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# Age Estimation Using Exfoliative Cytology and CBCT – A Comparative Study

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## ABSTRACT

**Introduction:** One of the most crucial steps in establishing someone's identification is making an educated guess about their age. One method is exfoliate cytology (EC), which involves painlessly and easily collecting unharmed cells from the oral cavity for microscopic examination. Pulp-to-tooth area ratio (PTR) measurements utilising cone beam computer tomography (CBCT), which is a 3D image of teeth in living humans, are another innovative nondestructive way of age estimate

The motivation behind this study is to decide a singular's age by estimating the pulp-to-tooth area ratio (PTR) of maxillary canines through the utilization of three-layered cone beam computed tomography (CBCT),(CBCT),and by analysing the average cell size of buccal smears through the use of image morphometric software in exfoliative cytology.

From 100 patients who seemed to be in good health, buccal swabs were obtained. The pulp and tooth regions were marked using the Kodak 9600 3D digital imaging device on the CBCT images of the mandibular canines.

**Results:** Using a paired t test, we found that there was no significant difference between the predicted age and the actual age using cell size and the pulp-tooth area ratio ($P > 0.05$). In the current investigation, CBCT and EC both yielded similarly accurate ages based on the pulp-tooth area ratio.

**Keywords:** *Age estimate, exfoliation cytology, cone beam computed tomography, and pulp-tooth area ratio*

## INTRODUCTION

An essential aspect of the study of criminal justice is assessing a person's age, whether they are living or deceased. A subspecialty of forensic medicine called forensic age estimate aims to ascertain as precisely as possible the sequential age of an individual whose age is obscure however who is engaged with a legitimate or judicial proceeding1. Age assessment of living individuals is now becoming more and more necessary to resolve legal concerns.

Adoption, child marriage, elections, pedopornography, and the age of consent for rape all include minors in some way. When dealing with civil cases concerning the retirement age, undocumented immigrants, refugees, and asylum seekers, adults should take this into account.[2] It takes a multidisciplinary method that includes professionals from anthropology, forensic medicine, forensic dentistry, and radiology to identify the age of a live individual. Given the correlation between dental health and ageing, forensic dentistry procedures have been created.[3]

Dental wear, root dentin translucency, annulations in the cementum, the degree of racemization of aspartic corrosive in the dentin, and the thickness of the tooth finish may all be used as indicators of age. For ethical, cultural, and scientific grounds, these invasive treatments [1] that require tooth manipulation or extraction may not be suitable. Based on the pulp-to-tooth area ratio, which decreases with age as a result of secondary dentin deposition, radiography can be used to quickly and painlessly identify an individual's age.[2]

Exfoliative cytology (EC), a non-invasive technique, is one of the more recent approaches to age estimation. It includes rapidly, painlessly, and intactly collecting oral epithelial cells from various levels for microscopic examination.[1] Despite the fact that many research have been carried out to date to evaluate the nuclear and cytomorphological alterations in disease states that enable diagnosis, very few publications have been identified on the study of normal epithelial cells.

### *Aim*

on the same individual, to use cone beam computed tomography to analyse the pulp-tooth area ratio in the mandibular canine and to use image morphometric tools to calculate the typical cell size.

### *Objectives*

A person's age may be roughly calculated from their CBCT scan by analysing the pulp-to-tooth area ratio of their mandibular canine. Comparing the ages predicted by CBCT pulp tooth area ratio and EC to the actual ages of the subjects.

## MATERIALS AND METHODS

100 participants (54 females and 46 males) ages 18 to 40 who visited our college's outpatient department were included in the study after receiving ethical approval from the Institutional Review Board. The study's participants had to be clinically free of any endocrine, developmental, or nutritional abnormalities as well as any systemic diseases. The study eliminated participants who reported using cigarettes or alcohol, as well as those who had impacted canines, severely damaged teeth, repaired canines, and twisted or misaligned canines. The study sample also did not include any radiographs that were unclear or that contained artefacts. Spreads were taken from the right buccal mucosa utilizing a wet wooden spatula and consistently spread out on a spotless, stamped glass slide after obtaining informed consent. To prepare cytological smears, 2 drops of NaCl (0.09%) were placed onto slides. The cells were fixed using a 3:1 solution of acetic acid and methanol. Feulgen-Rossenbeck was altered and stained. The prepared slides were submerged in 5 N HCl for 15 minutes to maintain room temperature. After 15 minutes of distilled water rinsing, the slide was stained using Schiff's reagent. After 90 minutes of staining, 1% Fast Green stain was used to counterstain the slide for 1 minute. 20 cells with a clear definition on average were selected from each smear, and then

