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Title: - Exploring the relationship between age, sex and pulp tooth size in canines. A CBCT analysis

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ABSTRACT

Radiographic methods using pulp tooth volume ratio (PTVR) are important for dental age estimation. According to previous studies, using PTVR possess different relationships with age in males and females but none of the studies have used a homogenous (approximately equal numbers of individuals in each age range) age distribution to assess this relationship and the effect of sex as predictor on age estimation.

This study was performed on Cone beam computed tomography images of 521 left maxillary and 681 left mandibular canines of 719 subjects of Pakistani origin (368 females and 349 males) aged from 15-65 years. Planmeca Romexis® software was used to trace the outline of the pulp cavity and tooth and to calculate respective volumes. Subsequently, Microsoft® Office Excel 2016 was used to calculate the ratios.

Regression analysis was performed to assess the relationship between PTVR factoring sex in as a predictor for age estimation. The obtained results showed that including sex as predictor with maxillary PTVR ($R^2=0.46$) have the highest predictive power. The relationship between maxillary PTVR including sex with chronological age demonstrates a non-linear relationship.

The conclusion is that including sex as predictor with maxillary PTVR produced the best estimate of chronological age.

Introduction

Once teeth are fully erupted, they undergo various physiological age related changes and secondary dentine deposition is one of them (1). Secondary dentine deposition is a continuous process that decreases the size of the pulp cavity with age. Bodecker first investigated this relationship in 1925 however it was not until 1952, that Gustafson introduced a method of age estimation. By sectioning teeth he was able to identify six age-related changes; attrition, periodontosis, cementum apposition, root resorption, transparency of the root and which also included a measure of secondary dentine deposition (1, 2).

Quantification of Gustafson's six age related changes requires sectioning of extracted teeth, which makes its applicability unethical in living individuals, therefore non-invasive techniques which rely on radiological imaging have been developed to estimate the age in living individuals. Pulp and teeth area and volume are measured on 2-D (two-dimensional) and 3-D (three-dimensional) radiographic images and converted into ratio, for use as a predictor of age estimation (3-5). Throughout a life time, pulp and tooth behave differently: - the pulp size decreases in size; whereas teeth remain less affected with age. Therefore, researchers use a ratio of these two tissues as a predictor to estimate age (3, 4). The ratio was chosen to diminish the magnification and angulation issues associated with radiographs and to overcome, variations evident in different populations and tooth morphology and size (4, 6).

In the literature, dental age has been successfully estimated from 2-D images by using the ratio of pulp tooth area and linear measurements. However, these 2-D images are the accumulation of horizontal or parallel aspects of the tooth, therefore, the ratios calculated from measurements never represent the entire 3-D morphological changes in teeth. The introduction of 3-D images in dentistry has provided comprehensive knowledge of teeth particularly the pulp cavity thus measurements based on these images truly reflect structural changes with age.

The relationship between pulp tooth volume ratio (PTVR) and age is not necessarily a straight forward one. The majority of studies using small to moderate sample sizes have reported a linear relationship (5-16). However, studies using moderate to large sample size have reported a non-linear relationship between PTVR and age (17, 18). This difference has made it potentially challenging for researchers to understand the utility and applicability of PTVR to age estimation purposes.

Previous 3-D studies evaluating PTVR differences between males and females observed inconsistent results. Several studies report statistically no significant difference between PTVR of males and females (11-13, 17, 19, 20). Despite of no significant difference some studies have reported that PTVR of females showed stronger association with age (6, 18, 21) whereas, others found that the PTVR of males demonstrated a stronger association with age (8, 16, 22). Conversely, some studies found statistically significant difference between PTVR of males and females. However some studies reported that PTVR of females has stronger association with age (5, 15) whereas, others found that PTVR of males more closely associated with age (9, 14).

Age estimation research is highly affected by the number of individuals in each group and the selected age range (9, 23). Historically, these studies have some common limitations, such as lack of uniform age distribution of subjects and small sample sizes. If the objective of a model is to estimate age, it makes sense that the sample used to generate that model should not be biased toward any one age group. Since age is not evenly distributed in the general population, more “naturalistic” sampling techniques will accordingly introduce potential bias into models. Therefore, a large sample size characterized by a homogenous age distribution of subjects is a sensible modification to better understand age estimation. Using Cone Beam Computed tomography (CBCT) technique additionally allows for 3D volumetric analysis. In addition,

these factors will provide a better understanding of the relationship of PTVR with age and with sex, which will further aid for age estimation.

