

# Age estimation using exfoliative cytology and radiovisiography: A comparative study

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## Abstract

**Introduction:** Age estimation is one of the essential factors in establishing the identity of an individual. Among various methods, exfoliative cytology (EC) is a unique, noninvasive technique, involving simple, and pain-free collection of intact cells from the oral cavity for microscopic examination. **Objective:** The study was undertaken with an aim to estimate the age of an individual from the average cell size of their buccal smears calculated using image analysis morphometric software and the pulp-tooth area ratio in mandibular canine of the same individual using radiovisiography (RVG). **Materials and Methods:** Buccal smears were collected from 100 apparently healthy individuals. After fixation in 95% alcohol, the smears were stained using Papanicolaou stain. The average cell size was measured using image analysis software (Image-Pro Insight 8.0). The RVG images of mandibular canines were obtained, pulp and tooth areas were traced using AutoCAD 2010 software, and area ratio was calculated. The estimated age was then calculated using regression analysis. **Results:** The paired *t*-test between chronological age and estimated age by cell size and pulp-tooth area ratio was statistically nonsignificant ( $P > 0.05$ ). **Conclusion:** In the present study, age estimated by pulp-tooth area ratio and EC yielded good results.

**Key words:** Age estimation, exfoliative cytology, pulp-tooth area ratio, radiovisiography

## Introduction

Age estimation is an intimidating task in forensic investigation as it helps in establishing the identity of an individual. Forensic age estimation 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.<sup>[1]</sup> Identification of human remains during mass disasters. In the present scenario, an increasing demand exists for age estimation in living individuals to unravel judicial

problems. In case of minors, it is of importance at the time of adoption, child marriages, elections, pedopornography, and age of consent in rape. In adults, it is of importance in civil issues on pensionable age, immigrants who lack proper documentation, refugees, and asylum seekers.<sup>[2]</sup>

Determining the age of a living person requires a multidisciplinary approach involving experts in anthropology, forensic medicine, forensic dentistry, and radiology. In forensic dentistry, techniques have been

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developed based on the correlation between age and dental structures.<sup>[3]</sup>

Methods of age estimation are thus based upon tooth wear, root dentin translucency, tooth cementum annulations, racemization of aspartic acid in dentin or tooth enamel, and so on.<sup>[1]</sup> However, these methods are invasive and require manipulation or extraction of tooth, which may not be tolerable for ethical, cultural, and scientific reasons. Radiography being a simple and nondestructive method, it can be used to assess the age of an individual based on pulp-tooth area ratio which decreases with increase in age due to secondary dentin deposition.<sup>[2]</sup>

One of the recent approaches for age estimation is the exfoliative cytology (EC) which is a noninvasive technique, involving simple, and pain-free collection of intact cells from different layers within the oral epithelium for microscopic examination.<sup>[1]</sup> So far, several studies were performed to assess the nuclear and cytomorphological variations in diseased conditions which enable diagnosis, and only very few articles were found on the analysis of the normal epithelial cells.

### Aim

This study was undertaken with an aim to estimate the age of an individual from their buccal smears by calculating the average cell size using image analysis morphometric software and evaluating the pulp-tooth area ratio in the mandibular canine by radiovisiography (RVG) of the same individual.

### Objectives

- To estimate the age of an individual using EC
- To estimate the age of an individual using pulp-tooth area ratio in mandibular canine by RVG of the same individual
- To compare the chronological age with the estimated age by EC and with estimated age by pulp-tooth area ratio.

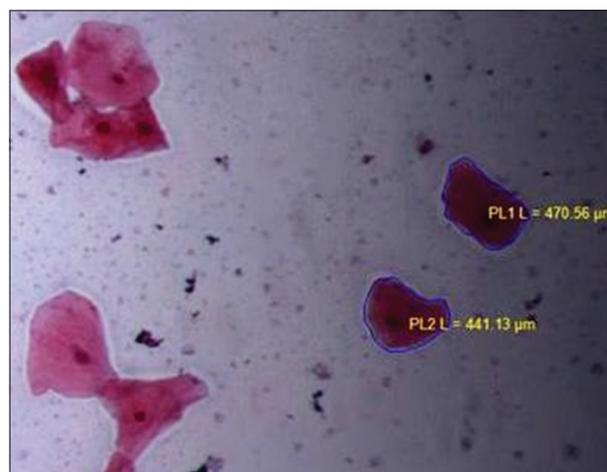
### Materials and Methods

After obtaining ethical clearance from Institutional Review Board, 100 individuals (54 females and 46 males) visiting the outpatient department of our college in the age group of 18–40 years were included for the purpose of this study.

