

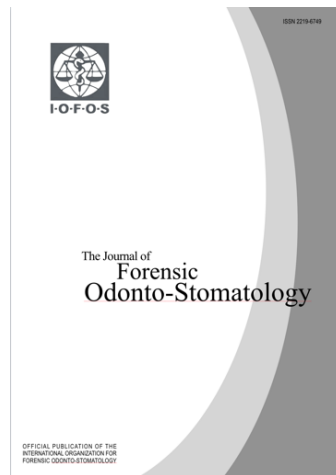


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Age estimation in north east Brazilians by measurement of open apices

Lidiane Gonçalves do Nascimento¹,
Rachel Lima Ribeiro Tinoco²,
Ane Polline Lacerda Protasio³,
Isabella Lima Arrais Ribeiro⁴,
Bianca Marques Santiago¹
Roberto Cameriere⁵

¹Department of Clinics and Social Dentistry, Federal University of Paraíba, Brazil

²Department of Anthropology, National Museum, Federal University of Rio de Janeiro, Brazil

³Centre of Exact and Natural Sciences, Federal University of Paraíba, Brazil

⁴Department of Social Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

⁵Department of Legal Medicine, University of Macerata, Macerata, Italy.

Corresponding author:
lidianegn@hotmail.com.br

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ABSTRACT

Dental age (DA) estimation is an extensively investigated resource used by forensic science. This study aimed to evaluate the applicability of the Measurement of Open Apices for DA estimation in north east Brazilians. A total of 429 orthopantomographs of individuals aged 5 to 14.99 years were used. The sample was distributed according to the age groups 5-6.99, 7-8.99, 9-10.99, 11-12.99 and 13-14.99 years, and the data were analyzed descriptively and by linear regression ($\alpha = 5\%$). The majority of the radiographs were from females ($n = 241$; 56.2%), with an overall mean age of 12 years (± 2.12). A significant difference was observed between DA and chronological age (CA) in the total sample and specifically in females and males. The method underestimated CA by 0.31 year (total sample) and by 0.3 and 0.32 year in females and males, respectively. In contrast, the method overestimated CA in the groups 5-6.99 and 7-8.99 years, with a mean difference (MD) of 0.48 year ($p = 0.007$) and 0.17 year ($p = 0.182$), respectively. In the other groups, DA was predicted to be below CA, with a significant difference in the group 13-14.99 (0.75 year). Based on the regression analysis, a correction factor was proposed from the original formula for this population, thereby reaching a predictive power of approximately 80%. To conclude, this method is applicable to the study population aged 5 to 13 years as the estimates obtained did not exceed the error limit of ± 1 year.

INTRODUCTION

Dental age (DA) estimation has been considered a valuable resource in areas such as the forensic disciplines, assisting with human identification, criminal investigations and mass disasters, as well as in biomedical areas such as paediatric endocrinology and orthodontics.^{1,2} Age estimation for forensic purposes is of interest in the civil, criminal and administrative settings. The estimation of age during criminal investigations has been decisive in cases of rape, abduction and identification of the age of criminal responsibility.³ In addition, age estimates can be utilized in cases of child adoption, preparation of documents, illegal immigration, and for retirement purposes. Therefore, age estimation tools have been consistently proven useful for documentation and law enforcement.⁴

The physiological age is determined by the degree of maturation of the individual's biological systems, and that can be used to define the progress of a child towards complete development or maturity. Instead, DA is based specifically on

the maturation of teeth, which is relevant if one considers that children of the same chronological age (CA) may present alterations in the development course of biological systems other than their teeth. Over the last decades, several indices have been developed to estimate age based on body maturation. Of these, DA estimation methods are the most used as teeth are not subject to the same external stimuli as other biological systems.⁵ Accordingly, the literature reports that teeth-related age estimation methods are more reliable, since they are not as much influenced by ethnicity and environmental factors as are skeletal methods.⁶ The method proposed by Cameriere et al.¹ is based on the measurement of open apices. Briefly, it estimates the age of children through the relationship between CA and the measurement of open apices in incompletely formed teeth. These authors proposed a method through a mathematical formula in line with previous studies addressing other age estimation approaches and with the purpose of improving the accuracy of this estimate. They tested the method in a sample of 455 Italian children aged 5 to 15 years and obtained satisfactory results with discrete age underestimation in both males and females, with a residual error of -0.0035 year.¹ Methods that are based on mathematical formulae have a relevant question which is the accuracy that the formula provides in the samples tested. The applicability of an age estimation method is also proportional to the accuracy and precision it demonstrates. Although the terms look similar, in statistics they have different gradations. Accuracy assesses the degree of conformity of a measured or calculated quantity to its actual value, whereas precision defines how many calculated values are the same or similar to the actual value. Thus, accuracy and precision are inherent concepts in order to reach the determination of the applicability of a method. It is known that the same population living in separate regions can present distinct age estimates due to cultural variations, eating habits, environmental factors and ethnic miscegenation, which, in Brazil, can be considered even more complex due to its great territorial area. As a result, the same method may not suit the whole population in distinct geographical regions, which reinforces the need to test several approaches in order to reduce distortions in the method and increase their clinical and/or forensic

usefulness. Thus, the objective of this study was to test the reproducibility and applicability of the Cameriere et al.'s method¹ in a population of north east Brazilians.

MATERIAL AND METHODS

Study design

This was a documentary, retrospective and descriptive study using secondary data from digital panoramic radiographs obtained between January and December 2016. The images were from individuals aged 5 to 15 years (N = 2,623) and were provided by a private radiology clinic located in the city of João Pessoa, Paraíba, in the north east of Brazil.

Ethical issues

This study was previously approved by the Research Ethics Committee of the Centre for Health Sciences, Federal University of Paraíba, under protocol CAAE 63928516.9.0000.5188, with exemption of the informed consent form.

Intra- and inter-examiner agreement

A pilot study was carried out with fifteen radiographs to check for internal agreement of the examiner (intra-examiner agreement) and comparable agreement with an experienced professional (inter-examiner agreement). Both examiners evaluated the same radiographs at two different moments, with a one week time interval in between. The intra- and inter-examiner concordances were analyzed by Student's paired *t* test and Intraclass Correlation Coefficient (ICC). The results of the pilot study did not indicate a statistically significant difference in the internal and external analyses, hence demonstrating satisfactory agreement. The ICC indicated excellent inter-examiner (0.891, 95% CI: 0.671-0.957) and intra-examiner agreement (0.975, 95% CI: 0.926-0.991).

Sample size and eligibility criteria

The pilot study showed 82.4% agreement between the estimated and the actual CA. Therefore, the magnitude of the effect or the difference between the estimated and the actual age by the complementary value (1 - concordance) was predicted to be 17.6%. A sample calculation with statistical power set at 82% indicated a sample size of 429 radiographs, which were randomly selected by means of a

simple draw with the use of a random number table. An additional 20% of radiographs were included as the sample had yet to be screened for eligibility criteria.

Radiographs of individuals aged 5 to 14.99 years of both sexes, obtained for clinical and/or orthodontic purposes, were included in this study. Distorted radiographic images with noticeable pathological alterations and/or changes in the number or shape of teeth, were excluded from the analysis. In addition, panoramic radiographs with images show pulp involvement in the lower left teeth (except 3rd molars) were also excluded from the study due to the possibility of necrosis with consequent interruption of mineralization.

Data collection

The data were collected by a single previously trained and calibrated examiner. Adobe® Photoshop® CC (PS®CC) image editing software was used to perform the measurements in pixels. After importing the images into the software, brightness, contrast and zoom features were adjusted for better visualization of the lower left teeth, and the pen tool was selected to make markings before measurement. With the pen tool, a line tangent to the incisal border or to the uppermost cusp(s) was marked as well as a line tangent to the lower extremities of the teeth root(s). Next, the midpoints of these two lines were marked and corresponded to the total length of the tooth. This same procedure was used to measure the inner sides of the apices by marking with the pen the distance between the walls of each root. Measurements were taken with the ruler tool after all markings with the pen had been made in pixels. In cases where a tooth on the left side was compromised for any reason, the corresponding tooth on the opposite hemiarch would be used if it was present and in good condition for analysis.

The radiographs were previously coded so that the examiner did not have access to the individual's CA. The seven lower left teeth were considered for analysis, except the third molars. The number of teeth with closed apex was determined (N). In teeth with open apices, the distance between the inner root walls was measured (A_i , where i corresponds to the tooth). In teeth with two roots, the distances of the lateral walls of each root were totaled. To avoid distortions due to the possible difference in

radiographic magnification and angulation, the measure A was divided by tooth length (L), then $X_i = A_i/L_i$.

The measurements obtained were used to estimate CA according to the following formula from Cameriere et al.:¹

$$\text{Age} = 8.971 + 0.375G + 1.631X_5 + 0.674N - 1.034S - 0.176SN$$

Data analysis

The sex, DA and CA data of each individual were tabulated and treated as variables. CA was obtained from the subtraction between the date of birth and the date of the radiographic examination.

The data were analyzed by descriptive and inferential statistics in IBM SPSS software (Statistical Package for Social Sciences) version 20.0 and in R software (Bell Laboratories, version 3.4.2). The Kolmogorov-Smirnov test indicated that DA and CA data did not present a normal distribution (p-value < 0.001), and non-parametric tests were used for data analysis. The Wilcoxon's test was used to compare the means of chronological age with dental age. Linear regression was used in the analysis of age estimation, and in the evaluation of assumptions from the residuals analysis for the equations. To all the tests, a significance level of 5% was adopted.

RESULTS

A total of 600 radiographs were randomly selected and screened for the eligibility criteria, resulting in a final sample of 429 radiographs. The sample had a mean CA of 12.02 years (± 2.06), with 56.18% (n = 241) of female subjects. The reasons for excluding some of the images (n = 171) were diverse, mainly: older than 14.99 years (n = 99), low image sharpness (n = 48) and, to a lesser extent, atypical number of teeth (n = 1) (Table 1).

The distribution of the sample by sex and CA group (in years) can be found in Table 2.

The mean CA by sex is shown in Table 3.

There was a statistically significant difference between DA and CA in the total sample (Wilcoxon test, p-value < 0.001), as well as specifically in females (p-value < 0.001) and males (p-value < 0.001) (Table 4). The method underestimated the actual age by 0.3 year in females and by 0.32 year in males.

Table 1. Absolute and relative frequencies of the reasons for sample exclusions

Exclusion reason	N	%
Older than 14.99 years	99	57.89
Poor image quality	48	28.07
Missing tooth	16	9.36
Pulp calcification	3	1.6
Abnormal tooth shape	2	1.2
Abnormal tooth position	2	1.2
Atypical number of teeth	1	0.5
Total	171	100.0

Table 2. Sample distribution by sex and age group (chronological age is expressed in years)

Chronological age (years)	Sex				Total	
	Female		Male		Total	
	n	%	n	%	n	%
5	0	--	1	0.5	1	0.2
6	5	2.1	4	2.1	9	2.1
7	6	2.5	3	1.6	9	2.1
8	20	8.3	7	3.7	27	6.3
9	17	7.0	14	7.4	31	7.2
10	19	7.9	20	10.6	39	9.1
11	26	10.8	24	12.8	50	11.6
12	58	24.1	42	22.3	100	23.3
13	47	19.5	37	19.7	84	19.6
14	43	17.8	36	19.1	79	18.4
Total	241	100.0	188	100.0	429	100.0

Table 3. Mean dental and chronological ages expressed in years by sex

	Number of cases	Mean chronological age (\pmstandard deviation)	Mean dental age (\pmstandard deviation)	Mean difference	p-value
Male	188	12.09 (\pm 1.99)	11.77 (\pm 1.95)	-0.32	0.000*
Female	241	11.97 (\pm 2.12)	11.68 (\pm 1.90)	-0.30	0.000*
Total	429	12.02 (\pm 2.06)	11.71 (\pm 1.93)	-0.31	0.000*

*Statistically significant difference (Wilcoxon test)

Table 4. Mean dental and chronological ages expressed in years by age group

	Number of cases	Mean chronological age (\pmstandard deviation)	Mean dental age (\pmstandard deviation)	Mean difference	p-value
5-6.99 years	10	6.34 (\pm 0.46)	6.82 (\pm 0.50)	0.48	0.007*
7-8.99 years	36	8.28 (\pm 0.48)	8.45 (\pm 0.1)	0.17	0.182
9-10.99 years	70	10.07 (\pm 0.62)	10.01 (\pm 0.99)	-0.06	0.302
11-12.99 years	150	12.13 (\pm 0.53)	12.02 (\pm 1.1)	-0.11	0.174
13-14.99 years	163	13.94 (\pm 0.8)	13.19 (\pm 0.8)	-0.75	0.000*
Total	429	12.02 (\pm 2.1)	11.71 (\pm 1.93)	-0.31	0.000*

*Statistically significant difference (Wilcoxon test)

As shown in Table 5, the analysis of the age groups revealed that DA was above CA in the groups 5-6.99 and 7-8.99 years, with a statistically significant difference in the former (p -value $<$ 0.001). On the other hand, DA was found to be below CA in the other groups, with a statistically significant difference found only in the group 13-14.99 years (p -value $<$ 0.001).

Overall, the method underestimated the age in 89.28% of the cases as opposed to only 11.72% of overestimation. The mean differences observed in Table 5 indicate overestimation in groups 5-6.99 and 7-8.99 years and underestimation in

the other groups. The method considerably underestimated the age of individuals within the range 13 to 14.99 years, which clearly shows reduced applicability near the age of 15 (Figure 1). Table 6 shows the correction factors for age estimation in both sexes and specifically in females and males, with the corresponding determination (R^2) and correlation (r) coefficients and estimation error of the models. The coefficients of determination presented satisfactory values for age estimation, with approximately 80.0% accuracy. The model for both sexes and the one for males can be

considered more accurate as they showed higher R^2 and less significant estimation errors.

Figure 2 presents a graph panel on the predictive diagnoses of the models generated by linear regression analysis for age estimation in the total sample ($n = 429$) and only in females ($n = 241$) and males ($n = 188$).

The homoscedasticity analysis of the different models indicated that the points in the graphs are distributed randomly and uniformly, therefore the errors are independent and have constant variance. The Kolmogorov-Smirnov normality test indicated that the residues have a normal distribution. The Durbin-Watson test confirmed what can be seen in the homoscedasticity graphs – the errors are independent. Lastly, the analysis of Figure 2 suggests that the residues are distributed

linearly, that is, the proposed models have adequate linear adjustments.

Once the proposed models are consistent with all assumptions, one should opt for those with higher coefficients of determination (R^2) and less significant errors. There is a greater number of errors near 14 to 15 years of age in the correction factor generated for both sexes and only for females or males. Therefore, as shown in Table 6, age estimation in females should be performed preferably through the general equation for both sexes, despite the small difference in the method's accuracy between the models for both sexes and females only. In contrast, age estimation in males should preferably be performed through the equation generated specifically for males.