Materials and Methods

The retrospective cross-sectional study was approved by the Ethical Committee of Advance Digital Imaging Centre Lahore Pakistan (Reference: 16062017/2). All canines with caries, restoration, excessive wear and attrition and other pathologies were excluded from measurements. After screening, sample consisted of 258 left maxillary and 313 mandibular canines of females and 263 left maxillary canines and 307 mandibular canines of males from 717 individuals (349 males and 368 females) of known age ranging from 15-65 years (Fig 1 and 2). For optimal distribution of subjects, the sample was divided into 50 age groups with an interval of 1 year with an ideal maximum 8 females and 8 males in each age range (Fig3).

All images were obtained from Planmeca Promax 3D classic CBCT unit using the following parameters: 200 μ m, 90-kVp tube current, 8-mA tube voltage, field of selection $\text{Ø}8.0 \times 8.0$ cm (401 \times 401 \times 401), and 0.5mm focal spot.

DICOM data files were imported to the Planmeca Romexis software for pulp and tooth volumetric measurements. Each image was oriented into axial, coronal and sagittal planes in software. For volumetric measurements of the tooth, a coronal plane was selected and a sagittal plane for pulp measurements. The 'Free region grow' tool was selected from the annotation tools section to manually measure the borders of the pulp and tooth. A minimum of ten points were selected for measuring the pulp and at least twenty points for the tooth (Fig 4).

Both the pulp and tooth measurements were done in cubic centimetre. Data was tabulated in an Excel sheet and an Excel calculator was used to calculate the PTVR. Based on the PTVR, six predictive models (1-6) were tabulated and sex was only included in models 4, 5 and 6 (Table 1).

Before the study, pulp and teeth volume slice thickness and interval were selected. The pulp slice thickness and interval paired with the scan voxel size of 0.2mm. To select the most suitable slice interval for teeth volumes, a small study was carried out between 0.3mm and 0.5mm slice intervals. For this purpose, 99 teeth volumes from 30 CBCT images were selected for the slice interval analysis.

Since no significant difference was found between the 0.3mm and 0.5mm slice intervals of teeth therefore 0.3mm slice interval of tooth was selected in the study. An intra-class correlation coefficients (ICC 2, 1-consistency) analysis was carried out to investigate the test-retest and inter-rater reliability measurements. For test-retest reliability, 105 canines from 85 CBCT scans were randomly selected and measured for inter-rater reliability, 48 canines from 30 CBCT scans were randomly selected and measured by another expert examiner.

The 6 regression models mentioned previously were created and R^2 was calculated for each of the models to assess the strength of the relationship between the predictors and age. The model with the strongest relationship was selected for further analysis.

Results

The correlation coefficient value obtained from 0.3mm and 0.5mm slice intervals of teeth volumes ranged above ≥ 0.9 . The obtained correlation values were sufficiently high and acceptable. For ease, 0.5mm slice intervals were selected for the tooth volume measurements. Furthermore, test-retest values were above ≥ 0.9 and inter-rater reliability values 0.8-0.9. These results suggest high levels of repeatability and reproducibility of measurements and a high degree of agreement between two examiners. Linear regression analysis showed significant association between models and chronological age. Furthermore, the strength of correlation value for Model 3 was higher therefore, it was selected for further analysis. The relationship between Model 3 and age was described by using scatter plot and results showed a clear non-linear relationship between them.

Using Table 3, a basic calculator was developed in Microsoft Excel to assess whether a given score is consistent with membership of a given age group and sex. This calculator is included in the supplementary material.

The equation from the regression analysis derived for age estimation was

$$\text{Estimated age} = 72.7 + \text{upper PTVR} \times -691.4 + \text{Sex} \times 3.8.$$

The equation adds 3.8 to a person's estimated age when they are males, (i.e. females are coded as 0 and males are coded as 1 in the model).

Based on the sex, an independent t-test results showed significant difference ($p=0.00$) between PTVR of males and females. This difference existed from the start of the juvenile age and remained throughout the age. However, in old age the difference becomes narrowed.