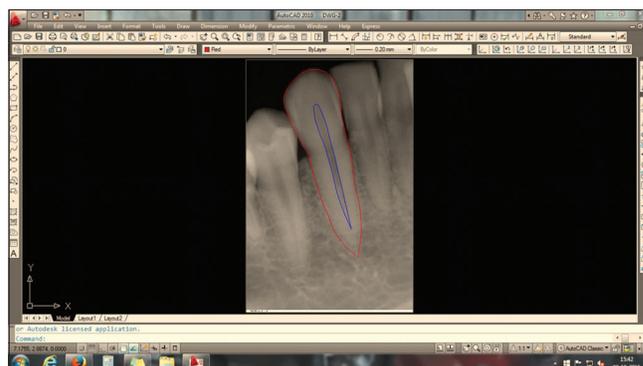
Participants clinically free from developmental, endocrine, or nutritional disorder and any other systemic illness were included in the study. Participants who presented with tobacco or alcohol consumption and also who presented with impacted canine, grossly destructed teeth, restored canines, and rotated or malaligned canines were excluded from the study. Furthermore, unclear radiographs or radiographs with artifacts were also excluded from the study sample.

Informed consent was obtained, and smears were collected from the right buccal mucosa using moistened wooden spatula and spread evenly on a clean labeled glass slide. Fixation with 95% ethanol for 15 min was done followed by routine Papanicolaou (PAP) staining given by George Nicholas PAP.<sup>[4]</sup> The stained smears were observed in a stepwise manner by trinocular research microscope (Olympus CX41, Olympus corporation, Tokyo, Japan, Made in Philippines) using image analysis software (Image-Pro Insight 8.0). An average of 20 clearly defined cells was selected in each smear followed by measurement of cell size and then the images were captured [Figure 1].

RVG of the right mandibular canines of the same individuals was obtained, and the radiographic images of the canine were then processed using computer-aided drafting program (AutoCAD 2010). Measurements of the pulp and tooth areas were done according to Cameriere *et al.*<sup>[5]</sup> The pulp space outline and tooth outline were identified and connected with line tool on AutoCAD's Draw Toolbox to evaluate pulp and tooth areas, respectively [Figure 2]. Then, pulp-tooth area ratio was calculated by dividing the pulp



**Figure 1:** Papanicolaou stained cytological smear showing assessment of average cell size (x40)



**Figure 2:** Radiovisiography of the right mandibular canine introduced into AutoCAD 2010 software for measuring pulp tooth area

area by the tooth area. Obtained results were tabulated and subjected to *t*-test and Karl Pearson's correlation coefficient method.

## Results

Karl Pearson's correlation coefficient was calculated between the chronological age of the patient and the cell size and the respective pulp-tooth area ratio values [Table 1].

A simple linear regression model was then developed to estimate the age of the patient using formulae:

$$\text{Estimated age} = -0.0516 (\text{cell size}) + 57.363.$$

$$\text{Estimated age} = -242.48 (\text{pulp-tooth area ratio}) + 49.711.$$

Chronological age was compared to the age estimated using cell size by paired *t*-test, and there is no statistically significant difference ( $P = 1$ ) between them suggesting that cell size decreases with increasing age. Similarly, chronological age was compared to the age estimated using pulp-tooth area ratio by paired *t*-test and found statistically insignificant ( $P = 1$ ) results [Table 2]. This suggests that age estimated using cell size and pulp-tooth area ratio are comparable to that of chronological age.

## Discussion

Forensic investigators use number of tools for age estimation which include both invasive and noninvasive methods. In the last few decades, a number of methods have been developed for age estimation among which age estimation in teenagers and adolescents claim relatively accurate estimates.<sup>[6]</sup>

The EC is the study of exfoliated superficial cells from mucous membrane of oral cavity, esophagus, and genital

mucosa. The history of EC dates back to 1860 when Bhale described the morphology of malignant cells in sputum of oropharyngeal carcinoma.<sup>[7]</sup> The normal EC of the oral epithelium was in detail studied by Miller and Montgomery in 1951. Ever since there are only few studies on normal buccal mucosal smears. The histological structure of the normal oral epithelium is a stratified squamous type, and these cells, as a part of normal physiologic turnover, undergo continuous renewal. They migrate from the basal layer to the surface and are exfoliated.<sup>[8]</sup> The direct scrapings of the surface epithelium may dislodge all the layers including the basal cells. Using EC, various parameters such as nuclear and cellular size, nuclear and cellular pleomorphism, and nuclear-cytoplasmic ratio can be analyzed.<sup>[9]</sup> The cellular activity, cellular organelles, and the epithelial turnover rate decrease as age advances which could be the reason for the decrease in cell size.<sup>[1]</sup>