Table 5. Equations generated for estimation of age in the Brazilian population

Population	Equation	Determination coefficient (R^2)	Correlation coefficient (r)	Error
Total	Age = 0.82 + 0.95X + 0.93	79.32%	0.89	0.93
Female	Age = 0.39 + 0.99X + 0.97	78.95%	0.88	0.97
Male	Age = 1.35 + 0.91X + 0.89	80.06%	0.89	0.89

X = age estimated by the Cameriere et al.'s method (2006).

All estimates meet the assumptions of normality, linearity, homoscedasticity and independence of errors.

Figure 1. Mean differences between the dental and chronological ages expressed in years

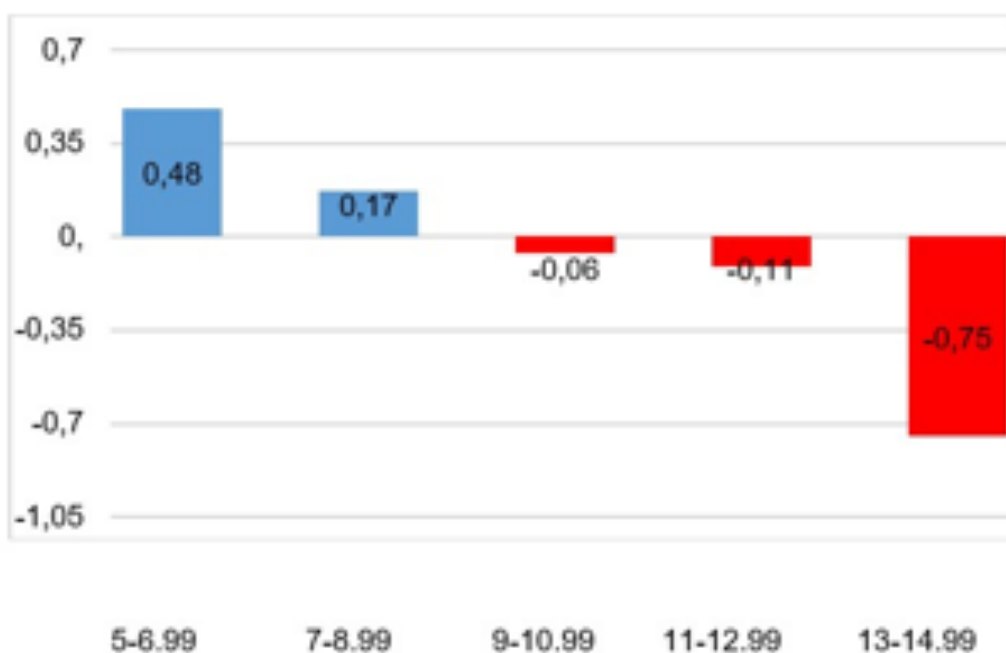
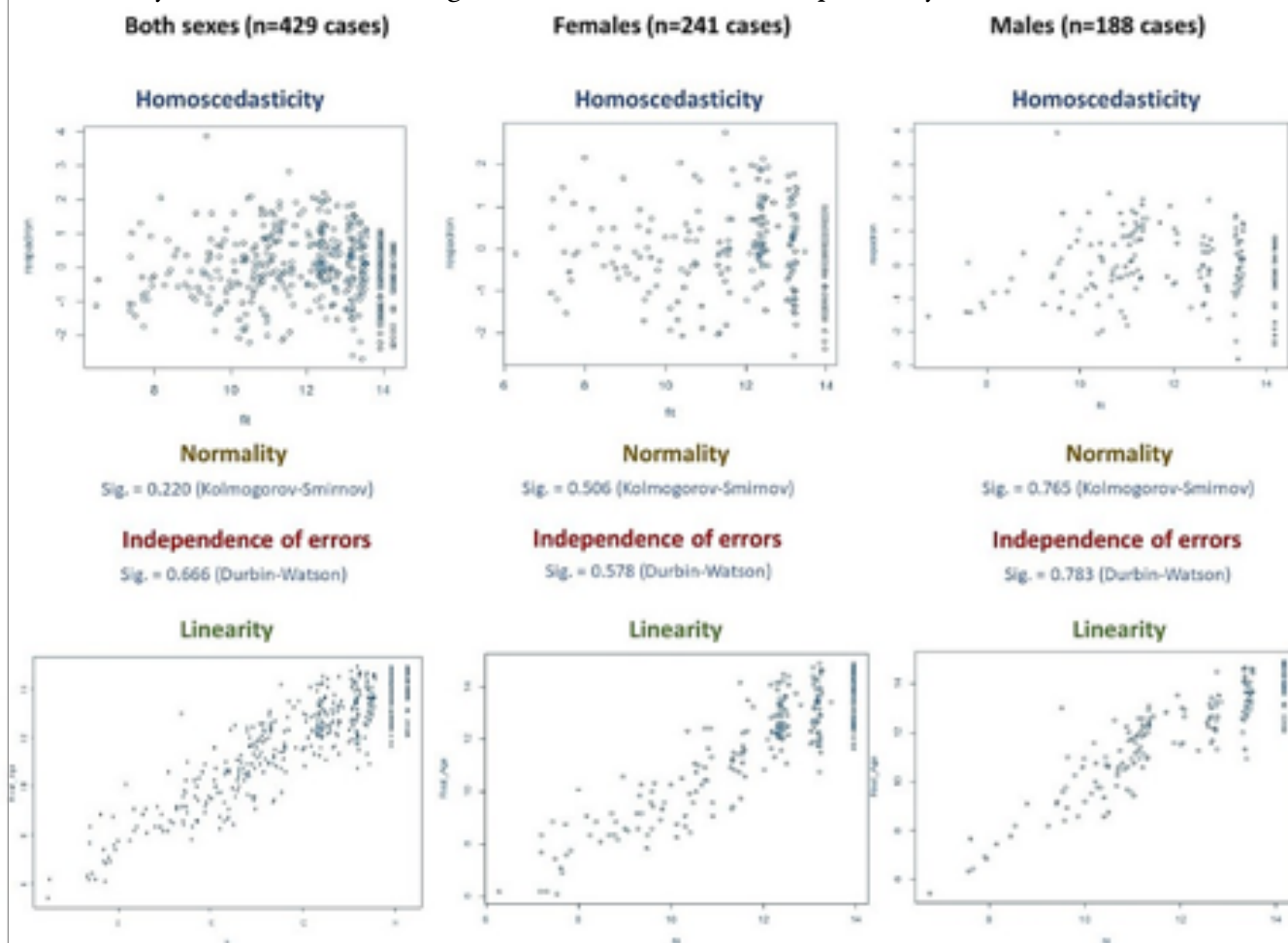


Figure 2. Graphical representation of the assumptions (homoscedasticity, normality and linearity) for correction factors generated for both sexes, and specifically for females and males



DISCUSSION

As previously reported by other authors, the limitations of this study comprise cases of age underestimation or overestimation. According to literature reports, the acceptable estimation error margin of the Cameriere et al.'s method¹ is up to one year. Beyond this, the method is considered not applicable to the study population and should therefore be combined with other approaches to estimate the individual's age.^{7,8} Initially tested in a population aged 5 to 15 years, the Cameriere et al.'s method¹ underestimated the age of individuals between 12 and 15 years. Hence, this method is not universally applicable to individuals of any age, which may also be considered a shortcoming of the study. Another possible limitation refers to the sample, which was defined by a completely random probabilistic method, yet not balanced for age. This is because in Brazil it is uncommon to request orthopantomographic examinations of young

children, generating a situation with few young children's radiographs.

In the present study, the method overestimated the individuals' age within the range 5-6.99 and 7-8.99 years, whereas it underestimated the age in the other groups. These findings corroborate the study by Fernandes et al.,² who investigated the applicability of the Cameriere et al.'s method¹ in a population of south east Brazil aged 5-15 years. The authors did not find a statistically significant difference between CA and DA ($p=0.603$), which disagrees with our findings showing statistical difference in the total sample ($p<0.001$). In Fernandes et al.'s study,² DA was found to be significantly above CA in 5-10-year-olds (overestimation) and significantly below in 11-14-year-olds (underestimation). These results corroborate ours with respect to the groups in which age was over- or underestimated, except in 10-year-old individuals whose DA was underestimated herein. The mean difference

between CA and DA indicated an overestimate of 0.48 year among 5-6.99-year-olds and of 0.17 year among 7-8.99-year-olds. In the subsequent age groups, underestimates of 0.06 and 0.11 years were observed in the groups 9-10.99 and 11-12.99 years, respectively. The highest age underestimate (0.75 year) was found in the group 13-14.99 years. By using the same method, Fernandes et al.² reported that the CA of Brazilian south east individuals was under- and overestimated in 54.4% and 45.6% of the cases, respectively. Here, the method underestimated the individuals' age in 89.28% of the cases and overestimated it in only 11.72% of the cases. Despite agreeing with the previous report, this study showed a predominance of underestimates in more than 75% of the sample. This discrepancy may be a result of the number of radiographs examined, that is, 160 panoramic radiographs analyzed by Fernandes et al.² and 429 analyzed in our study; or due to sample size, which may lead to statistical significance or non-significance; in addition to individual and regional differences in each population sample.

Cameriere et al.¹ reported a mild CA underestimation and pointed out that 90% of the absolute value of residual errors obtained were less than 1 year. This fact corroborates the findings of the present study, since the largest mean difference found was -0.75 year in the group 13-14.99 years.

When comparing two age estimation methods, one of which being the Cameriere et al.'s method,¹ Wolf et al.⁹ observed DA overestimation in males aged 6 to 11 years and underestimation at 12 to 14 years. In females, DA overestimation was observed in individuals aged 6 to 10 years and underestimation at 11 to 14 years. Cameriere et al.¹⁰ showed a clear lack of accuracy of the method for individuals aged 12 to 14 years. Gulsahi et al.¹¹ reported that DA decreases progressively as CA increases. However, this could not be confirmed by Wolf et al.,⁹ since the authors observed loss of accuracy of the method from the age of 12, i.e., DA remained stagnant and did not continue to decrease with the increase of CA. This fact was also observed in our study, from the age of 14 though.

Gulsahi et al.¹¹ found an overall underestimate of 0.35 year in a Turkish sample of both sexes. The age underestimate was 0.24 years in females and 0.47 year in males. They observed that the difference between DA and CA in different age

groups was accompanied by a greater underestimate with the increase of CA. The authors also stated that the Cameriere et al.'s method¹ is more accurate for females than it is for males. The authors reasoned that pre-puberty and puberty phases may take place at the age of 8 to 15, with particular growth changes occurring in females. With this, the variation in maturity and development can affect both sexes differently, including tooth development. The present study is in line with what has been described by Gulsahi et al.¹¹ regarding age underestimation in the total sample, with a mean difference of 0.31 year between CA and DA.

Our findings agree with other studies in the literature. In a sample of Malaysian children, DA was underestimated by 0.41 year in the total sample, 0.44 year in males and 0.39 year in females, thus indicating a slight difference between the sexes, although with greater underestimation observed in males.¹² When analyzing 259 individuals aged 5-15 years in India, Rai, Cameriere and Ferrante⁷ observed that the method overestimated by 1 year the age of 20% and 25% of the female and male samples, respectively.

In Egypt, El-Bakary, Hammad and Mohammed¹³ also showed similar findings, with an underestimate of 0.26 year in females, 0.49 year in males, and 0.29 year in the total sample. In a Mexican sample aged 5 to 15 years, De Luca et al.¹⁴ found a significant positive correlation ($p = 0.001$) between DA and CA in both sexes. The authors reported mild underestimation of age by 0.10 year in females and 0.00 in males (100% accuracy). In Italy, Pinchi et al.⁸ reported underestimates of 0.96 year and 1.07 year in a sample of female and male children, respectively. These data are not consistent with those of Balla et al.,³ who observed age underestimates of 0.51 year in males and 0.7 year in females. Guo et al.¹⁵ reported underestimates of 0.03 year in females (non-significant, $p > 0.05$) and of 0.43 year in males (significant, $p < 0.05$). In the present study, a mean DA underestimate of 0.32 year was observed in males ($p < 0.05$) as compared to 0.3 year in females, which indicates accuracy due to their proximity to CA.

When analyzing 2,630 panoramic radiographs of an Italian population aged 4 to 17 years, Cameriere et al.¹⁰ observed an overestimate of 0.72 year in males and 0.73 year in females. In 2006, Cameriere et al.¹ found different results,

that is, underestimation of the total sample of 0.035 year. In the 2006 study, the authors analyzed an Italian sample (213 males and 242 females) aged 5 to 15 years. The study by Cameriere et al.¹⁰ differs from the present study since the former observed age overestimation in the total sample. This can be explained due to variations in different countries, such as race and culture, hormonal factors, climatic factors, genetic influence, among others. The method is not considered universal; therefore, it needs to be tested in a certain population before it is deemed valid.

Mazzilli et al.¹⁶ applied the Cameriere et al.'s method[†] in a population of south east Brazilians aged 4 to 16 years. The authors observed a mean DA of 8.76 years, which did not match the CA of 10.00 years, indicating total underestimation of 1.24 year. When the authors applied a correction for the European formula, the mean DA was found to be 10.04, thus demonstrating greater similarity to the CA and better adaptation to the use of the adjusted formula. Here, a regression analysis was performed to generate predictive power data, which represented the possibility of predicting DA in approximately 80% of the total sample.

The results observed in this study using the measurement of open apices corroborate others reported in the literature. The variations between under- and overestimation of age can be explained by existing regional differences, even within the same country. Racial miscegenation is a factor that may contribute to different region-based outcomes.^{2,11} Different findings from age estimation studies with several populations have to do with the accuracy and relevance of the selected method, ethnic diversity, sample age, sample size, biological variation, and statistical approach.¹⁷ In forensic practice, the knowledge

about all these variables is of utmost importance, given that previous information on an age estimation method allows dental experts and anthropologists to make the choice for a reliable tool and thus prevent the occurrence of technical errors.¹⁸

The quest for an accurate age estimation method should consist of the validation of several existing methods in the literature. Importantly, the study of the applicability of estimation tools should contemplate the rich miscegenation existing in each country. In clinical and forensic practice, a single method does not provide reliable information, that being the reason for validation of the Cameriere et al.'s method[†] in this Brazilian population. Herein, a correction factor was proposed from the original formula, but it still needs to be further tested for cross-validation.

CONCLUSIONS

The Cameriere et al.'s method[†] can accurately estimate the age of the Brazilian north east population aged 5 to 14.99 years, since the mean difference between the estimated dental age and the actual chronological age was close to zero. Although the method underestimated age in 89.28% of the cases and overestimated it in 11.72% of the cases, the mean difference between the dental and chronological age indicated an acceptable error of 0.3 year (underestimate) and 0.32 year (overestimate). For the age group 13-14.99 years, specifically, it is recommended to combine the method with other approaches that have been validated in this population in order to improve the accuracy of the age estimate. Furthermore, the correction factor proposed from the original formula for this study population may provide a greater predictive power and lower estimation error.