Diagnostic test and assumptions carried out for Model 3. Firstly, the studentized residuals calculated between the expected and observed values. No values exceeding ± 3 were found so the data was considered to be free from outliers. A graph plotted between residuals and fitted values and graph showed the evidence of non-linearity (the distinctive wedge shape shown in Fig 5). Several transformations like square root, log, and reciprocal attempted to correct the non-linearity but none were found to improve it.

Cooks distance was used to find any overly influential outliers in the data. No values ≥ 0.0223 were found, thus indicating that no individual score negativity affected the model causing bias to the results. Similarly, a Durbin Watson test conducted to measure the autocorrelation in the residuals. Results showed that the test statistic was less than 1 and statistically significant, which indicated a serious problem with non-independence. The errors of Model 3 are not random: the predictions tended to overestimate the age of the young and underestimate the age of the old. Finally, multicollinearity was assessed using Variance Inflation Factors, but no problems were found.

Given that applying a linear regression function to this clearly non-linear relationship is probably unwise, the descriptive statistics for Model 3 in each group are reported here. Using these values, a basic calculator was created in Excel to assess whether a given score is consistent with membership of a given age group and sex. This calculator is included in the Appendix. The calculator includes the polynomial linear model but also includes the means and Standard deviation. By putting the calculated PV simply it calculates the region where estimated age falls in the expected 95 % of scores to fall (2 standard deviations on either side of the mean) for each age group and it checks whether a particular target score falls within that region.

The data has a slight nonlinear variation in the start and in the end. Since the straight line in the linear regression unable to capture the pattern of the data therefore to overcome the under and over fitting in the data poly nominal regression was carried out for Model 3. In order to generate Poly nominal regression equation, powers such as $PTVR^2$ squared, 3 cubic and 4 quadratic were added to the linear regression equation.

The quadratic (2 predictor) and cubic (3 predictor) models showed statistically significant improvement over the standard linear model. However it did still suffer from autocorrelation/non-independence, just like linear Model 3.

A cubic polynomial with the curve provided a better visual fit than the linear line. Additionally, the R^2 of linear model of Model 3 was 0.46 which also slightly increased to 0.48 in quadratic polynomial regression. The relationship between Model 3 and chronological age is shown in Fig 6.

Discussion

A non-linear relationship was found between PTVR and age in this study, which is very similar to other 3-D studies. However, contrary to the findings of several studies, this relationship was not a simple linear one (17, 18). While linear models can be fitted to this data and be informative, the fit is not optimal. PTVR decreases in young age, then becomes stable, and again rapidly decreases again in old age. Regarding non-linearity, the direction and strength of the relationship is similar to other 3-D studies but different in shape, as this study found a sigmoid (S-shaped) relationship between PTVR and age. PTVR decreases in young age, then becomes stable, and again rapidly decreases again in old age. Regarding non-linearity, the direction and strength of the relationship is similar to other 3-D studies but different in shape, as this study found a sigmoid (S-shaped) relationship between PTVR and age.

The non-linearity in the data went away when our sample was partitioned into smaller subsets, which strongly suggests that this sigmoid function is only discoverable when using a very large sample size. Other 3-D studies which used large sample sizes also observed non-linear tendencies in their data, but those were not sigmoid functions. However, the difference in shape could be due to the balance in the number of individuals in each group and age range of this

study, as other 3–D studies lacked a uniform distribution of the sample size, especially in old adults (17, 18).

A significant difference ($p=0.00$) was found between the PTVR of males and females. This study found a strong relationship between PTVR among males. As is evident from the (Fig 6), the difference between the sexes was apparent from a young age. The significant difference between the PTVR of males and females remains consistent throughout life. The difference narrows after the age of 55; therefore, it can be supposed that the role of sex as a predictor is less helpful in old age.

Interestingly, this present study suggests that with the sex as a predictor improves the predictive power of Model 3. Significant differences between males and females, with various ranges of R^2 for age were found by researchers (6, 8, 16, 18, 21, 22). This present study found a higher R^2 value than other CBCT studies, but lower than in μ CT studies. These result can be attributed not only to a large and uniform distribution sample (even distribution of age), but also to other factors. When considering model R^2 values, the obtained results from μ CT are superior to CBCT. Higher resolution images are obtained from μ CT than CBCT. Drawing measurement of pulp tooth volumes more accurately, especially the border between the pulp and dentine. However, due to the high radiation dose, longer scan time, and the need for an extracted tooth, these factors make the process unusable in living individuals.

