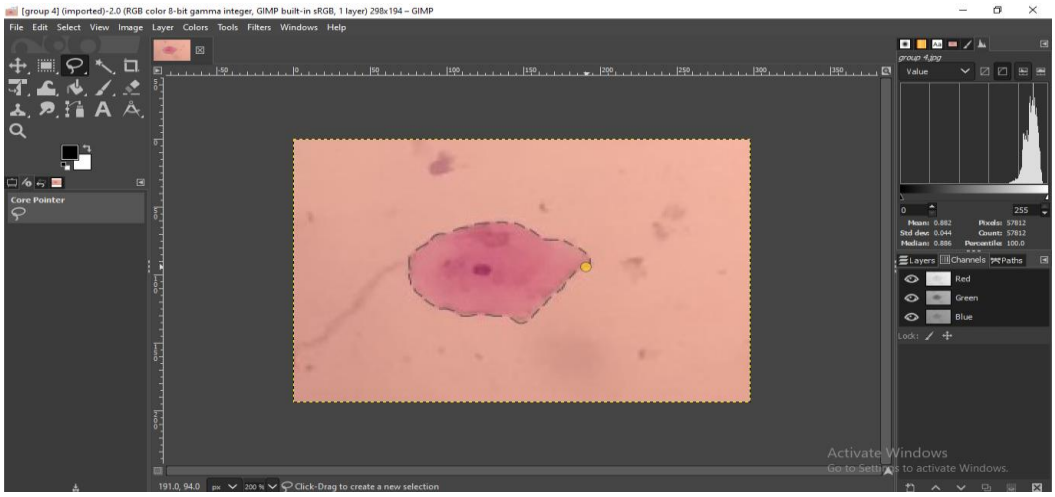

Figure 2: Figure depicts the estimation cell size using GIMP 2.10 image analysis software

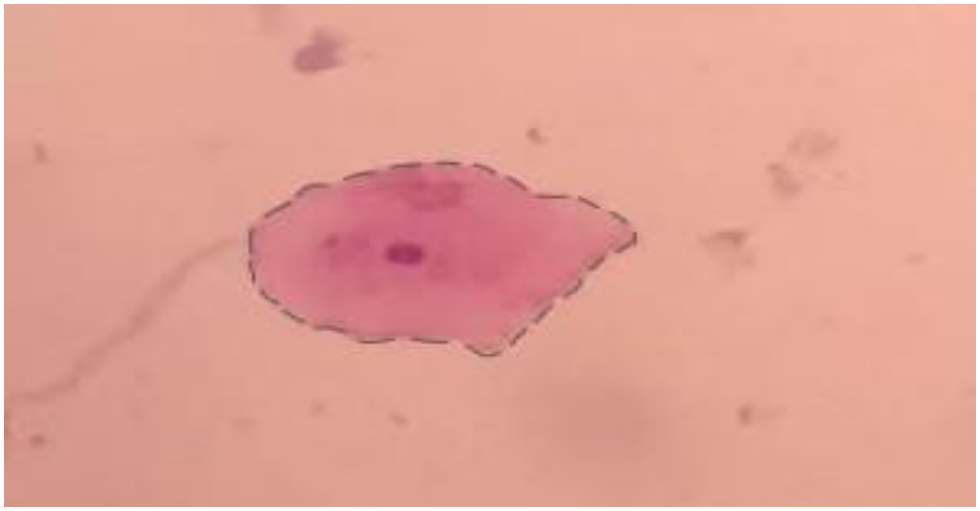

Figure 3: Exfoliative cell being analyzed by the image analysis software to calculate the cell size.

STATISTICAL ANALYSIS

The average cell size values were obtained for each case and statistically analyzed using one-way ANOVA, Bonferroni comparison tests using IBM SPSS Statistics 23 software.

RESULTS

S.NO	AGE GROUPS	MEAN	STANDARD DEVIATION
1.	10-20 years	0.11mm	0.050
2.	20-30 years	0.0832	0.00607
3.	30-40 years	0.0778	0.00045
4.	40-50 years	0.0759	0.00211
5.	50-60 years	0.0700	0.0053
6.	60-70 years	0.0677	0.00098

Table 3: Table represents the mean and standard deviation of the average cell size of all the age groups.