The Kodak Carestream CS 9600 3D digital imaging equipment was used for the CBCT exams. The maxillary canines were placed in the centre of the scan volume. Images with a 0.7 mm3 voxel size were captured at 120 kV, 6.3 mA, and 19 seconds. From the area of interest, a 3D image with a small volume (50 mm 50 mm) was captured. The acquired volumes were reorganised into pictures with a thickness of 200 m. Used was the Windows edition of KODAK Dental Imaging Software 3.10.10 from Carestream Health Inc. in New York, USA. Three-dimensional slices of the CBCT image data were made [Figure 1]. We looked at three different parts of the root in the axial plane: the middle of the root, a quarter of the way down the root from the cementoenamel junction (CEJ), and the CEJ itself. The sagittal plane delineated arcs that followed the shape of the maxillary arch. In order to locate the mid-sagittal plane, the cursor was adjusted opposite to the long pivot of the maxillary canine. To get the central coronal section of the tooth, an oblique slice through it

was made, following the tooth's long axis with respect to the coronal plane. To get them into AutoCAD 2004, we first had to export them as 8-bit TIFF files, analyse them in Adobe Photoshop® CS2, and then save them as high-resolution JPEG files. Each tooth's form was traced with at least 30 points, and the pulp's contour was traced with at least 10 points, using the point tool in AutoCAD's Draw Toolbox [Figure 2]. When the points in [Figure 3] were joined using the line tool, the area including the tooth and pulp was obtained. These comprehensive treatments were applied to all five parts of each tooth. Using information input into an Excel spreadsheet created in Microsoft Office 2007, PTR was calculated for each of the five CBCT portions for each tooth. Regression analysis was utilized to decide the strength of the relationship (r) between the PTR information and ordered age (y), and the subsequent evaluations were utilized to process the SEE for every one of the five segments (across the three planes).A numerous relapse examination was also carried out using the whole set of the five independent variables to examine whether the correlation and examine were enhanced. Ten of the sixty individuals had their measurements taken twice by the primary examiner on each of the five sections (across the three planes) to account for any possible intra-observer error. The results were then submitted to a paired t-test. An application called SPSS 17.0 was used for all statistical calculations.

## RESULTS

The patient's chronological age, cell size, and the related pulp to-tooth region proportion measurements were used to calculate Karl Pearson's correlation coefficient (see Table 1).

The patient's age was then estimated using a straightforward linear regression model, which is + 57.362. The age estimated from cell size and chronological age were compared using a paired t-test, and no statistically significant difference (P = 1) was found between the two. This suggests that as people age, cell size declines. Similar results were obtained when a paired The t-test was utilized to think about the assessed age from the pulp tooth region proportion to the ordered age, and the outcomes were measurably irrelevant (P = 1) [Table 2]. This proposes that the proportion of pulp to tooth and cell size both predict ageing in a manner similar to chronological age. According to the regression analysis [Table 2], the PTR kept in the sagittal, coronal, and two center planes were little and measurably irrelevant (P > 0.05) at the root and mid-root levels, which is one-fourth the distance from the CEJ. The PTR assessed in the center plane at the CEJ alone showed a measurably huge affiliation (P 0.05) although also being small [Table 2]. For this variable, running quadratic and cubic regression analyses led to somewhat larger correlations that were also statistically significant (P 0.05). A moderate association (R = 0.42) was found for the cubic function alone. The next four parts' quadratic and cubic regression analyses failed to produce a statistically significant association, so those findings are not included in this article.

## DISCUSSION

Forensic investigators employ a variety of tools, both intrusive and non-invasive, to estimate age. As of late, various age assessment approaches have been made, with age assessment in teenagers and young people professing to offer evaluations that are remarkably trustworthy.[6] As part of regular physiological turnover, the ordinary oral epithelium has a delineated squamous histological design, and these cells are routinely recharged. They peel as they move from the basal layer to the surface.[8] By cleaning the surface epithelium straightforwardly, every one of the layers, including the basal cells, might be moved. Atomic and cell size, atomic and cell pleomorphism, and the atomic cytoplasmic proportion are some of the variables that may be analysed with EC.Cellular activity, the number of cell organelles, and the pace of epithelial turnover are all thought to decrease with age [9], which might explain why cells are becoming smaller.[1]

Cowpe et al. studied smears taken from the buccal mucosa, the floor of the mouth, and the palate, among other areas of the oral cavity. According to their research, the diameter of cells did not change with age, but the diameter of nuclear spheres did vary significantly.[10] Contrary to popular belief, a recent research found that as people age, cell size reduces.