There was a diverse variety of PTVR within each age group in this present study, which was also observed in other studies. The patterns of tooth size and dental dimensions vary between different ethnic groups. Regarding variability and patterning, significant differences in mesial–distal crown dimensions and patterns of crown sizes were observed. In relation to mesio-distal and buccolingual crown dimensions, the largest teeth were measured from Australians and regarding tooth size, western Europeans have the smallest teeth (24). Similarly, differentiation

within and diversity among the population can be due to environmental causes. Tooth wear occurs with age and contributes to the differences in tooth dimensions. There are specific factors that can affect tooth wear, such as nature of the diet, masticatory forces, non-chewing parafunctional activities, and usage of teeth as a tool. A potential contributor to tooth wear is a tough fibrous diet, which requires prolonged mastication, especially in old age. These factors reflect the contribution of genetic and environmental influences to tooth size within and between different populations (25).

Using various types of teeth for age estimation could be another reason for the diversity in the statistical correlation results. Additionally, differences in tooth shape and size cannot be ignored. Mandibular central incisors have been selected by some authors due to having the lowest morphological diversity in human permanent teeth (26).

Using PTVR from multiple teeth or a single tooth as predictors is also a matter of concern. Star et al. used anterior teeth collectively and individually, and their results suggest that individual teeth provide a higher R^2 (6). However, the results of a study by Nima et al. suggest that there is no difference in the predictive powers using teeth collectively or individually for age estimation (9). Regarding, the comparison between different teeth and age, maxillary central incisor was found the strongest relationship with age (9, 13, 14, 22).

Secondary dentine is not laid down homogeneously in the pulp cavity; it differs by site in relation to bucco-lingual and mesial-distal widths and in incisal apical direction. Someda et al. measured different regions of the tooth and found strong relationship between whole PTVR and age (5). In contrast, to these findings, Asif et al. found that pulp chamber volume and crown ratio provided a higher R^2 value (7). Similarly, Aboshi et al. measured secondary dentine at different levels, and their results suggest that the coronal-one third of the root is highly

correlated with age (27). Thus, measuring these specific sites can cause variations in the predictive power.

Voxel resolution can be an important factor affecting volumetric measurements. Different voxel sizes have been used for pulp tooth volume measurements because CBCT scans with different voxel sizes have been used according to differing diagnostic needs. Asif et al suggest using a voxel size of 0.3mm to nullify the partial volume averaging effect of voxels on volumetric measurements (7). The findings of Maret et al. suggest that measurements using voxel sizes of $\geq 0.3\text{mm}$ are significantly underestimated (28). Waltrick et al. suggest that a 0.3mm voxel size is ideal and a good compromise between radiation dose and image quality (29). Similarly, Damstra et al. state that no improvement was evident in the accuracy of measurements by increasing the voxel size from 0.25mm to 0.4mm (30). The results of Adisen et al. suggest that is no statistically significant difference between measurements of 0.2mm and 0.4mm voxel sizes; thus, 0.4 mm can reliably be used for age estimation (31).

Both 2-D and 3-D images have been widely used in non-destructive methods for measuring the size of pulp and tooth. Radiographic methodologies applied to 2-D and 3-D images are the same which are based on relationship between age and ratios of the width, height, various pulp tooth locations, and whole pulp tooth.

In the literature, dental age has been successfully estimated from 2-D images by using the ratios between pupal size and tooth size. However, these 2-D images are the accumulation of horizontal or parallel aspects of the tooth, therefore, the ratios calculated from measurements never represent the entire 3-D morphological changes in teeth.

Usefully, 3-D CBCT images of living individuals can be acquired from dental archives thus, providing the opportunity for volumetric studies for dental age estimation. The present study

investigated the relationship between PTVR, and age among Pakistani adults. An S-shaped non-linear relationship was found between PTVR, and age but was only evident because of the large sample employed. Moreover, with sex as predictor improves the predicative power.

Employing PTVR in a large sample size with a uniform age distribution has demonstrated a sigmoid S-shaped relationship. The conclusion is that including sex as predictor with maxillary PTVR produced the best estimate of chronological age. Using CBCT volumetric measurements of various parts of tooth can be useful for dental age estimation in living subjects.

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