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Morphological analysis of palatal rugae patterns in a population of Maharashtrian ancestry: a cross-sectional study

Vibhuti Shreesh Mhatre¹,
Jigna Pathak²,
Shilpa Patel¹,
Leela S. Poonja¹,
Niharika Swain¹,
Kamlesh Dekate¹,
Amit Bhandarwar¹

¹ Department of Oral & Maxillofacial Pathology, MGM Dental College & Hospital, Navi Mumbai - India

² Department of Oral & Maxillofacial Pathology, MGM Dental College & Hospital and MGMIHS, Navi Mumbai - India

Corresponding author:
drjignapathak@gmail.com

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ABSTRACT

Aim: To analyze the morphological parameters of palatal rugae in a population of Maharashtrian ancestry.

Material and methods: This study was conducted on 1000 subjects of Maharashtrian ancestry with at least 3 generations on the mother's and father's side. Their palatal impressions were obtained with alginate and the casts were analyzed for length, shape and direction of palatal rugae.

Results: Our results showed that the most predominant rugae were primary followed by secondary and fragmentary with significant differences between them. The most prevalent rugae shapes found were straight followed by wavy followed by curved with significant differences between them. According to direction, forward rugae were significantly higher than perpendicular rugae and backward rugae.

Conclusion: The rugae are considered to have population specific configurations. This baseline data of patterns of palatal rugae in a sample of Maharashtrian ancestry may serve 'as an accessory tool' for population identification in Forensic Dentistry.

INTRODUCTION

Palatoscopy or palatal rugoscopy is the name given to the study of palatal rugae in order to establish a person's identity. Palatal rugae (rugae palatinae or plicae palatinae transversae) are defined, according to the Glossary of Prosthodontics Terms, as anatomical folds or wrinkles; the irregular ridges of folds of fibrous connective tissue located on the anterior third of the palate behind the incisive papilla. Rugae are secured at an internal position in the oral cavity and are well protected by the lips, cheeks, buccal pad of fat, tongue, teeth and bone and hence are protected from trauma and high temperatures. Their uniqueness, stability and resistance to damage facilitate their use in forensic investigations.

When it is difficult to identify the individual by conventional methods such as fingerprints or DNA analysis, particularly in cases of fragmented bodies in mass disasters, palatal rugoscopy can serve as an alternative method in human identification. There seems to be a remarkable association between rugae forms and ethnicity of a person. Previous literature states that palatal rugae patterns may be specific to racial groups facilitating population identification.¹ The few studies which are done to find this association, show that specific patterns are predominant in specified populations.¹ Hence this study was undertaken to analyze the morphological parameters of palatal rugae and determine the predominant palatal rugae pattern in a population of Maharashtrian ancestry visiting our dental institution.

MATERIAL AND METHODS

This cross-sectional study was conducted on 1000 subjects of Maharashtrian ancestry with at least 3 generations on the mother's and father's side on the basis of convenient sampling. Our sample was equally divided between males and females. Informed consent was obtained from all the participants.

Inclusion Criteria:

- Maharashtrians with at least 3 generations on the mother's and father's side visiting our dental institution.
- Individuals between 18-60 years of age.
- Healthy individuals without any pathology in the palatal region.

Exclusion Criteria:

- Individuals with any palatal pathology.
- Individuals not willing to participate in the study
- Individuals who have undergone / undergoing orthodontic treatment.
- Individuals with extreme finger sucking habit during childhood.
- Subjects with edentulous maxillary arch.
- Individuals wearing maxillary denture.

The palatal impressions were obtained using alginate impression material and the casts were poured with type III dental stone. In the present study, dental casts were used due to their simplicity, easy production, low cost and reliability. The rugae were highlighted using sharp graphite pencils and analysis of rugae

patterns in terms of number, length, shape and direction was done.

The most accepted classification systems are by Lysell in 1955³ and Thomas & Kotze in 1983.^{4,6} Lysell classified palatal rugae according to length as primary rugae - rugae which are more than 5 mm in length, secondary rugae - rugae which are 3-5 mm in length and tertiary or fragmentary rugae - rugae which are 2-3 mm in length. (Fig.1) The length of an individual ruga is measured from its starting point near the mid-palatine raphe to its end point transversely. Rugae with lengths of less than 2 mm are not considered. In the current study, the method of rugae classification used was that followed by Ahmed (2015)⁵, as we found it to be very detailed. The shapes of the rugae were categorized into five major types: straight, curved (Fig.2), wavy (Fig.3), circular (Fig.4) and angular (Fig.5). The united rugae were categorized into unification (Figs.6 and 7), branching (Fig.3), and crosslink (Fig.8). The unification was further subclassified into diverging (Fig.6) and converging rugae (Fig.7). Any rugae shape that did not fit this classification was considered as nonspecific (Fig.9). The direction of each primary ruga was classified according to the angle between the line joining its origin and termination with a line perpendicular to the median palatal raphae. Forward-directed rugae corresponded with positive angles. Backward-directed rugae corresponded with negative angles, and perpendicular rugae corresponded with no angle (Fig.10).⁵

Figure 1. a: Primary rugae, b: Secondary rugae, c: Fragmentary rugae

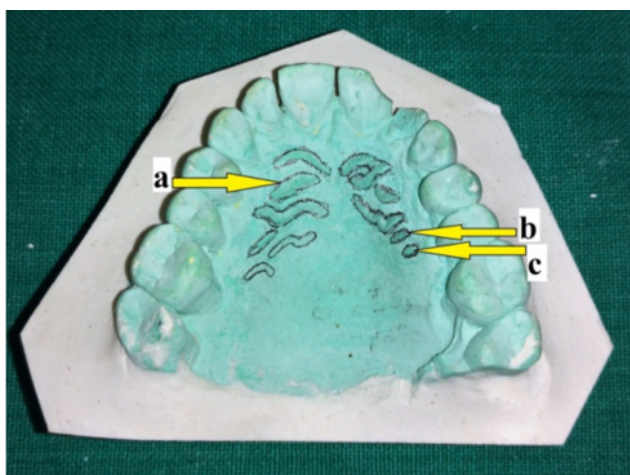


Figure 2. d: Curved rugae, e: Straight rugae

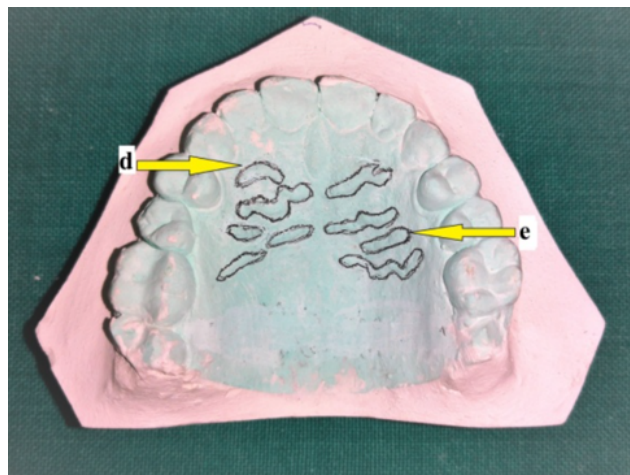


Figure 3. f: Wavy rugae, g: Branched rugae

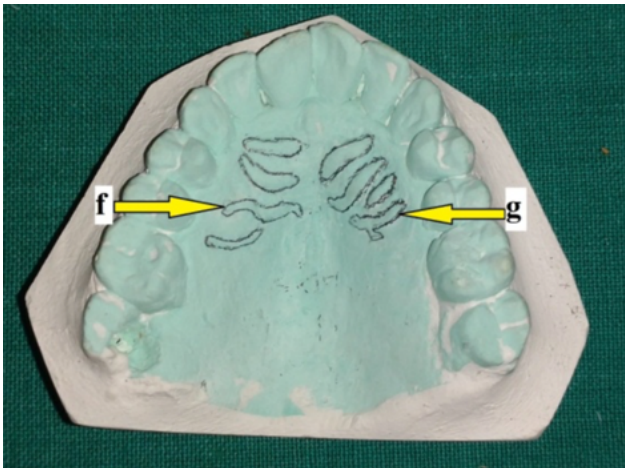


Figure 4. h: Circular rugae



Figure 5. i: Angular rugae

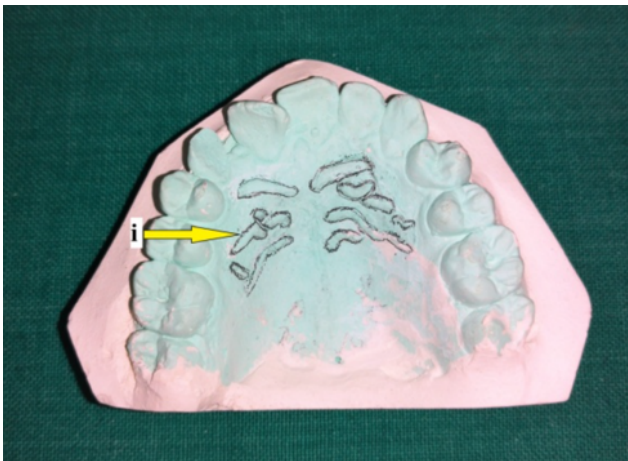


Figure 6. j: Diverging rugae



Figure 7. k: Converging rugae

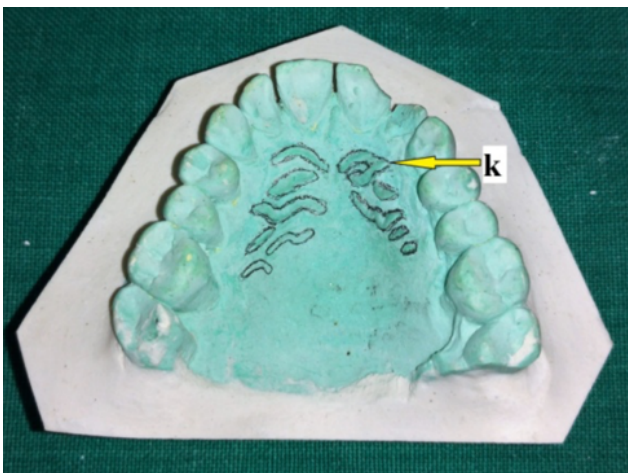


Figure 8. l: Crosslink rugae

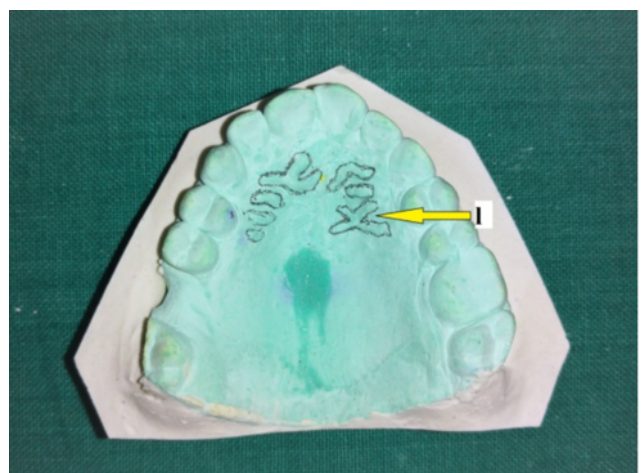
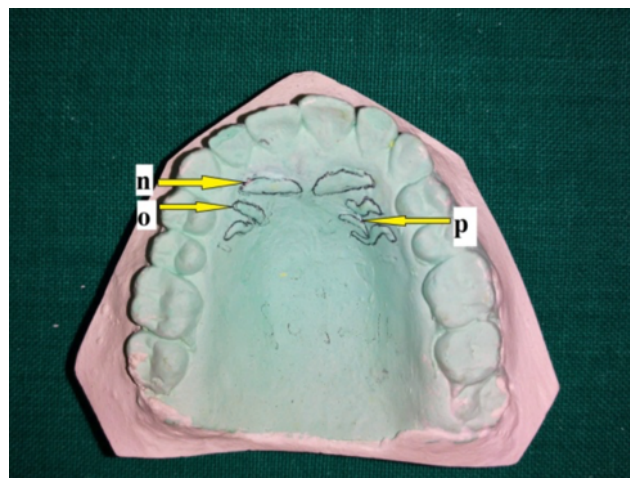


Figure 9. m: Nonspecific rugae



Figure 10. n: Perpendicular rugae, o: Forward rugae, p: Backward rugae



RESULTS

The casts were analyzed by one independent observer and the numbers of rugae were counted in all the casts. Results on continuous measurements were presented as Mean ± SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant. The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data.

The results of the present study showed that the mean of the total number of rugae was 9.49 in males (n=500) and 9.08 in females (n=500). Statistical comparison of the total number of rugae in terms of the mean among males and females performed using unpaired t-

test revealed significant difference (p<0.001). (Table 1) el (-0.92 years) and 1 tooth model (-0.86 years).

Overall comparison of the mean number of palatal rugae patterns according to length i.e. primary, secondary and fragmentary also showed statistically significant differences (p<0.001). (Table 2) Gender wise comparison of the mean number of rugae according to length showed that the mean number of primary rugae was the highest followed by secondary and fragmentary rugae in both males and females. However, the mean number of only primary rugae was significantly higher in males than that in females. (Table 2A)

Table 1. Comparison of total no of rugae in terms of {Mean (SD)} among males and females using unpaired t-test

Gender	N	Mean	Std. Deviation	t value	P value
Male	500	9.49	1.763	3.715	<0.001**
Female	500	9.08	1.710		

Table 2. Comparison of the palatal rugae patterns {length – Primary, Secondary and Fragmentary} in terms of {Mean (SD)} using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Primary	1000	7.78	1.493	12552.7540	<0.001**
Secondary	1000	1.32	1.226		
Fragmentary	1000	0.19	0.525		

(Tukey's post hoc analysis)

	<i>Primary</i>	<i>Secondary</i>	<i>Fragmentary</i>
<i>Primary</i>	-	<0.001**	<0.001**
<i>Secondary</i>	<0.001**	-	<0.001**
<i>Fragmentary</i>	<0.001**	<0.001**	-

Table 2A. Comparison of the palatal rugae patterns {length – Primary and Secondary} in terms of {Mean (SD)} among males and females using unpaired t-test and comparison of the palatal rugae patterns {length - fragmentary} among males and females using Mann Whitney U-test

	Gender	N	Mean	Std. Deviation	t value	P value
Primary	Male	500	8.04	1.460	5.657	<0.001**
	Female	500	7.51	1.480		
Secondary	Male	500	1.28	1.242	1.135	0.257
	Female	500	1.36	1.209		
(p < 0.05 - Significant*, p < 0.001 - Highly significant**)						
	Gender	N	Mean	Std. Deviation	Z value	P value
Fragmentary	Male	500	0.18	0.485	0.499	0.618
	Female	500	0.21	0.563		

Evaluation of the mean number of rugae according to shape showed that straight rugae (mean = 3.55) was the highest followed by wavy rugae (mean = 2.85), followed by curved rugae (mean = 1.85). For statistical comparisons, only straight, wavy and curved rugae were considered as they were higher in number and the majority of studies have also considered these three shapes. Overall

comparison of the mean number of palatal rugae patterns according to shape also showed statistically significant differences (p<0.001). (Table 3) Gender wise statistical comparison of the mean number of rugae patterns according to shape (straight, wavy, curved) was done using unpaired t-test, which revealed that the mean number of wavy rugae was significantly higher in males than that in females. (Table 3A)