Cowpe *et al.* conducted a study on smears obtained from different sites of oral cavity such as buccal mucosa, floor of mouth, and palate. Their results showed a significant variation in nuclear diameter with age, but there was no variation in cell diameter.<sup>[10]</sup> This is in contrast to the present study where there is decrease in cell size with increasing age. Patel *et al.* conducted a cytomorphometric study in normal exfoliated gingival cells, and the results revealed an age-related significant variation in nuclear area, cytoplasmic area, and nuclear-cytoplasmic ratio, irrespective of gender which is on par with the present study where the cell size showed variation with increasing age irrespective of gender.<sup>[11]</sup>

A study conducted by Shetty *et al.* in the normal buccal mucosal smears showed a significant decrease in average cell size of individual with increasing age which is in accordance with the present study.<sup>[1]</sup>

Secondary dentin apposition is a significant morphological dental age predictor. It is defined as the formation of dentin after the completion of the primary dentin and starts at the moment the tooth formation is completed. Due to the formation of secondary dentin, the area and the volume of the pulp chamber are reduced. Therefore, the area changes of the pulp chamber in intact teeth can be considered as reliable dental age predictor.<sup>[12]</sup>

In 2004, a pioneer study was conducted by Cameriere *et al.* to estimate age using pulp-tooth area ratio in 100

**Table 1: Correlation between chronological age with cell size and pulp-tooth area ratio by Karl Pearson's correlation coefficient method**

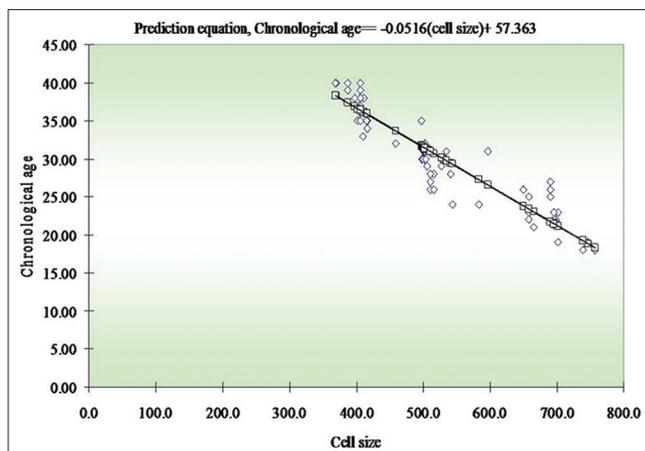
Variables	Correlation between chronological age with		
	<i>r</i>	<i>t</i>	<i>P</i>
Cell size	-0.9341	-25.9094	0.00001*
Pulp-tooth area ratio	-0.7600	-11.5745	0.00001*

\* $P < 0.05$

**Table 2: Comparison of chronological age with estimated age by cell size and pulp-tooth area ratio by paired *t*-test**

Variables	Mean	SD	Mean different	SD different	Paired <i>t</i>	<i>P</i>
Chronological age	30.07	6.60	0.00	2.35	0.0000	1.0000
Estimated age by cell size	30.07	6.16				
Chronological age	30.07	6.60	0.00	4.29	0.0000	1.0000
Estimated age by pulp-tooth area ratio	30.07	5.01				

SD: Standard deviation



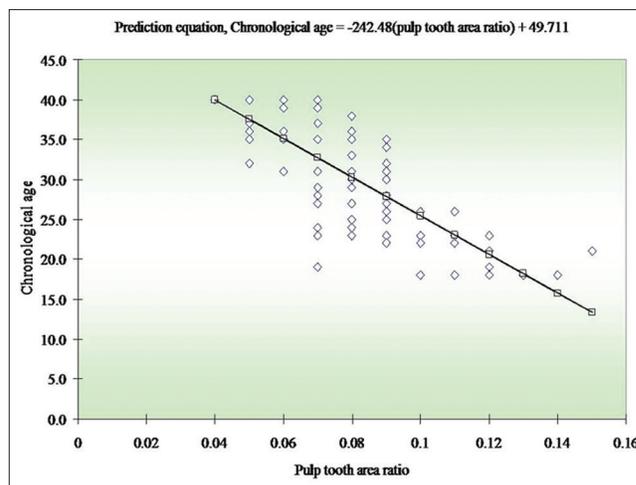
**Figure 3:** Plot between the chronological age and cell size indicating that as the age of the patient increases the cell size decreases

individuals aged between 18 and 72 years in the right maxillary canines using orthopantomographs, and they proved that the estimated age can be closely correlated with participants' chronological age.<sup>[5]</sup> Since then, many studies were done on pulp-tooth area ratio using different radiographic techniques on anterior as well as posterior teeth. Compared to the other techniques, RVG produces sharper images with higher resolution which helps in delineating pulpal and tooth outline with more precision. Hence, in the present study, RVG was chosen. Canines were chosen as they are often present in old age, less likely than other anterior teeth to suffer attrition or abrasion, and they are the single-rooted teeth with the largest pulp area which helps in easier analysis.<sup>[13]</sup>