Regardless of gender, atomic region, cytoplasmic region, and atomic cytoplasmic proportion dramatically changed with age, according to a

cytomorphometric investigation of typical peeled gingival cells by Patel et al. This finding is consistent with the current study's finding that cell size varied with age, regardless of gender.[11]

The best coefficient of assurance (R2) values between the squash/tooth volume extent and successive age were found in the maxillary right central incisors (R2 = 0.70),as per an exploration by Muhammad et al.

Shetty et al.'s investigation of typical buccal mucosal smears revealed that average cell size dropped noticeably with ageing, according to the current research.[1]

A strong indicator of dental age in terms of morphology is secondary dentin apposition. It begins as soon as tooth production is finished and is characterized as the development of dentin following the finishing of the essential dentin. The pulp chamber's area and volume are diminished as a result of the development of auxiliary dentin. The locale changes of the pound chamber in healthy teeth are therefore a reliable measure of dental age.[12]

Despite its beginnings as cutting-edge imaging, CBCT technology has entered the standard of dentistry and is consistently becoming open to dental specialists. In order to determine an individual's age utilising PTR of the maxillary canines, the current study investigated the potential application of this, now nearly standard, technology in the world of forensics.

The results of the investigation showed that the coronal plane PTR to age correlation was not statistically significant. It's possible that the issue stems from the difficulty in choosing the representative portion, which didn't show the pulp and tooth's full shape in one area. Even though the mid-coronal section was created using oblique sections that were aligned with the tooth's long axis, It was difficult to make a part that showed the entire state of the tooth and the mash in one piece. The bucco-palatal plane's example of optional dentine testimony and the direction of the mash chamber suggested a significant relationship between age and size.

So, we suggest that PTR be performed in a few segments in the sagittal plane in the future, which may provide a greater connection to age. A greater 3D quantification may be necessary in this case. According to this research, the only statistically significant relationship between age furthermore, the hub plane at the CEJ was demonstrated to be the best one. This shows how CBCT has its cutoff points concerning the cone bar projection calculation, indicator responsiveness, and difference goal. Each time an establishment picture is anticipated, a huge volume is presented to radiation in light of the fact that to the cone bar projection math. It additionally results in significant picture noise and an increase in Compton scattering interaction, both of which degrade the image's quality. The delicate tissue area (mash) shrivels comparative with the dentine and cementum that encompass it at more profound apical root levels from the CEJ, increasing the amount of imaging noise in these sections.

When compared to the current study, the Pearson's correlation coefficient (r) in earlier studies that used PTR to estimate age has a substantially higher value.[6,8] But the r value of the present study is similar to other studies on Indian populations,[9,20,21] highlighting the likely uttermost compasses of radiographic assessment of helper dentinal improvement in this country. The results of the current investigation confirm previous claims that Indians may have small correlations that prevent accurate age estimation[22].

Use of PTR using CBCT should be done cautiously because of substantial observer variance (P 0.05) for the one variable that demonstrated statistically significant association to age (PTR acquired in an axial plane measured at the CEJ).

## CONCLUSION

CBCT technology offers 3D visualisation and more intricate, precise images than analogue and digital radiography. The availability of an imaging technique that can create a 3D image of the teeth and jaws is expanding, providing forensic odontologists access to it. The benefits of this technology may be used in a variety of ways to the study of forensic dentistry. The results of the present research demonstrate that age estimate by EC and pulp tooth area is equivalent to age estimation by chronological age, however EC is a much more accurate approach than pulp-tooth area ratio. Additionally, higher sample size research are required for drawing a firm conclusion.

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Authors have clearly stated that they do not have any commercial interest and financial interest. The research costs were easily covered by the researchers.

### *Author Contributions*

Conceptualization, Dr. Ishwariya Krishnan and Dr. Kalaiselvi Santhosh, methodology, Dr. Anu Sushanth A., Dr. Sasirekha. B., and Dr. Ishwariya Krishnan., validation, K.S, and N.R., and resources Writing: creation of the first draught, and K.S; writing: review and editing, K.S, and A.S; writing: supervision, N.R, and K.S; writing: project administration, K.S. Each author has given credit to the published text version.

## CONFLICTS OF INTEREST

All authors have disclosed no relevant financial relationships.