Table 3. Comparison of the palatal rugae patterns {Curved, Wavy, Straight} in terms of {Mean (SD)} using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Curved	1000	1.85	1.360	276.4974	<0.001**
Wavy	1000	2.85	1.595		
Straight	1000	3.55	1.878		

(Tukey’s post hoc analysis)

	<i>Primary</i>	<i>Secondary</i>	<i>Fragmentary</i>
<i>Primary</i>	-	<0.001**	<0.001**
<i>Secondary</i>	<0.001**	-	<0.001**
<i>Fragmentary</i>	<0.001**	<0.001**	-

Table 3A. Comparison of the palatal rugae patterns {shape - straight, wavy, curved} in terms of {Mean (SD)} among males and females using unpaired t-test

	Gender	N	Mean	Std. Deviation	t value	p value
Straight	Male	500	3.51	1.902	0.673	0.501
	Female	500	3.59	1.855		
Wavy	Male	500	3.08	1.636	4.443	<0.001**
	Female	500	2.63	1.522		
Curved	Male	500	1.80	1.315	1.139	0.255
	Female	500	1.90	1.404		

Evaluation of the mean number of rugae according to direction showed that forward rugae (mean = 3.83) was the highest followed by perpendicular rugae (mean = 2.78), followed by backward rugae (mean = 2.67). The mean number of forward rugae was significantly higher than the mean number of backward and perpendicular rugae. (Table 4)

Gender wise comparison of the mean number of rugae patterns according to direction (forward, backward, perpendicular) was done using unpaired t-test, which revealed that the mean number of perpendicular rugae was significantly higher amongst males (mean = 2.92) than females (mean = 2.64). (Table 4A)

Table 4. Comparison of the palatal rugae patterns {Forward, Backward and Perpendicular} in terms of {Mean (SD)} using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Forward	1000	3.83	2.259	106.2640	<0.001**
Backward	1000	2.67	1.798		
Perpendicular	1000	2.78	1.800		

(Tukey’s post hoc analysis)

	<i>Primary</i>	<i>Secondary</i>	<i>Fragmentary</i>
<i>Primary</i>	-	<0.001**	<0.001**
<i>Secondary</i>	<0.001**	-	0.4226
<i>Fragmentary</i>	<0.001**	0.4226	-

Table 4A. Comparison of the palatal rugae patterns {direction - forward, backward, perpendicular} in terms of {Mean (SD)} among males and females using unpaired t-test

	Gender	N	Mean	Std. Deviation	t value	p value
Forward	Male	500	3.89	2.294	0.756	0.450
	Female	500	3.78	2.223		
Backward	Male	500	2.68	1.864	0.158	0.874
	Female	500	2.66	1.730		
Perpendicular	Male	500	2.92	1.890	2.483	0.013*
	Female	500	2.64	1.696		

($p < 0.05$ - Significant*, $p < 0.001$ - Highly significant**)

DISCUSSION

The variations in rugae patterns observed may be associated with the interracial genetic differences. Although rugae pattern morphology may be impacted by environmental factors, it is also genetically controlled. It was hypothesized that various genes govern the orientation of collagen fibres within rugae connective tissue during embryogenesis and post-natal growth, which in turn is responsible for differences in rugae patterns in different racial groups. The research performed on twins further ascertains the fact of genetic influence playing a role in determining rugae pattern.^{3,7} Since palatal rugae are specific to racial groups, they may be used for population identification. We designed this study to explore the unique patterns of palatal rugae in a homogenous population of Maharashtrians with 3 generations on the mother's and father's side.

The results of the present study showed that the mean number of rugae was 9.49 in males ($n=500$) and 9.08 in females ($n=500$) and the difference between them was statistically significant. Our results in terms of the mean number of rugae were comparable to the studies done on Gujarati and Uttar Pradesh populations. The number of rugae were less than compared to Central Indian population, Coastal Andhra population and Sudanese populations⁵, whereas it was more than in Jordanian population, Lucknow Population, the Australian aborigines¹, Meerut Population and in the Indian population. Our results in terms of the number of rugae amongst males and females were evaluated and the difference was statistically significant as in Central Indian population¹² and also in a study by Gautam N.⁸⁻²¹

The mean number of primary rugae was highest followed by secondary followed by fragmentary rugae in the total study population ($n=1000$) and this difference was statistically significant ($p < 0.001$). These results were comparable to Jordanian population¹⁴, Egyptian population, Coastal Andhra Population¹³ and in Yeroba and Igbo populations of Nigeria when both populations were combined. On gender wise statistical evaluation, the mean number of only primary rugae was found to be significantly higher in males. Primary rugae in males were significantly higher than females in Sudanese population⁵, West Godavari population and in a study by Balgi as well as by Gautam¹⁸. Our results were contradictory to the study performed on Nalgonda paediatric population which showed significantly higher primary rugae in females than males and significantly higher secondary rugae in males than females. The more primary rugae in males than females can be due to variations in palatal width, as males have larger palates than females, allowing them to have lengthier rugae.

In our study, evaluation of the mean number of rugae according to shape showed that straight rugae was the highest followed by wavy rugae followed by curved rugae and this difference was statistically significant. The least common was crosslink rugae in the total sample as well as in both males and females. For statistical comparisons, only straight, wavy and curved rugae were considered as they were higher in number and the majority of studies have also considered these three shapes. Our results were in accordance with Egyptian¹⁹ population, Tharu population, Gujarati population¹⁰, Andhra

population, Malayalees. Crosslink rugae which were the least common type which was similar to the results in Sudanese populations⁵ in which the sample selection criteria were similar to the one chosen in our study (3 generations on the mother's and father's side).²²⁻²⁶ Gender wise statistical comparison showed that the mean number of wavy rugae was significantly higher in males than that in female as in a study on Lucknow population¹⁵. Study on Telangana population and in a study by Gautam¹⁸, found significantly higher wavy rugae pattern in females than males. Our results were contradictory to study on Malayalees²⁷ and by Balgi²² which showed that males had significantly higher straight rugae than females.

Evaluation of the mean number of rugae according to direction showed that forward rugae was the highest followed by perpendicular rugae followed by backward rugae. The mean number of forward rugae was significantly higher than the mean number of backward and perpendicular rugae. Statistically significant differences in all rugae patterns according to directions were also found in Egyptian¹⁹, Jordanian¹⁴ and Sudanese⁵ populations and contrary to the results seen in Gujarati¹⁰ population where perpendicular rugae was highest. Gender wise evaluation of the mean number of rugae in males (n=500) according to direction showed that forward rugae was the highest followed by perpendicular and backward and in females (n=500), forward rugae was the highest followed by backward rugae and perpendicular rugae. On statistical evaluation, the mean number of perpendicular rugae was significantly higher amongst males than females. To the best of our knowledge, no other study showed statistically significant difference in the mean number of perpendicular rugae among males and females.

Various studies are carried out in the literature where straight, wavy and curved are predominantly found. Amongst Indian population studies, wavy is the most common and circular is the least common (<5%). However, in our study, circular shape was even less, only 0.6%. (Table 5) In our study, straight is more common and crosslink is the least common. (Table 5) In Indians, straight is the most predominant only in Gujarati¹⁰ and Andhra populations²⁶ where sample size considered was only 100 subjects. Thus, the reliability of these studies is questionable. Sample size of 1000 subjects was

seen in only one other study on Lucknow population¹⁵. Genetic basis (3 generations on maternal and paternal side) to obtain a homogenous sample was considered in only two other studies conducted on Nigerian and Sudanese populations⁵ and on no studies on Indian population. Discrepancy in the results of various studies could be attributed to sample selection, sample size and various classification methods used.

Table 5. Rugae shapes and their percentage

Rugae shape	Overall percentage (%)
Curved	19.48
Wavy	30.73
Straight	38.22
Circular	0.6
Branched	2.56
Crosslink	0.6

Since a mixture of various patterns is obtained in our study and similar patterns maybe seen in different population groups, this study does not preclude identification based on palatal rugae patterns of an individual unless the pattern has been stored as a previous ante-mortem record in the form of a dental cast or its image. Rugoscopy is highly individualistic and can only be used as a supplementary tool for personal identification and sex determination. Importantly, palatal rugoscopy is not legitimate as evidence in a court of law currently.¹¹ The reliability and validity of research on tools for population identification, using racial / ancestral background, can be authenticated only if concrete criteria and guidelines can be formulated for homogenous sample selection which should be followed by all researchers for comparable results. Only then, the credibility of using a combination of all accessory tools in population identification of a specific racial group could be emphasized as a cheaper and reliable modality as compared to genetic analysis.

There were certain limitations of our study scanning digital technology would provide three-dimensional records which could be obtained faster and would be more precise

Multiple combinations of geometric variables maybe of more value than individual parameters

Molecular forensics, when available, defy the usage of morphological methods such as palatal rugae, which obviously have not occurred in routine forensic and medico-legal assessment.¹

CONCLUSIONS

The palatal rugae patterns were analyzed in a population of Maharashtra ancestry, which showed that the most predominant rugae were primary, and the most prevalent rugae shapes found were straight followed by wavy followed by curved. Also, forward rugae were the highest followed by perpendicular rugae followed by backward

rugae. Since rugae are said to be genetically controlled, a homogenous sample selection based on ancestry should be emphasized. A larger sample size will give a more predictable database. There is a need for a standardized classification system to have comparable data. The least common shape may serve as a better population indicator. Preliminary studies should be carried out on a large homogenous sample to provide cut-off values for the total number of rugae in males and females in a specific population selected on the basis of ancestry. This baseline data may thus be valuable for future research on population based studies.

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Testing the accuracy of Bedek et al's new models based on 1-to-7 mandibular teeth for age estimation in 7-15 year old south Indian children

Sultan Omer Sheriff ¹,
Rama Haranath Reddy
Medapati ²,
Srikanth Aryasri Ankiseti ³,
Venkat Ram Reddy Gurrula ⁴,
Haritha K. ⁴,
Swethasree Pulijala ⁵,
Sudheer B. Balla ⁶

¹ Clinical dentistry division, School of Dentistry International Medical University, Malaysia

² Department of Oral & Maxillofacial Surgery, Panineeya Mahavidyalaya Institute of Dental Sciences, Hyderabad

³ Department of Orthodontics & Dentofacial Orthopedics, Panineeya Mahavidyalaya Institute of Dental Sciences, Hyderabad

⁴ Department of Oral Medicine & Radiology, Panineeya Mahavidyalaya Institute of Dental Sciences, Hyderabad

⁵ Department of Oral Pathology & Microbiology, Panineeya Mahavidyalaya Institute of Dental Sciences, Hyderabad.

⁶ Department of Forensic Odontology, Panineeya Mahavidyalaya Institute of Dental Sciences, Hyderabad, Telangana

Corresponding author:

forensics.sudheer@gmail.com

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KEYWORDS

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ABSTRACT

The goal of long term research on age assessment is to focus on the strengths and weaknesses of existing reliable methods of age estimation. In cases of age estimation when all teeth are present, maximum accuracy can be obtained using a 7 tooth model. Demirjian's system and Willems models require all seven mandibular teeth in the lower left quadrant for age assessment. Unfortunately, these methods cannot be applied in children with hypodontia. In 2019, Bedek et al., from Croatia, developed new models of age estimation based on a combination of one to seven mandibular teeth. In the present study, we tested the accuracy of the newly developed models for age estimation in South Indian children. Tested in parallel with Willems models, the accuracy of the new models was tested in terms of mean difference, mean absolute error (MAE) and percentage of correct estimations within intervals of ± 0.5 and ± 1 years. In terms of mean difference between chronological age (CA) and estimated dental age (DA), all models along with Willems models have underestimated the CA except Bedek et al's 6 tooth model where overestimation of CA was seen in boys. For MAE and percentage of correct estimations, the new models performed better than Willems models. With regards to our results, it can be concluded that the new models for dental age calculation are accurate and suitable. Therefore, we may encourage their use for age estimation in South Indian children, particularly in individuals with hypodontia or when multiple teeth are missing.

INTRODUCTION

The need for accurate age estimation exists when the chronological age (CA) of the individual who is in conflict with the law and who was not known or could not be documented.¹ In the majority of countries, court officials rely either on forensic practitioners or health authorities (in UAE) to provide specific age estimations in an attempt to reduce inappropriate judicial ruling and to carry out age-dependent legal procedures in accordance with the law.¹⁻³ Adapting an appropriate and accurate age assessment method for any population group in need is vital in forensic practice. Many methods which are accepted all over the world are based on the analyses of Europeans and North Americans, pointing out the scarcity and underrepresentation of literature for scientific standards for the populations most in need.⁴ Owing to the changes in modern population such as global migrations, increased number of intermarriages and other environmental factors,

there is a need to set new contemporary global standards, as a large proportion of available methods were formulated several generations ago.

In 1973, Demirjian and co-workers derived dental maturity scores by evaluating the dental development of children of French-Canadian origin.⁵ Three years later (1976), the same authors updated their work by increasing the sample size to incorporate dental maturity scores for additional tooth development stages. They also presented scoring systems and percentile standards for two different sets of four teeth.⁶ To date, Demirjian's method is considered to be the oldest and most frequently used method for dental age estimation in children. Later, a multitude of validation studies exploring the reliability and accuracy of Demirjian's system in populations foreign to the original reference sample, resulted in an overestimation of CA.⁷⁻⁹ In their original work, Demirjian et al., reported a possibility that their method may not be valid in other populations and perhaps adaptations should be made. Considering this, Willems et al., in 2001, presented a modified scoring system based on a Belgian Caucasian sample, which is a relatively simple system. Using these models, dental age (DA) is calculated by summing scores assigned to each stage of a single tooth.¹⁰ When its accuracy was tested in different comparative studies, it revealed greater accuracy consistently in estimating CA.¹¹⁻¹³

The most significant disadvantage of Demirjian's system and Willems models, is that neither of these methods can be used in children with multiple missing teeth or those with mandibular hypodontia. To date, no method in the literature was put forward to assess CA in living individuals or incomplete human remains with multiple missing mandibular teeth. Bedek et al., highlighted the absolute necessity of age estimation methods, particularly in cases where there is an absence of several teeth as in hypodontia or incomplete human remains with multiple missing teeth.¹⁴ They developed and tested new models for dental age estimation based on a combination of one to seven mandibular teeth using univariate regression and regression with forward and backward elimination.

In the same study, Bedek et al., also tested new age estimation models by comparing their performance in parallel with Willems models.

Results showed that the newly developed models significantly surpass the accuracy of Willems models.¹⁴ To the best of our knowledge, no studies are available in the dental literature that tested the accuracy of Bedek et al's new models of age estimation. Therefore, this study was aimed to evaluate the accuracy of Bedek et al's new models (gender specific scores) for age estimation of 7 to 15 year old South Indian children and to compare their accuracy with Willems models.