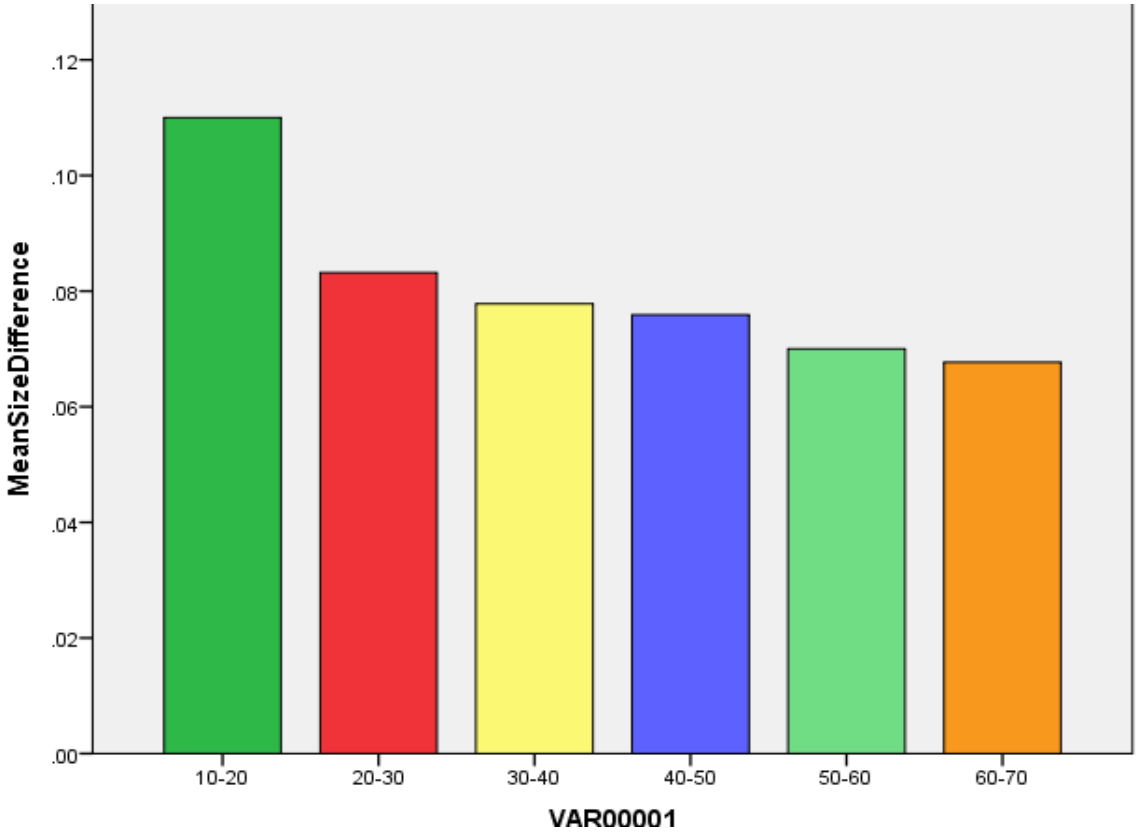

Figure 4: Bar graph representing the relationship between the average cell sizes over various age groups. There is a steady decrease in the average cell size with increase in age.

Figure 4 depicts a bar graph which represents the relationship between the average cell sizes over various age groups. There is a steady decrease in the average cell size with increase in age. The mean cell size in group 1 was 0.11 +/- 0.050 mm/sq. In group 2, the mean cell size was 0.0832 +/- 0.0067 mm/sq. In group 3, the mean cell size was 0.0778 +/- 0.00045 mm/sq. In group 4, the mean cell size was 0.0759 +/- 0.00211 mm/sq. In group 5 the mean cell size was 0.0700 +/- 0.0053 mm/sq. In group 6 the mean cell size was 0.0677 +/- 0.00098 mm/sq. Table 1 shows the mean value and standard deviation of the average cell size of all the age groups. Statistical analyses were done using one way ANOVA and post Hoc Bonferroni tests. Statistical significance was found when Group 1 was compared with Group 2 and 3 ($p<0.05$). Group 1 compared with Group 3,4,5 and 6 produced insignificant results($p>0.05$).

DISCUSSION

The estimation of the age and sex of the human body is important to the investigators since it can reduce the time taken to compare the body with the missing person's reports and find a possible match(Shetty *et al.*, 2015).unlike gender determination, determination of age is not as simple as it sounds, since gender discrimination can be done using various methods including morphometric analysis of the skulls, Barr body analysis and DNA analysis(Sakuma *et al.*, 2012). Estimation of age of children and young adults can be done using radiological examination of skeletal development on various parts of the skeleton like epiphyseal plate and the most common method would be estimation of age using dental eruption status. In adults, age estimation can be quite difficult and more often less accurate(Willems, 2001). The various methods for age estimation for adults that are currently used include skeletal and dental morphology, teeth wear faucets, root dentin transparency, secondary dentin apposition, pulp tooth area ratio of maxillary canines, tooth cementum annulations etc.. Other methods include laboratory techniques that are less commonly used such as radiocarbon dating of the tooth, but these are comparatively expensive(Sakuma *et al.*, 2012).