Singaraju *et al.* (2009) conducted a study for assessing the chronological age based on the relationship between age and measurement of the pulp-tooth area ratio on single-rooted teeth, using orthopantomographs and a computer-aided drafting program AutoCAD 2000. They concluded that there is no significant difference between estimated age and chronological age which is in accordance to the present study. This study is in consistency with the previous studies conducted by Kvaal and Solheim and Bosmans *et al.* which showed that changes in pulp chamber can be used in age estimation.<sup>[2]</sup>

In 2013, Joseph *et al.* conducted a study to estimate age using pulp-tooth area ratio of the mandibular premolars by RVG, and they found no statistically significant difference between estimated age and chronological age and that gender had no significant influence on age. In the present study, similar results were obtained.<sup>[6]</sup>

In the present study, age estimated using cell size and pulp-tooth area ratio are comparable to that of chronological age. The plot between the chronological age and cell size showed a significant correlation with only a few points lying



**Figure 4:** Plot between the chronological age and pulp/tooth area ratio indicating that as the age of the patient increases the pulp/tooth area ratio decreases

outside the correlation line [Figure 3]. Moreover, the plot between the chronological age and pulp-tooth area ratio also shows a significant correlation between them with a relatively more deviation of points from the correlation line [Figure 4]. This suggests that age estimated both by cell size and pulp-tooth area ratio are comparable to that of chronological age, but the age estimated using cell size from buccal mucosal smears is of more accuracy as compared to that of pulp-tooth area ratio.

## Conclusion

The present study elucidates that age estimation done using EC and pulp tooth area are comparable to that of chronological age, but EC is a relatively more accurate procedure as to that of pulp-tooth area ratio. Further, studies on larger sample size are necessary for deriving definitive conclusion.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

## References

- Shetty DC, Wadhwan V, Khanna KS, Jain A, Gupta A. Exfoliative cytology: A possible tool in age estimation in forensic odontology. *J Forensic Dent Sci* 2015;7:63-6.
- Juneja M, Devi YB, Rakesh N, Juneja S. Age estimation using pulp/tooth area ratio in maxillary canines-A digital image analysis. *J Forensic Dent Sci* 2014;6:160-5.
- Azevedo Ade C, Alves NZ, Michel-Crosato E, Rocha M, Cameriere R, Biazevic MG. Dental age estimation in a Brazilian adult population using Cameriere's method. *Braz Oral Res* 2015;29. pii: S1806-83242015000100215.

4. Papanicolaou GN. A new procedure for staining vaginal smears. *Science* 1942;95:438-9.
5. Cameriere R, Ferrante L, Cingolani M. Variations in pulp/tooth area ratio as an indicator of age: A preliminary study. *J Forensic Sci* 2004;49:317-9.
6. Joseph CC, Reddy BH, Cherian NM, Kannan SK, George G, Jose S. Intraoral digital radiography for adult age estimation: A reliable technique. *J Indian Acad Oral Med Radiol* 2013;25:287-90.
7. Kumaresan GD, Jagannathan N. Exfoliative cytology – A predictive diagnostic tool. *Int J Pharm Sci* 2014;6:1-3.
8. Anuradha A, Sivapathasundharam B. Image analysis of normal exfoliated gingival cells. *Indian J Dent Res* 2007;18:63-6.
9. Reddy VS, Kumar GS, Vezhavendhan N, Priya S. Cytomorphometric analysis of normal exfoliative cells from buccal mucosa in different age groups. *Int J Clin Dent Sci* 2011;2:53-6.
10. Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: An age, site and sex-related survey. *J R Soc Med* 1985;78:995-1004.
11. Patel PV, Kumar S, Kumar V, Vidya G. Quantitative cytomorphometric analysis of exfoliated normal gingival cells. *J Cytol* 2011;28:66-72.
12. Star H, Thevissen P, Jacobs R, Fieuws S, Solheim T, Willems G. Human dental age estimation by calculation of pulp-tooth volume ratios yielded on clinically acquired cone beam computed tomography images of monoradicular teeth. *J Forensic Sci* 2011;56 Suppl 1:S77-82.
13. Afify MM, Zayet MK, Mahmoud NF, Ragab AR. Age estimation from pulp/tooth area ratio in three mandibular teeth by panoramic radiographs: Study of an Egyptian sample. *J Forensic Res* 2014;5:1-5.