MATERIAL AND METHODS

Materials

Six hundred and fifty orthopantomographs (OPGs) of children (275 boys and 375 girls) aged between 7 and 15 years were collected retrospectively from four different states of Southern India: Telangana, Andhra Pradesh, Chennai and Bangalore. Table 1 shows the age and gender distribution of the sample. All the OPGs were obtained from private clinical practitioners, taken for the purpose of radiological diagnosis. No OPG was taken for the sole purpose of this investigation. Ethics approval to undertake this investigation was granted by the Institutional ethics committee. OPGs from individuals of South Indian origin, aged between 7 to 15 years, with no apparent dental pathology on the left side of the mandible, all permanent teeth present in the lower left quadrant (except third molars) were included. Exclusion criteria were OPGs with incomplete details, dental pathology of permanent teeth, low quality radiographs, history of systemic diseases and congenital anomalies.

All OPGs were digitalised and each OPG was coded with unique identification, with relevant information about date of birth (DOB) and date of exposure (DOE). Chronological age (CA) for each individual was then calculated by subtracting DOB from DOE of the radiograph, which was then converted into decimal points (years and months) as a fraction of twelve months.

Methods

The developmental stages of seven permanent mandibular teeth on the left side of the jaw were evaluated using Demirjian's method.⁵ Details of the combination of mandibular teeth to derive new models of age estimation for both genders

were presented in table 2. Coefficients were assigned gender wise based on the developmental stages of the teeth. The coefficients of individual teeth were summed up. The age of each individual was calculated by the formula:

Dental age= Intercept + coefficients assigned to the tooth stages in the model

Table 1. Age and Gender distribution of the sample (n=650)

Age groups	Boys	Girls	Total
7- 7.9	7	10	17
8- 8.9	14	16	30
9- 9.9	24	22	46
10- 10.9	28	37	65
11- 11.9	36	94	130
12- 12.9	49	79	128
13- 13.9	53	53	106
14- 14.9	64	64	128
Total	275	375	650

Table 3 show the details of the formulae for different tooth models for boys and girls separately.

All the OPGs were examined for staging by a single examiner (single blind approach), a forensic odontologist (SB Balla), who had six years of experience in evaluating radiographic images and age estimation. The second examiner was a dentist with a Master’s degree in orthodontics (SA Ankisetti). To test intra- and inter- examiner agreement, 100 OPGs were selected randomly and re-assessed after an interval of one month.

Statistical analysis

Statistical and mathematical analyses were carried out using SPSS software version 20.0 for Windows (SPSS Inc, Chicago, IL, USA). Kappa statistics were applied to determine intra- and inter-examiner agreement. A Paired sample t-test

Table 2. Combination of one to seven mandibular teeth used to derive new models of dental age estimation by Bedek et al., in both genders

Bedek et al., New models of dental age estimation	Combination of teeth†	
	Boys	Girls
Seven-teeth model	31-37	31-37
Six-teeth model	31 33 34 35 36 37	32 33 34 35 36 37
Five-teeth model	31 33 34 36 37	32 33 34 36 37
Four-teeth model	31 33 34 37	32 33 34 37
Three-teeth model	33 34 37	32 34 37
Two-teeth model	34 37	34 37
One-tooth model	34	37

†FDI notation

was performed to quantify statistical significance of the difference between the CA and estimated dental age (DA) among all age groups in each sex for all models. For all tested models, differences at the individual level were evaluated by subtracting CA from DA, expressed in mean error (ME), that indicates the direction of the error (over- vs. underestimation).¹⁵ We also calculated absolute mean difference or mean absolute error (MAE), which expresses the magnitude of the error for all models in each sex. The results of Bedek et al’s new models were compared with Willems models, and their accuracy was determined by the percentage of correct estimations within specified intervals i.e., ±0.5 years and ±1 year. If the obtained p-value was less than 0.05, the results were considered statistically significant.

Table 3. Calculation of dental age (DA) using newly developed models in Boys and Girls, separately

Model	Formula
Boys	
31-37 (Seven- teeth)	DA= 4.396 + Sum of coefficients assigned to the stages of teeth in the model
31 33 34 35 36 37 (Six- teeth)	DA= 4.544 + Sum of coefficients assigned to the stages of teeth in the model
31 33 34 36 37 (Five- teeth)	DA= 4.664 + Sum of coefficients assigned to the stages of teeth in the model
31 33 34 37 (Four- teeth)	DA= 5.451 + Sum of coefficients assigned to the stages of teeth in the model
33 34 37 (Three- teeth)	DA= 6.069 + Sum of coefficients assigned to the stages of teeth in the model
34 37 (Two- teeth)	DA= 5.370 + Sum of coefficients assigned to the stages of teeth in the model
34 (One- tooth)	DA= 5.828 + Sum of coefficients assigned to the stages of teeth in the model
Girls	
31-37 (Seven- teeth)	DA= 5.095 + Sum of coefficients assigned to the stages of teeth in the model
32 33 34 35 36 37 (Six- teeth)	DA= 5.077 + Sum of coefficients assigned to the stages of teeth in the model
32 33 34 36 37 (Five- teeth)	DA= 5.079 + Sum of coefficients assigned to the stages of teeth in the model
32 33 34 37 (Four- teeth)	DA= 5.122 + Sum of coefficients assigned to the stages of teeth in the model
32 34 37 (Three- teeth)	DA= 5.350 + Sum of coefficients assigned to the stages of teeth in the model
34 37 (Two- teeth)	DA= 5.350 + Sum of coefficients assigned to the stages of teeth in the model
37 (One- tooth)	DA= 5.771 + Sum of coefficients assigned to the stages of teeth in the model

RESULTS

Kappa statistics revealed that there were no statistically significant differences between intra- and inter-examiner agreement. Kappa values of intra-examiner agreement varied from 0.76 for first molar to 0.91 for first premolar, while inter-examiner agreement varied from 0.73 for second premolar to 0.9 for the central incisor (Table 4).

Boys

Group differences

Tables 5 - 12 showed the results of mean comparisons of estimated DA with CA for each age group for both Willems models and Bedek et al's newly developed models. When the mean differences between DA and CA for individual age groups were evaluated, it shows that Willems models have the least number of significantly different age groups (i.e., 4 out of 8). Among the new models of Bedek et al's 3 to 7 tooth models

have 7 out of 8 significantly different age groups, whereas 1 and 2 tooth models have all significantly different age groups.

When the mean data of all age groups was calculated, except Willems models, all newly developed models showed statistically significant differences between the DA and CA; Willems models ($t=-0.468$; $p=0.640$), Bedek et al's 7 tooth model ($t=-8.476$; $p=0.000$), 6 tooth model ($t=13.249$; $p=0.000$), 5 tooth model ($t=-8.941$; $p=0.000$), 4 tooth model ($t=-8.900$; $p=0.000$), 3 tooth model ($t=-9.249$; $p=0.000$), 2 tooth model ($t=-12.542$; $p=0.000$) and 1 tooth model ($t=-10.709$; $p=0.000$). When the overall mean age deviations were calculated, Willems models had the smallest mean error of -0.03 years, for Bedek et al's 7 tooth model (-0.55 years), 6 tooth model (0.75 years), 5 tooth model (-0.59 years), 4 tooth model (-0.59 years), 3 tooth model (-0.67 years), 2 tooth model (-0.92 years) and 1 tooth model (-0.86 years).

Table 4. Intraexaminer and Interexaminer agreement of Demirjian’s stages of tooth development

Tooth	31	32	33	34	35	36	37	Mean
Intra	0.84	0.87	0.89	0.91	0.83	0.76	0.79	0.84
Inter	0.9	0.88	0.78	0.82	0.73	0.78	0.85	0.82

Table 5. Comparison between chronological age and dental age estimated using Willems gender specific scores in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	7.24 (0.46)	-0.11 (0.46)	-0.54 to 0.31	-0.66 (6)	0.530
8- 8.9 (14)	8.51 (0.29)	7.99 (0.57)	-0.52 (0.64)	-0.89 to -0.15	-3.05 (13)	0.009*
9- 9.9 (24)	9.37 (0.26)	8.86 (1.04)	-0.51 (1.08)	-0.96 to -0.05	-2.03 (23)	0.030*
10- 10.9 (28)	10.39 (0.26)	9.73 (0.72)	-0.65 (0.63)	-0.91 to -0.41	-5.44 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	11.49 (1.25)	0.09 (1.19)	-0.31 to 0.49	0.47 (35)	0.641
12- 12.9 (49)	12.47 (0.27)	12.29 (1.64)	-0.17 (1.6)	-0.63 to 0.28	-0.77 (48)	0.444
13- 13.9 (53)	13.51 (0.29)	13.74 (1.61)	0.23 (1.49)	-0.17 to 0.65	1.17 (52)	0.247
14- 14.9 (64)	14.44 (0.29)	14.77 (1.32)	0.33 (1.27)	0.01 to 0.64	2.08 (63)	0.041*
7- 14.9 (275)	12.17 (2.01)	12.13 (2.63)	-0.03 (0.07)	-0.19 to 0.11	-0.46 (274)	0.640
Girls						
7- 7.9 (10)	7.52 (0.31)	7.71 (1.27)	0.18 (0.36)	-0.63 to 1.01	0.51 (9)	0.617
8- 8.9 (16)	8.34 (0.25)	7.6 (0.9)	-0.74 (0.96)	-1.25 to -0.22	-3.07 (15)	0.008*
9- 9.9 (22)	9.45 (0.25)	8.89 (2.07)	-0.56 (2.07)	-1.48 to 0.35	-1.27 (21)	0.218
10- 10.9 (37)	10.42 (0.25)	9.55 (1.52)	-0.87 (1.46)	-1.36 to -0.38	-3.63 (36)	0.001*
11- 11.9 (94)	11.41 (0.28)	10.58 (1.43)	-0.82 (1.43)	-1.11 to -0.52	-5.54 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	12.06 (1.51)	-0.36 (1.49)	-0.71 to -0.03	-2.18 (78)	0.032*
13- 13.9 (53)	13.46 (0.29)	13.73 (1.52)	0.26 (1.5)	-0.14 to 0.68	1.30 (52)	0.198
14- 14.9 (64)	14.46 (0.23)	15.16 (1.06)	0.71 (1.02)	0.44 to 0.96	5.15 (63)	0.000*
7- 14.9 (375)	11.99 (1.8)	11.72 (2.66)	-0.27 (0.07)	-0.42 to -0.11	-3.43 (374)	0.001*

*Statistically significant (p<0.05); SD Standard deviation; SE Standard error; df degree of freedom

Table 6. Comparison between chronological age and dental age estimated using Bedek et al., 7-teeth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA- CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	6.58 (0.35)	-0.77 (0.13)	-1.11 to -0.43	-5.57 (6)	0.001*
8- 8.9 (14)	8.51 (0.29)	7.17 (0.69)	-1.33 (0.21)	-1.77 to -0.89	-6.6 (13)	0.000*
9- 9.9 (24)	9.37 (0.26)	8.5 (1.38)	-0.87 (0.28)	-1.46 to -0.27	-3.01 (23)	0.006*
10- 10.9 (28)	10.39 (0.26)	9.72 (0.93)	-0.66 (0.87)	-1.01 to -0.32	-4.01 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	11.31 (1.01)	-0.08 (0.16)	-0.41 to 0.23	-0.55 (35)	0.582
12- 12.9 (49)	12.47 (0.27)	11.9 (0.19)	-0.56 (0.18)	-0.94 to -0.18	-3.01 (48)	0.004*
13- 13.9 (53)	13.51 (0.29)	13.07 (1.24)	-0.43 (0.15)	-0.74 to -0.11	-2.77 (52)	0.008*
14- 14.9 (64)	14.44 (0.29)	13.88 (0.9)	-0.55 (0.11)	-0.77 to -0.34	-5.13 (63)	0.000*
7- 14.9 (275)	12.17 (2.01)	11.61 (2.38)	-0.55 (0.06)	-0.68 to -0.42	-8.47 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	7.93 (1.49)	0.41 (0.45)	-0.61 to 1.43	0.91 (9)	0.389
8- 8.9 (16)	8.34 (0.25)	7.86 (0.91)	-0.48 (0.25)	-1.02 to 0.06	-1.89 (15)	0.078
9- 9.9 (22)	9.45 (0.25)	9.22 (1.86)	-0.22 (0.39)	-1.05 to 0.59	-0.57 (21)	0.570
10- 10.9 (37)	10.42 (0.25)	10.01 (1.17)	-0.41 (0.18)	-0.78 to -0.04	-2.26 (36)	0.030*
11- 11.9 (94)	11.41 (0.28)	10.88 (1.12)	-0.52 (0.11)	-0.75 to -0.29	-4.50 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	12.01 (1.16)	-0.42 (0.12)	-0.67 to -0.16	-3.29 (78)	0.001*
13- 13.9 (53)	13.46 (0.29)	13.23 (1.06)	-0.22 (0.14)	-0.51 to 0.06	-1.59 (52)	0.118
14- 14.9 (64)	14.46 (0.23)	14.16 (0.75)	-0.29 (0.09)	-0.47 to -0.11	-3.24 (63)	0.002*
7- 14.9 (375)	11.99 (1.8)	11.62 (2.11)	-0.36 (0.05)	-0.48 to -0.25	-6.32 (374)	0.000*

*Statistically significant ($p < 0.05$); SD Standard deviation; SE Standard error; df degree of freedom

Table 7. Comparison between chronological age and dental age estimated using Bedek et al., 6-teeth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	8.51 (0.36)	1.15 (0.16)	0.75 to 1.55	7.08 (6)	0.000*
8- 8.9 (14)	8.51 (0.29)	9.14 (0.61)	0.63 (0.18)	0.23 to 1.03	3.43 (13)	0.004*
9- 9.9 (24)	9.37 (0.26)	10.36 (1.26)	0.98 (0.26)	0.43 to 1.53	3.72 (23)	0.001*
10- 10.9 (28)	10.39 (0.26)	11.48 (0.96)	1.09 (0.17)	0.73 to 1.45	6.29 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	12.88 (0.81)	1.47 (0.13)	1.2 to 1.74	11.51 (35)	0.000*
12- 12.9 (49)	12.47 (0.27)	13.35 (1.1)	0.88 (0.15)	0.56 to 1.19	5.64 (48)	0.000*
13- 13.9 (53)	13.51 (0.29)	14.12 (0.82)	0.62 (0.11)	0.42 to 0.82	6.16 (52)	0.000*
14- 14.9 (64)	14.44 (0.29)	14.56 (0.47)	0.12 (0.06)	-0.01 to 0.25	1.81 (63)	0.075
7- 14.9 (275)	12.17 (2.01)	12.93 (1.91)	0.75 (0.05)	0.64 to 0.87	13.24 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	7.63 (1.79)	0.11 (0.54)	-1.11 to 1.33	0.19 (9)	0.848
8- 8.9 (16)	8.34 (0.25)	7.65 (1.27)	-0.68 (0.33)	-1.39 to 0.02	-2.06 (15)	0.057
9- 9.9 (22)	9.45 (0.25)	9.17 (1.96)	-0.28 (0.41)	-1.14 to 0.58	-0.67 (21)	0.508
10- 10.9 (37)	10.42 (0.25)	10.01 (1.17)	-0.41 (0.18)	-0.78 to -0.04	-2.25 (36)	0.030*
11- 11.9 (94)	11.41 (0.28)	10.88 (1.12)	-0.52 (0.11)	-0.75 to -0.29	-4.49 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	12.01 (1.16)	-0.41 (0.12)	-0.67 to -0.16	-3.29 (78)	0.001*
13- 13.9 (53)	13.46 (0.29)	13.23 (1.06)	-0.22 (0.14)	-0.51 to 0.06	-1.59 (52)	0.118
14- 14.9 (64)	14.46 (0.23)	14.16 (0.75)	-0.29 (0.09)	-0.47 to -0.11	-3.24 (63)	0.002*
7- 14.9 (375)	11.99 (1.8)	11.6 (2.16)	-0.38 (0.05)	-0.5 to -0.26	-6.49 (374)	0.000*