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malignant oral epithelium. The use of oral exfoliative cytology in the past was restricted due to its high rate of false-negative results and the subjective nature of its interpretations(Sakuma *et al.*, 2012). These limitations were later overcomed by the use of quantitative methods such as image analysis systems.

The epithelium of the aged people is usually atrophied which is seen as a decrease in the cell size and reduced functional activity. Local atrophy of the oral epithelium usually undergoes various changes which can range from simple, degenerative or numerical. There is a overall decrease in size in the simple variety('Harrow, Stone. Borek, Wagreich, And Mazur. Laboratory manual of biochemistry. Philadelphia: W. B. Saunders Company, 1940. 119 p. $1.50', 1941; Sakuma *et al.*, 2012).

Shetty et al in their study have obtained buccal smears from 100 patients which later underwent papanicolaou stain. In this study, we have included oral smears from six sites namely right buccal mucosa, left buccal mucosa, junction of the hard and soft palate, dorsum surface of the tongue, floor of the mouth and lower labial region. This gives a panoramic view to the cell size of the entire oral cavity. Different sites in the oral will have variable cell sizes according to their function(Sawke, Sawke and Parmar, 2015). Likewise epithelium of various sites has different turnover rates(Ogden, Cowpe and Green, 1990; Babuta*et al.*, 2014). This leads to collection of different amounts of exfoliative cells at a given time. Application of such a technique gives a more comprehensive result to the calculation of average cell size.

Radhika et al in their study have used wooden spatulas to collect buccal smears for exfoliative cytology. In this study we have used wooden spatula and cytobrush to collect oral smears from six sites. Oral smears from three sites namely right buccal smear, junction of hard and soft palate and floor of the mouth, were collected by using wooden spatula. Oral smears from other three sites namely left buccal smear, dorsum of the tongue and lower labial mucosa were collected by using cytobrush. Difference in the collection equipment could improve the nature of the collected cells therefore giving a comprehensive result(Hegde, 2011)(Reddy *et al.*, 2012).

In this study, there is a steady decrease in the average cell size with increase in age. The mean cell size in group 1 was 0.11 +/- 0.050 mm/sq. In group 2, the mean cell size was 0.0832 +/- 0.0067 mm/sq. In group 3, the mean cell size was 0.0778 +/- 0.00045 mm/sq. In group 4, the mean cell size was 0.0759 +/- 0.00211 mm/sq. In group 5 the mean cell size was 0.0700 +/- 0.0053 mm/sq. In group 6 the mean cell size was 0.0677 +/- 0.00098 mm/sq. Statistical analyses were done using one way ANOVA and post Hoc Bonferroni tests. Statistical significance was found when Group 1 was compared with Group 2 and 3 ($p<0.05$). Group 1 compared with Group 3, 4, 5 and 6 produced insignificant results ($p>0.05$).

Shetty et al have discussed in their results that there is a decrease in the average cell size with increase in age. We obtained similar results in this study. There was a decrease in the average cell size of oral exfoliative cells with increase in age. There was a significant decrease in the cell size between group 1, 2 and 3. This is consistent with the previous articles. The cell size difference between group 4, 5, 6 and 7 were found insignificant which is not consistent with the previous articles(Radhika *et al.*, 2019)(Shetty *et al.*, 2015; Chaudhary *et al.*, 2018).

Radhika et al in their study have used Dewinter's image analysis software to calculate average cell size of the oral exfoliative cells. In this study we have used GIMP 2.10 image analysis software to calculate average cell size of the oral exfoliative cells. This difference in the software was to evaluate if similar results could be obtained with a change in the image analysis software. It was found that similar results could be obtained even with the change in image analysis software.

CONCLUSION

The cytomorphometric analysis of oral smears from various age groups showed a decrease in cell size from 0.11mm/sq to 0.0677mm/sq. The steady decrease in cell size can be due to increase in age, which leads to age related atrophy of tissues. Estimation of age for identification purpose can be of profound value during mass disasters and other calamity related destructions. Cytomorphometric analysis of exfoliated cells of oral mucosa using GIMP 2.10 image analysis software can serve as a potential alternative non-invasive procedure in evaluation of age of an individual compared to the other screening modalities, which are usually either expensive or invasive.

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CONFLICT OF INTEREST

There are no conflicts of interests.

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