### *Data availability*

Most of the time, the data sets are created and/or analysed during the whole inquiry, and they are available from the corresponding author upon reasonable request.

**TABLE 1**: shows the results of a correlation analysis between the ratio of pulp to tooth area and age using Karl Pearson's method of calculating the correlation coefficient.

| Variables | Correlation between chronological age with r t P | | |
|---|---|---|---|
| | r | t | p |
| Cell size | 0.9242 | −25.9082 | 0.00001 |
| Pulp– tooth area ratio | −0.7500 | −11.5643 | 0.00001 |

*P<0.05
Estimated age = −0.0526 (cell size) + 57.362.
Estimated age = −242.37 (pulp–tooth area ratio) + 49.701.

**TABLE 2:** consists of the regression equations for the different ratios, the standard error of the estimate, the F values, and the Pearson correlation coefficient (r).

| Parameter/variable | Correlation coefficient (r/R) | Regression equation | SEE (years) | F |
|---|---|---|---|---|
| Sagittal | 0.10 | Age=54.444+(−45.72×sagittal PTR) | ±14.4 | 0.69 |
| Coronal | 0.05 | Age=41.34+(23.03×coronal | ±16.0 | 0.14 |

| | | | | |
|---|---|---|---|---|
| Axial (CEJ) | 0.05 | Age=41.34+(23.013×coronal PTR) ±13.5 0.13 Axial (CEJ) 0.22 Age=62.947+(−233.041×axial CEJ PTR) ±14.7 6.39* | ±14.7 | 5.09* |

Significant statistically (P 0.05). PTR, CEJ, and SEE stand for Pulp to Tooth Area Ratio, Cementoenamel Junction, and Standard Error of the Estimate, respectively.

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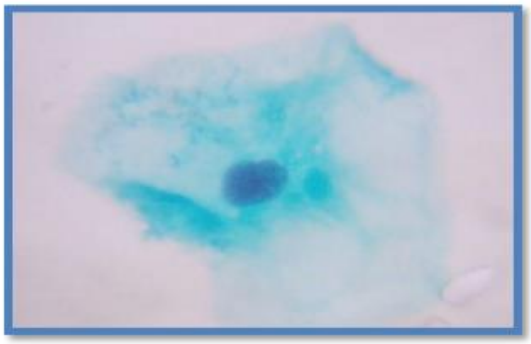

**FIG 1:** Histologic features demonstrating micronucleated cell (Feulgen— fast green stain at 1,000× magnification)

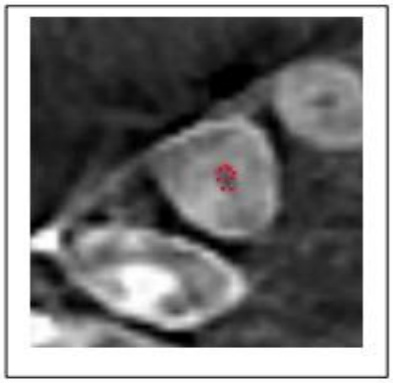

**FIG 2:** The axial section image at cementoenamel junction level seen in AutoCAD 2023 computer-aided drafting program with more than 10 points marked on the pulp outline which were connected using the line tool on the draw toolbox

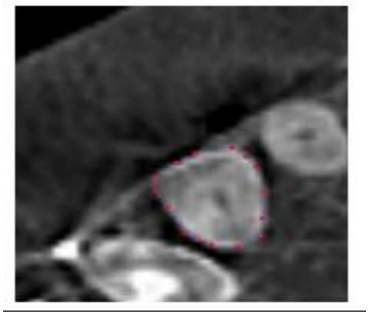

**FIG 3:** The axial section image at cementoenamel junction level seen in AutoCAD 2022 computer-aided drafting program with more than 30 points marked on the outline of the tooth using the point tool on AutoCAD's draw toolbox

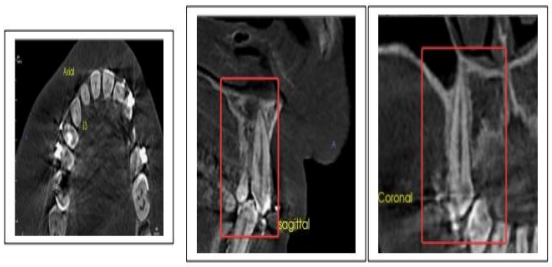


**FIG 4:** a,b,c Cone beam computed tomography images of maxillary right arch obtained for the study subjects are shown in three-dimensional, axial, coronal, and sagittal views

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