*Statistically significant ($p < 0.05$); SD Standard deviation; SE Standard error; df degree of freedom

Table 8. Comparison between chronological age and dental age estimated using Bedek et al., 5-teeth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	6.5 (0.41)	-0.85 (0.16)	-1.26 to -0.44	-5.12 (6)	0.002*
8- 8.9 (14)	8.51 (0.29)	7.24 (0.77)	-1.27 (0.22)	-1.75 to -0.78	-5.7 (13)	0.000*
9- 9.9 (24)	9.37 (0.26)	8.46 (1.37)	-0.91 (0.28)	-1.49 to -0.31	-3.18 (23)	0.004*
10- 10.9 (28)	10.39 (0.26)	9.72 (0.93)	-0.67 (0.16)	-1.02 to -0.32	-4.03 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	11.25 (1.02)	-0.14 (0.16)	-0.48 to 0.18	-0.89 (35)	0.379
12- 12.9 (49)	12.47 (0.27)	11.85 (1.35)	-0.61 (0.18)	-0.99 to -0.23	-3.26 (48)	0.002*
13- 13.9 (53)	13.51 (0.29)	13.03 (1.25)	-0.46 (0.15)	-0.78 to -0.15	-2.99 (52)	0.004*
14- 14.9 (64)	14.44 (0.29)	13.84 (0.93)	-0.59 (0.11)	-0.81 to -0.37	-5.31 (63)	0.000*
7- 14.9 (275)	12.17 (2.01)	11.58 (2.38)	-0.59 (0.06)	-0.72 to -0.46	-8.94 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	7.65 (1.71)	0.13 (0.51)	-1.02 to 1.3	0.26 (9)	0.798
8- 8.9 (16)	8.34 (0.25)	7.67 (1.19)	-0.66 (0.31)	-1.33 to 0.01	-2.13 (15)	0.050
9- 9.9 (22)	9.45 (0.25)	9.11 (1.92)	-0.34 (0.41)	-1.19 to 0.51	-0.83 (21)	0.415
10- 10.9 (37)	10.42 (0.25)	9.83 (1.25)	-0.59 (0.19)	-0.99 to -0.19	-3.03 (36)	0.004*
11- 11.9 (94)	11.41 (0.28)	10.79 (1.21)	-0.61 (0.12)	-0.86 to -0.36	-4.86 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	12.01 (1.19)	-0.43 (0.13)	-0.69 to -0.17	-3.3 (78)	0.001*
13- 13.9 (53)	13.46 (0.29)	13.26 (1.04)	-0.21 (0.14)	-0.48 to 0.07	-1.47 (52)	0.147
14- 14.9 (64)	14.46 (0.23)	14.11 (0.81)	-0.34 (0.09)	-0.54 to -0.15	-3.56 (63)	0.001*
7- 14.9 (375)	11.99 (1.8)	11.55 (2.19)	-0.43 (1.18)	-0.55 to -0.31	-7.14 (374)	0.000*

*Statistically significant ($p < 0.05$); SD Standard deviation; SE Standard error; df degree of freedom

Table 9. Comparison between chronological age and dental age estimated using Bedek et al., 4- teeth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	6.54 (0.41)	-0.81 (0.17)	-1.24 to -0.38	-4.63 (6)	0.004*
8- 8.9 (14)	8.51 (0.29)	7.26 (0.82)	-1.25 (0.24)	-1.77 to -0.72	-5.15 (13)	0.000*
9- 9.9 (24)	9.37 (0.26)	8.48 (1.44)	-0.88 (0.3)	-1.51 to -0.26	-2.94 (23)	0.007*
10- 10.9 (28)	10.39 (0.26)	9.69 (0.9)	-0.7 (0.16)	-1.03 to -0.37	-4.35 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	11.22 (1.07)	-0.17 (0.17)	-0.52 to 0.17	-1.01 (35)	0.319
12- 12.9 (49)	12.47 (0.27)	11.84 (1.36)	-0.62 (0.19)	-1.01 to -0.24	-3.27 (48)	0.002*
13- 13.9 (53)	13.51 (0.29)	13.03 (1.25)	-0.46 (0.15)	-0.78 to -0.15	-3.01 (52)	0.004*
14- 14.9 (64)	14.44 (0.29)	13.84 (0.93)	-0.59 (0.11)	-0.82 to -0.37	-5.33 (63)	0.000*
7- 14.9 (275)	12.17 (2.01)	11.57 (2.38)	-0.59 (0.06)	-0.72 to -0.46	-8.90 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	8.06 (1.31)	0.54 (0.39)	-0.34 to 1.43	1.37 (9)	0.201
8- 8.9 (16)	8.34 (0.25)	7.96 (0.78)	-0.37 (0.22)	-0.86 to 0.11	-1.67 (15)	0.116
9- 9.9 (22)	9.45 (0.25)	9.17 (1.8)	-0.27 (0.38)	-1.07 to 0.51	-0.72 (21)	0.474
10- 10.9 (37)	10.42 (0.25)	9.82 (1.26)	-0.6 (0.19)	-1.01 to -0.19	-3.02 (36)	0.005*
11- 11.9 (94)	11.41 (0.28)	10.72 (1.23)	-0.62 (0.12)	-0.88 to -0.37	-4.89 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	12.01 (1.19)	-0.43 (0.12)	-0.68 to -0.17	-3.31 (78)	0.001*
13- 13.9 (53)	13.46 (0.29)	13.25 (1.03)	-0.21 (0.14)	-0.49 to 0.07	-1.49 (52)	0.142
14- 14.9 (64)	14.46 (0.23)	14.11 (0.81)	-0.34 (0.09)	-0.54 to -0.15	-3.59 (63)	0.001*
7- 14.9 (375)	11.99 (1.8)	11.57 (2.13)	-0.41 (0.05)	-0.53 to -0.29	-6.91 (374)	0.000*

*Statistically significant ($p < 0.05$); SD Standard deviation; SE Standard error; df degree of freedom

Table 10. Comparison between chronological age and dental age estimated using Bedek et al., 3-teeth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	6.11 (0.1)	-1.23 (0.09)	-1.46 to -1.01	-13.27 (6)	0.000*
8- 8.9 (14)	8.51 (0.29)	7.04 (0.89)	-1.46 (0.26)	-2.04 to -0.89	-5.49 (13)	0.000*
9- 9.9 (24)	9.37 (0.26)	8.01 (1.78)	-1.36 (0.37)	-2.13 to -0.59	-3.68 (23)	0.001*
10- 10.9 (28)	10.39 (0.26)	9.53 (1.14)	-0.86 (0.2)	-1.28 to -0.43	-4.18 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	11.22 (1.1)	-0.18 (0.17)	-0.54 to 0.17	-1.01 (35)	0.316
12- 12.9 (49)	12.47 (0.27)	11.83 (1.41)	-0.63 (0.19)	-1.03 to -0.23	-3.19 (48)	0.002*
13- 13.9 (53)	13.51 (0.29)	13.03 (1.25)	-0.46 (0.15)	-0.77 to -0.15	-2.98 (52)	0.004*
14- 14.9 (64)	14.44 (0.29)	13.84 (0.93)	-0.59 (0.11)	-0.82 to -0.37	-5.33 (63)	0.000*
7- 14.9 (275)	12.17 (2.01)	11.49 (2.53)	-0.67 (0.07)	-0.82 to -0.53	-9.24 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	8.02 (1.45)	0.49 (0.42)	-0.47 to 1.46	1.15 (9)	0.277
8- 8.9 (16)	8.34 (0.25)	7.86 (0.8)	-0.48 (0.22)	-0.96 to 0.001	-2.11 (15)	0.052
9- 9.9 (22)	9.45 (0.25)	9.02 (1.74)	-0.43 (0.37)	-1.2 to 0.33	-1.16 (21)	0.256
10- 10.9 (37)	10.42 (0.25)	9.75 (1.26)	-0.66 (0.19)	-1.07 to -0.26	-3.36 (36)	0.002*
11- 11.9 (94)	11.41 (0.28)	10.65 (1.14)	-0.74 (0.11)	-0.98 to -0.51	-6.22 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	11.83 (1.19)	-0.59 (0.13)	-0.85 to -0.33	-4.54 (78)	0.000*
13- 13.9 (53)	13.46 (0.29)	13.14 (1.12)	-0.31 (0.15)	-0.62 to -0.01	-2.1 (52)	0.040*
14- 14.9 (64)	14.46 (0.23)	14.08 (0.84)	-0.37 (0.1)	-0.57 to -0.17	-3.67 (63)	0.000*
7- 14.9 (375)	11.99 (1.8)	11.47 (2.14)	-0.51 (0.05)	-0.63 to -0.4	-8.69 (374)	0.000*

*Statistically significant ($p < 0.05$); SD Standard deviation; SE Standard error; df degree of freedom

Table 11. Comparison between chronological age and dental age estimated using Bedek et al., 2-teeth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	6.22 (0.09)	-1.13 (0.11)	-1.39 to -0.86	-10.52 (6)	0.000*
8- 8.9 (14)	8.51 (0.29)	6.96 (0.87)	-1.54 (0.25)	-2.1 to -0.98	-5.98 (13)	0.000*
9- 9.9 (24)	9.37 (0.26)	7.72 (1.56)	-1.64 (0.32)	-2.32 to -0.97	-5.04 (23)	0.000*
10- 10.9 (28)	10.39 (0.26)	8.99 (1.25)	-1.39 (0.21)	-1.84 to -0.94	-6.36 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	10.96 (1.16)	-0.43 (0.18)	-0.82 to -0.04	-2.29 (35)	0.028*
12- 12.9 (49)	12.47 (0.27)	11.54 (1.42)	-0.93 (0.19)	-1.32 to -0.53	-4.73 (48)	0.000*
13- 13.9 (53)	13.51 (0.29)	12.83 (1.25)	-0.67 (0.15)	-0.98 to -0.35	-4.27 (52)	0.000*
14- 14.9 (64)	14.44 (0.29)	13.66 (1.02)	-0.77 (0.12)	-1.01 to -0.52	-6.33 (63)	0.000*
7- 14.9 (275)	12.17 (2.01)	11.24 (2.55)	-0.92 (0.07)	-1.07 to -0.78	-12.54 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	7.62 (1.61)	0.09 (0.46)	-0.95 to 1.15	0.21 (9)	0.840
8- 8.9 (16)	8.34 (0.25)	7.24 (1.04)	-1.09 (0.27)	-1.68 to -0.5	-3.96 (15)	0.001*
9- 9.9 (22)	9.45 (0.25)	8.52 (2.04)	-0.93 (0.43)	-1.83 to -0.02	-2.13 (21)	0.044*
10- 10.9 (37)	10.42 (0.25)	9.42 (1.58)	-1.01 (0.25)	-1.51 to -0.48	-3.95 (36)	0.000*
11- 11.9 (94)	11.41 (0.28)	10.54 (1.33)	-0.87 (0.13)	-1.14 to -0.59	-6.24 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	11.83 (1.19)	-0.59 (0.13)	-0.85 to -0.33	-4.55 (78)	0.000*
13- 13.9 (53)	13.46 (0.29)	13.14 (1.12)	-0.31 (0.15)	-0.62 to -0.01	-2.1 (52)	0.040*
14- 14.9 (64)	14.46 (0.23)	14.08 (0.85)	-0.37 (0.1)	-0.57 to -0.17	-3.67 (63)	0.000*
7- 14.9 (375)	11.99 (1.8)	11.33 (2.34)	-0.65 (0.06)	-0.78 to -0.51	-9.76 (374)	0.000*

*Statistically significant (p<0.05); SD Standard deviation; SE Standard error; df degree of freedom

Table 12. Comparison between chronological age and dental age estimated using Bedek et al., 1-tooth formulae in boys and girls

Age Groups (n)	Mean (SD)			95% CI of age difference	t statistics (df)	p-value
	Chronological Age (CA)	Dental Age (DA)	DA-CA (SE)			
Boys						
7- 7.9 (7)	7.35 (0.29)	6.43 (0.0)	-0.92 (0.11)	-1.19 to -0.65	-8.32 (6)	0.000*
8- 8.9 (14)	8.51 (0.29)	7.2 (0.91)	-1.31 (0.26)	-1.89 to -0.72	-4.86 (13)	0.000*
9- 9.9 (24)	9.37 (0.26)	7.7 (1.41)	-1.67 (0.29)	-2.29 to -1.05	-5.57 (23)	0.000*
10- 10.9 (28)	10.39 (0.26)	8.8 (1.39)	-1.58 (1.32)	-2.1 to -1.07	-6.34 (27)	0.000*
11- 11.9 (36)	11.4 (0.29)	10.92 (1.35)	-0.47 (0.22)	-0.92 to -0.02	-2.13 (35)	0.040*
12- 12.9 (49)	12.47 (0.27)	11.65 (1.78)	-0.81 (0.24)	-1.31 to -0.31	-3.28 (48)	0.002*
13- 13.9 (53)	13.51 (0.29)	13.03 (1.32)	-0.46 (0.16)	-0.8 to -0.12	-2.77 (52)	0.008*
14- 14.9 (64)	14.44 (0.29)	13.72 (0.8)	-0.71 (0.1)	-0.92 to -0.51	-6.89 (63)	0.000*
7- 14.9 (275)	12.17 (2.01)	11.31 (2.62)	-0.86 (0.08)	-1.02 to -0.7	-10.71 (274)	0.000*
Girls						
7- 7.9 (10)	7.52 (0.31)	8.07 (1.61)	0.55 (0.46)	-0.48 to 1.6	1.02 (9)	0.260
8- 8.9 (16)	8.34 (0.25)	7.76 (1.07)	-0.57 (0.28)	-1.18 to 0.02	-2.03 (15)	0.060
9- 9.9 (22)	9.45 (0.25)	9.04 (1.85)	-0.41 (1.87)	-1.24 to 0.41	-1.03 (21)	0.312
10- 10.9 (37)	10.42 (0.25)	9.88 (1.53)	-0.54 (0.23)	-1.02 to -0.05	-2.26 (36)	0.030*
11- 11.9 (94)	11.41 (0.28)	10.69 (1.34)	-0.71 (0.13)	-0.99 to -0.44	-5.16 (93)	0.000*
12- 12.9 (79)	12.43 (0.28)	11.74 (1.14)	-0.68 (0.12)	-0.93 to -0.43	-5.37 (78)	0.000*
13- 13.9 (53)	13.46 (0.29)	13.05 (1.13)	-0.41 (0.15)	-0.71 to -0.11	-2.72 (52)	0.009*
14- 14.9 (64)	14.46 (0.23)	14.05 (0.86)	-0.41 (0.1)	-0.61 to -0.2	-4.01 (63)	0.000*
7- 14.9 (375)	11.99 (1.8)	11.45 (2.17)	-0.53 (0.06)	-0.66 to -0.41	-8.38 (374)	0.000*

*Statistically significant (p<0.05); SD Standard deviation; SE Standard error; df degree of freedom

Individual differences

The maximum and minimum deviations and their frequency were presented for all models in Figure 1. The individual difference between the DA and CA was found to be more frequent within 0 to +0.5 years (overestimation) for Bedek et al's 7 tooth, 5 tooth and 2 tooth models, within 0 to -0.5 years (underestimation) for Willems models, within 0.5 to 1 years (overestimation) for Bedek et al's 4 tooth, 3 tooth and 1 tooth models, within -0.5 to -1 year (underestimation) for Bedek et al's 6 tooth model.

Mean absolute error

Table 13 shows the output for gender as well as overall mean absolute error (MAE) for each tested model. The smallest MAE for boys was obtained for Bedek et al's 6 tooth model, 0.75 years, while the greatest MAE was for Bedek et al's 1 tooth model, 1.05 years. For the remaining models, MAE ranged from 0.85 to 1.02 years.

Figure 1. Individual differences for South Indian sample of boys using Willems model (GS) and Bedek et al., age estimation models

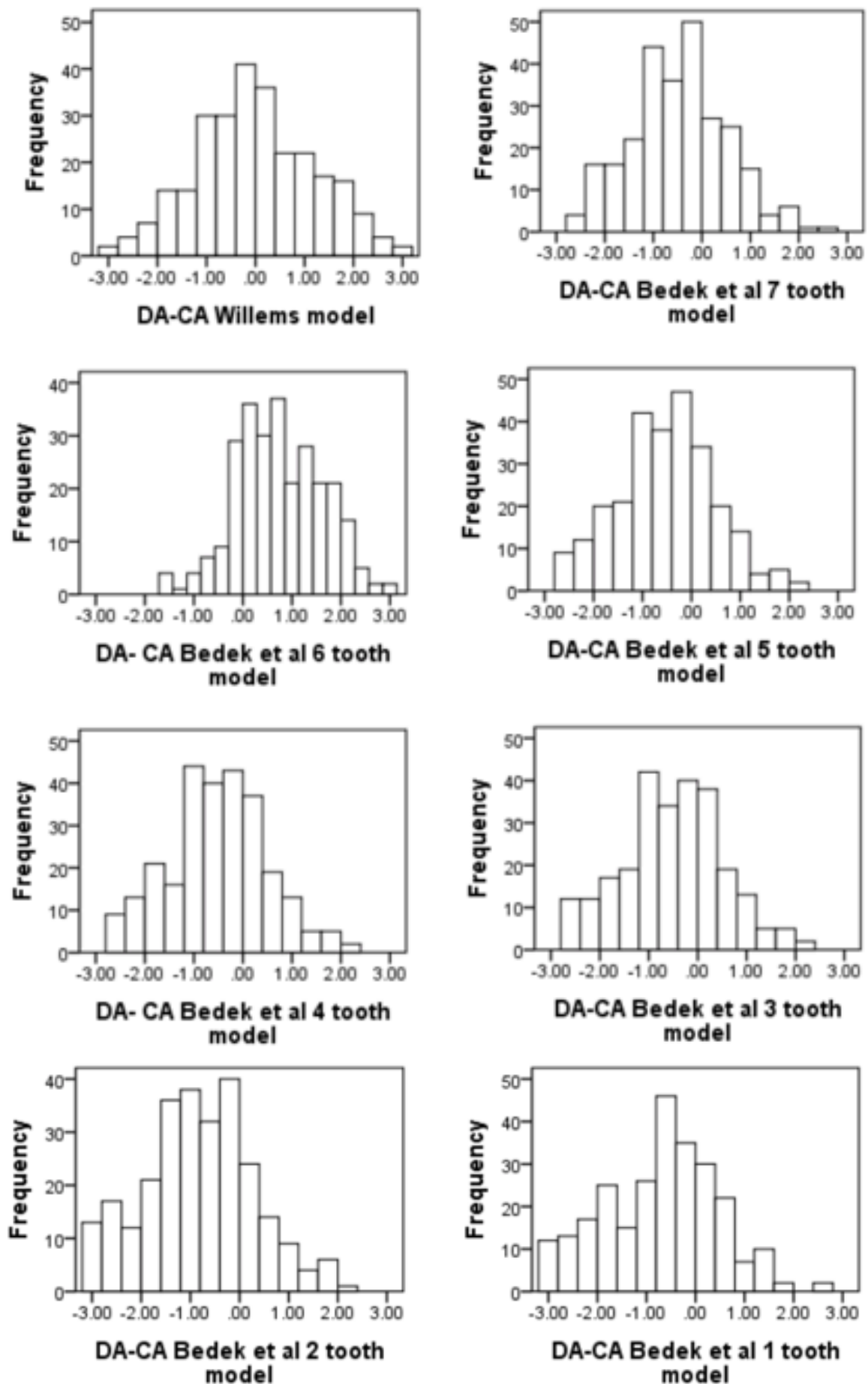


Table 13. Mean absolute error (MAE) for all tested methods in boys and girls respectively

Methods	Mean Absolute error/ deviation		
	Boys	Girls	Total
Willems	1.02	1.25	1.16
Bedek's seven teeth	0.85	0.88	0.86
Bedek's six teeth	0.75	0.9	0.94
Bedek's five teeth	0.85	0.94	0.9
Bedek's four teeth	0.86	0.92	0.89
Bedek's three teeth	0.95	0.91	0.93
Bedek's two teeth	0.96	1.01	0.99
Bedek's one tooth	1.05	0.98	1.01

Girls

Group differences

Comparisons of mean estimated DA and CA for all the tested models for girls were shown in tables 5 - 12. When the mean differences DA and CA for individual age groups were evaluated, Bedek et al's 7 to 4 tooth models have the least number of statistically significant different age groups (i.e., 4 out of 8). For Willems models, Bedek et al's 3 and 1 tooth models, statistically significant differences were seen in 5 out of 8 age groups, while the 2 tooth model has 7 out of 8 statistically significant age groups.

With respect to the mean data of all age groups, it is evident that all models significantly underestimated CA in girls; Willems models ($t=-3.437$; $p=0.001$), Bedek et al's 7 tooth model ($t=-6.322$; $p=0.000$), 6 tooth model ($t=-6.497$; $p=0.000$), 5 tooth model ($t=-7.144$; $p=0.000$), 4 tooth model ($t=-6.913$; $p=0.000$), 3 tooth model ($t=-8.694$; $p=0.000$), 2 tooth model ($t=-9.767$; $p=0.000$) and 1 tooth model ($t=-8.380$; $p=0.000$). Overall mean age deviations are larger for Bedek et al's 2 tooth model (-0.65 years) and the smallest deviation for Willems model (-0.27 years). For the remaining models, the mean age deviations are as follows; Bedek 7 tooth (-0.36 years), 6 tooth (-0.38 years), 5 tooth (-0.43 years), 4 tooth (-0.41 years), 3 tooth (-0.51 years), and 1 tooth (-0.53 years).

Individual differences

The maximum and minimum deviations and their frequency were presented for all models in Figure 2.

The individual difference between the CA and estimated DA was found to be more frequently within 0 to +0.5 years (overestimation) for Bedek et al's 1 tooth model, within 0 to -0.5 years (underestimation) for Bedek et al's 5 to 2 tooth models, within 0.5 to 1 years (overestimation) for Bedek et al's 7 and 6 tooth models, within -0.5 to -1 year (underestimation) for Willems models.

Mean absolute error

Results of MAE in girls shows that Bedek et al's 7 tooth model has the smallest value i.e., 0.88 years, while the greatest MAE was for Willems models, 1.25 years. And for remaining models of Bedek et al., MAE ranged between 0.9 to 1.01 years (Table 13).

Percentage of correct estimations

Table 14 presents the accuracy of dental age estimation for different models as a percentage of correct estimations within ± 0.5 years and ± 1 year interval. Our results show that Bedek et al's 7 tooth model has outperformed remaining models, 33.8% (220 out of 650) of age estimates fell within ± 0.5 years from CA, whereas 60.1% (391 out of 650) of age estimates were found to be within ± 1 year. For Willems models, 24.1% of age estimates were within ± 0.5 years and 45.3% were within ± 1 year, which is markedly less when compared to the remaining models of Bedek et al.

Figure 2. Individual differences for South Indian sample of girls using Willems model (GS) and Bedek et al., age estimation models

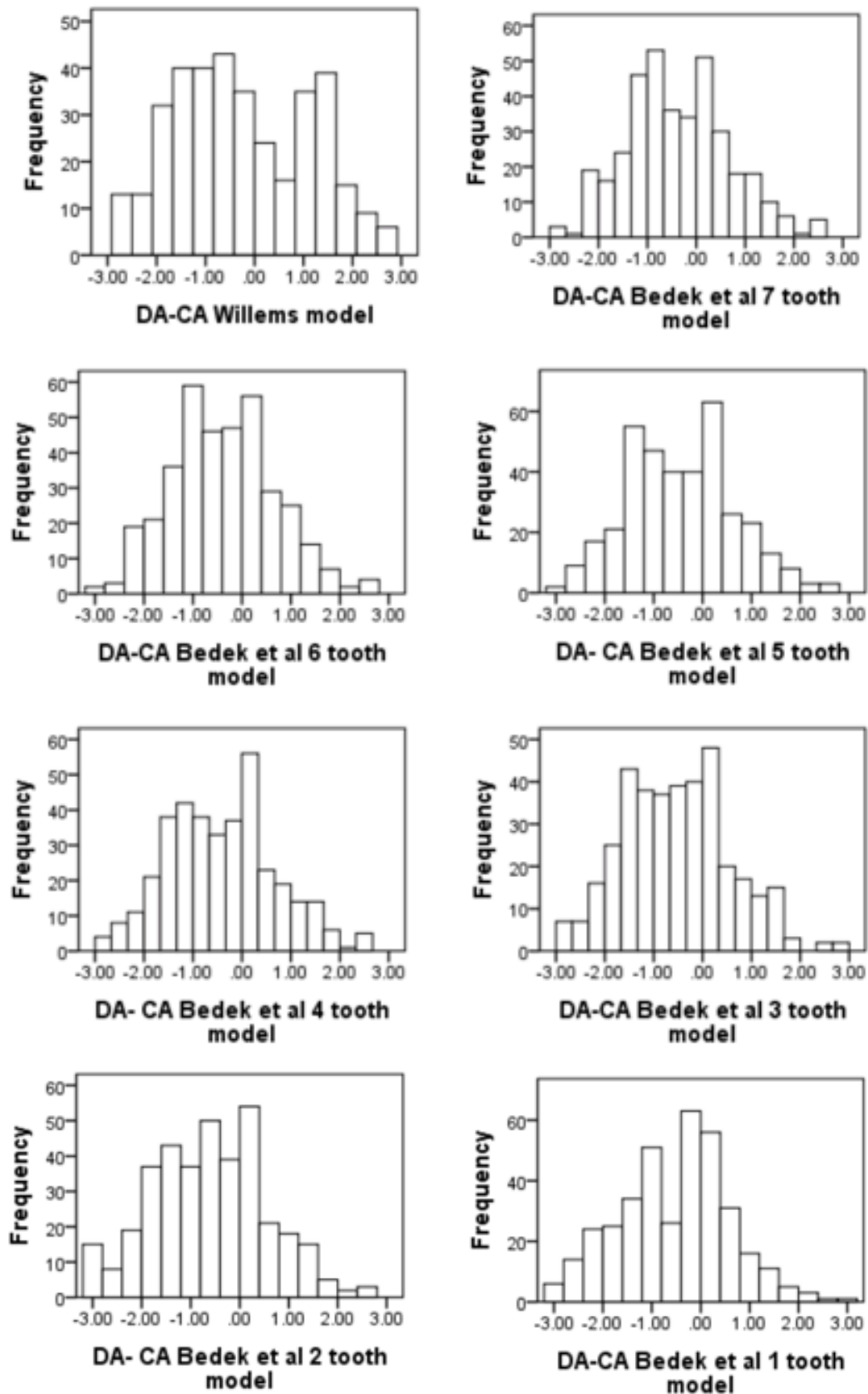


Table 14. Accuracy of age estimation using Willems method and Bedek et al., newly developed models based on 1 to 7 teeth for overall (percentage of correct estimations within interval)

Methods	Percentage of correct estimations			
	Boys		Girls	
	± 0.5 years n (%)	± 1 year n (%)	± 0.5 years n (%)	± 1 year n (%)
Willems	90 (32.7)	144 (52.3)	67 (17.8)	151 (40.2)
Bedek's seven teeth	102 (37.1)	171 (62.1)	118 (31.4)	220 (58.6)
Bedek's six teeth	91 (33.1)	164 (59.6)	121 (32.2)	217 (57.8)
Bedek's five teeth	98 (35.6)	170 (61.8)	122 (32.5)	202 (53.8)
Bedek's four teeth	95 (34.5)	174 (63.2)	124 (33.1)	204 (54.4)
Bedek's three teeth	94 (34.1)	165 (60)	113 (30.1)	200 (53.3)
Bedek's two teeth	71 (25.8)	135 (49.1)	110 (29.3)	190 (50.6)
Bedek's one tooth	84 (30.5)	149 (54.1)	132 (35.2)	205 (54.6)

DISCUSSION

Traditionally, Demirjian's system and Willems method require all seven mandibular teeth in the lower left quadrant to make an assessment. If any tooth/teeth is/are missing in the examining quadrant, the authors have suggested substituting an assessment of the matching tooth from the contralateral side, as there is high degree of lateral symmetry. If the same tooth from the contralateral side was also missing, then estimations may be obtained by inserting an average score of the remaining six teeth.⁵ Assigning score zero for all non-appearance teeth or calculating the average from the remaining teeth (less than seven) could result in a biased estimate at any particular age. This is a marked limitation for the use of maturity scores in Demirjian's system, and no allowance is made for missing data.¹⁶

Each case is specific in a forensic incident. Conducting age assessments in individuals with multiple missing teeth is a challenge for forensic experts. According to the reports from the survey by Endo et al., the most common symmetrical missing tooth could be the mandibular second premolars.^{17, 18} Wide variations in incidence and prevalence of hypodontia were reported in different studies conducted in various populations; 11.2% in Korea,¹⁹ 3% to 5.9% in Germany,²⁰ 6.3% in

Brazil,²¹ 3.31% in Spain,²² 11.01% in India.²³ Rakhshan in their systematic review and meta-analysis reported that congenitally missing teeth can range from 0.15% to 16.18% among different populations worldwide.²⁴ Badrov et al., indicated that age assessment methods may not be implemented in subjects with congenitally missing teeth, as lower permanent teeth are most likely to be affected with agenesis.²⁵ They also believed that hypodontia can impact results of dental age estimation, therefore researchers must account for the difference in the dental development of children with hypodontia when calculating DA.²⁵

Most of the studies published so far mentioned "radiographs that showed hypodontia or subjects with hypodontia were excluded" suggesting the unavailability of methods to assess dental age in such individuals. In 1976, Demirjian proposed two 4 tooth methods for age estimation, when tested in other populations, both 4 tooth methods were found to be relatively inaccurate, less frequently overestimate age and are not suitable.^{15, 26} As congenitally missing teeth are prevalent and methods to assess dental age are indispensable, Bedek et al., provided models for dental age estimation for forensic and clinical purposes in such subjects.¹⁴

Willems method

In the present study, Willems method underestimated CA by 0.03 years in boys and 0.27 years in girls. Consistent results were reported by various studies in the literature i.e., in British Caucasian and Bangladeshi, Korean and Brazilian children.²⁷⁻²⁹ On the other hand, many researchers have reported overestimations with Willems method.^{26, 30, 31} Similar to our findings, Hedge et al., also reported smaller underestimations of age when tested in Indian children.³² The MAE was 1.02 years, 1.25 years and 1.16 years for boys, girls and the total sample using Willems method.

Bedeck et al's new models

When considering the suitability of Bedeck et al's new models, generalised underestimation of age was seen in all age groups for both sexes except the 6 tooth model, where overestimation of CA was seen in boys.

In forensic anthropology, the mean difference between DA and CA is considered accurate if it falls within the range of ± 0.5 years, and a difference of ± 1.0 year is considered acceptable.^{33, 34} In the present study, in boys the 7 tooth model produced 37.1% cases within the ± 0.5 year interval, while the 4 tooth model produced 63.2% cases within the ± 1.0 -year interval. These findings were less when compared to the original Bedeck et al models where they observed 43.5% cases within ± 0.5 year interval and 72.2% cases within ± 1.0 -year interval, both for the 7 tooth model respectively.¹⁴ In girls, the 7 tooth model produced 33.1% cases within ± 0.5 year interval, while the 7 tooth model had 58.6% cases falling within ± 1.0 -year interval. Similar to boys, observations in girls were also less compared to original Bedeck et al's models where they observed 40.7% cases within ± 0.5 year (5 tooth model) and 71.6% cases within ± 1.0 -year interval (7 tooth model) respectively.¹⁴

As the phrase goes "Time is of the essence" and in forensic cases time is one important factor

that needs to be considered seriously. During criminal or civil investigations, the method of choice for forensic examiners will be the one that gives accurate results in a tested population within a short period of time. A method that quickly gives accurate results and is user-friendly satisfies judicial requirements.³⁵ It can be inferred from our results that Bedeck et al's models of age estimation are simple, accurate, produce maximum accuracy with a smaller number of teeth.

CONCLUSIONS

In cases of age estimation in children, maximum accuracy is usually obtained and prediction of DA is higher when all seven lower left mandibular teeth are available. Similarly, an equally effective and accurate method is needed when an individual has less than seven teeth. Our study results show that Bedeck et al's new models of age assessment could be valid for the tested population. The following are some advantages:

1. These new models are simple, reliable, accurate and easily applicable in cases of multiple missing teeth.
2. They can be used for simple clinical purposes (assessing age in individuals with hypodontia) as well as for complex forensic scenarios (incomplete human remains).
3. In the original study, it has been determined that models with two teeth are significantly more accurate than Willems models. When tested in the studied population, the 1 tooth model produced a higher percentage of correct estimations (± 0.5 and 1 year) than Willems models.
4. Maximum accuracy was also obtained using the models with less than seven teeth.

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Characterization of lip prints in a Portuguese twins' population

Susana Braga ¹,
Maria Lurdes Pereira ²,
Benedita Sampaio-Maia ²,
Inês Morais Caldas ².

¹ Faculdade de Medicina da
Universidade do Porto, Portugal

² Faculdade de Medicina Dentária da
Universidade do Porto, Portugal

Corresponding author:
icaldas@fmd.up.pt

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ABSTRACT

Lip print patterns are referred to as unique to each individual, but controversy exists surrounding twins. In this study, the lip prints of 19 pairs of monozygotic and 47 pairs of dizygotic twins were studied. The left lower lip was photographed and the furrows were classified using Renaud's classification. Results showed the same lip pattern was found only in one monozygotic pair (5.3%) and in 4 dizygotic pairs (8.5%), and no significant statistical differences were found between groups ($p > 0.05$). In monozygotic twins only type C furrows presence displayed statistical significant differences ($p = 0.034$). As for dizygotic twins, there were statistical significant differences in the frequency of type A ($p = 0.005$) and type G furrows ($p = 0.018$). As for the most common types, both groups displayed a higher prevalence of vertical furrows (type B: 97.4% and 96.8%, type A: 86.8% and 87.2%, in monozygotic and dizygotic, respectively). The least frequent furrow type was type I and type E in monozygotic (2.6% and 5.3%, respectively) and types E, F and I, in dizygotic (6.4%, 7.4% and 7.4%, respectively). Our results seem to point out that lip print patterns should be useful carefully in twins' identification.

INTRODUCTION

Lip prints usefulness in human identification has been widely explored. Their ability to distinguish among individuals is largely responsible for their use in forensic matters ^{1, 2}. Yet, some issues regarding singularity and inheritance have been raised, as some authors pointed out a positive resemblance in lip print patterns among family members ³ and others report no similarity between twins ^{4, 5} or with their parents.

Suzuki and Tsuchihashi, in 1971, ⁶ carried out the first twin study on lip prints, and analysed 18 pairs of monozygotic twins. Their results indicated lip prints of the twins were extremely similar and that their characteristics were inherited from either the father or the mother. Mc Donnel, in 1972, reported lip print patterns between two identical twins were quite different from each other ⁷. In 1974, Tsuchihashi extended the original research and reported that lip print patterns are unique to each individual. This researcher studied 1364 Japanese natives, including 49 pairs of identical twins, and concluded that it is possible to distinguish different persons through cheiloscopy examination. Actually, the Japanese researcher stated that a detailed comparison of twin pairs proved not to be identical despite the great similarity between the lip grooves. In this study, which occurred during a period of three years,

Tsuchihashi also demonstrated the unchangeability of lip print patterns². The same conclusions were reached by Hirthe et al. ⁸, analysing 76 families, including 22 pairs of monozygotic and 17 pairs of dizygotic twins. Similarly, Thakur et al. ⁹, referred to a comparative study performed by Schnuth and Marry Lee on 150 individuals including five pairs of identical twins; in this investigation the authors found that lip prints were not identical in the case of identical twins, but similarities of lip prints between parents and children were found. Further studies involving twins also revealed that lip prints were not exactly identical ¹⁰⁻¹² although some of the characteristics were inherited from the parents ¹⁰. However, other more recent studies concluded that lip print patterns were unique in monozygotic twins ¹³, and that no similarity was found with their parents.⁴

The aim of the present study was to perform a comparative study of lip print patterns in the monozygotic and dizygotic twins in a Portuguese population. It was intended to assess the intra-pair differences and variations of lip prints in monozygotic and dizygotic twins, and therefore contribute to the theory of uniqueness of lip print patterns.

MATERIAL AND METHODS

The studied sample had 19 pairs of monozygotic twins and 47 pairs of dizygotic twins. The sample’s distribution by sex is depicted in table 1.

Table 1. Sex distribution of the participants, n (%)

Sex	Monozygotic	Dizygotic
Male	24 (63.2)	50 (53.2)
Female	14 (36.8)	44 (46.8)

The 66 pairs of twins were part of the *Geração XXI*

(“Generation XXI”) cohort, from the Public Health Institute of the University of Porto. *Geração XXI* consists in the first cohort in Portugal, whose objective is prenatal characterization and the post-natal development, identifying determinants in Health with interest in childhood, adolescence and adulthood. The selected twins were of European ancestry and aged between 11 and 13 years old. This study included monozygotic and dizygotic twins, whose zygota was proven before its inclusion in the study. All participants who have congenital or acquired disorders, medically relevant conditions were excluded, as were children with a history of orofacial trauma.

Informed consent was obtained from all individual participants included in the study, and the investigation was submitted and approved by the Ethics Commission of the Faculty of Dental Medicine of Porto University (reference number 000030 – 10/01/2017) and by the Portuguese Data Protection Authority (reference number 64.567.634 – 13/10/2017).

The furrows on the left lower lip were photographed and analysed using Renaud’s classification¹⁴ (Figure 1 and Table 2). Each furrow was classified for its presence or absence. The analysis of the left lower lip alone was applied, as it simplifies the classification process, and has been applied previously. ^{15, 16}

Reproducibility or intra-observer error was evaluated assessing agreement between 20 randomly selected photographs examined twice by the same examiner 1 month apart. Cohen’s kappa (k) was used to evaluate the quality of the agreement, as suggested by Landis and Koch.¹⁷ The agreement was almost perfect with k =0.856. Repeatability or inter-observer error was evaluated by measuring the agreement between 20 randomly selected photographs examined by two different examiners. Again, the agreement was almost perfect with k =0.802.

Figure 1. Lip print types according Renaud's Classification

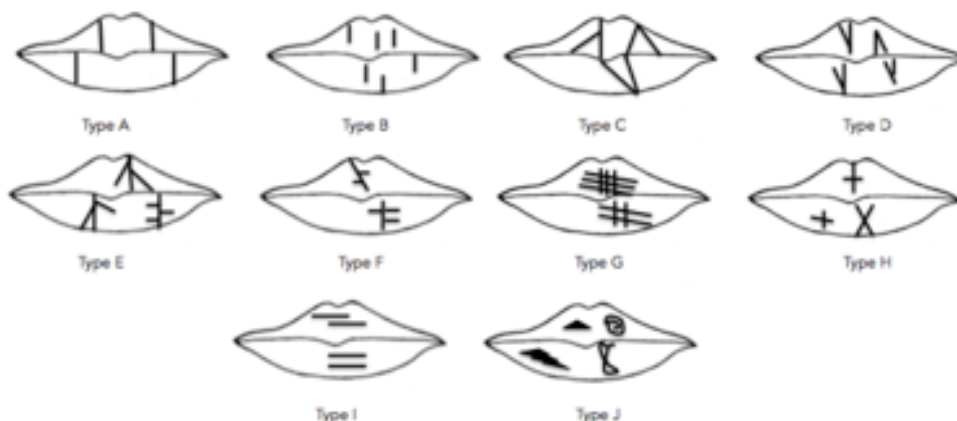


Table 2. Renaud's classification

Classification	Furrow type
A	Complete vertical
B	Incomplete vertical
C	Complete bifurcated
D	Incomplete bifurcated
E	Complete branched
F	Incomplete branched
G	Reticular pattern
H	X or coma form
I	Horizontal
J	Other forms (ellipse, triangle)

Statistical analysis was performed using SPSS 25.0 software (SPSS Inc., Chicago, IL). Pearson's Chi-square test was used to compare qualitative data and determine statistical significance. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

Lip furrows distribution in the left lower lip is depicted in table 3.

The furrows' frequency was quite similar in both groups. As a matter of fact, in both groups, the most prevalent furrow type was the vertical incomplete (type B), with percentages of frequency over 95% in both groups (97.4% and 96.8%, in monozygotic and dizygotic, respectively). The second most frequent furrow type was, in both groups, the type A (86.8% and 87.2%, in monozygotic and dizygotic, respectively). The least frequent furrow type was the horizontal type (type I) in monozygotic (2.6%) and type E in the dizygotic group (6.4%), although both types depicted low frequencies in both groups. Overall, vertical furrows were present in 46.7% of the total sample.

Overall, the same lip pattern was found only in one monozygotic pair (5.3%) and in 4 dizygotic pairs (8.5%), and no significant statistical differences were found between groups ($p > 0.05$).

As to dissimilarities among twins in the different groups, in monozygotic twins only type C furrows presence displayed statistically significant differences ($p = 0.034$) (table 4).

As for dizygotic twins, there were statistically significant differences in the frequency of type A ($p = 0.005$) and type G furrows ($p = 0.018$) (table 5).

Table 3. Type of lip furrows in monozygotic and dizygotic twins, n(%)

Type	Monozygotic, n=38	Dizygotic, n=94	TOTAL, n=520
A	33 (86.8)	82(87.2)	115(22.1)
B	37(97.4)	91(96.8)	128(24.6)
C	16(42.1)	30(31.9)	46(8.8)
D	20(52.6)	54(57.45)	74(14.2)
E	2(5.3)	6(6.4)	8(1.5)
F	3(7.9)	7(7.4)	10(1.9)
G	8(21.1)	31(33.0)	39(7.5)
H	8(21.1)	30(32.9)	38(7.3)
I	1(2.6)	7(7.4)	8(1.5)
J	14(36.8)	40(42.5)	54(10.4)

Table 4. Differences in lip furrows frequency in monozygotic twins, n(%); p value (significant values in bold) (n.c. – not calculated)

Type	Pair 1, n=19	Pair 2, n=19	p
A	17(89.5)	16(84.2)	0.110
B	19(100)	18(94.7)	n.c.
C	9(47.4)	5(26.3)	0.034
D	12(63.2)	7(36.8)	0.585
E	1(5.3)	1(5.3)	1
F	3(15.8)	14(73.7)	n.c.
G	5(26.3)	3(15.8)	1
H	3(15.8)	4(21.1)	1
I	1(5.3)	0(0)	n.c.
J	7(36.8)	7(36.8)	1

Table 5. Differences in lip furrows frequency in dizygotic twins, n(%); p value (significant values in bold)

Type	Pair 1, n=47	Pair 2, n=47	p
A	38(80.9)	44(93.6)	0.005
B	46(97.9)	45(95.7)	1.000
C	17(36.2)	12(25.5)	0.306
D	28(59.6)	25(53.2)	1.000
E	2(4.3)	4(8.5)	1.000
F	1(2.1)	6(12.8)	1.000
G	18(38.3)	13(27.7)	0.018
H	14(29.8)	15(31.9)	1.000
I	1(2.1)	5(10.6)	1.000
J	21(44.7)	19(40.4)	0.361

DISCUSSION

To the best of our knowledge, this is the first characterization of lip print patterns in a Portuguese twins' population.

Lip prints have been used as a genetic marker in many congenital and clinical diseases¹⁸, and some lip print patterns have been related with cleft lip and palate deformities.^{19, 20} Cardoso Fernandes and co-workers²¹ have linked a specific type of lip furrow (type I) with Down Syndrome individuals,

referring that statistical significant differences occurred between these individuals and their siblings ($p < 0.001$). According to the authors this link between type I furrows and Down Syndrome individuals may imply a lower potential of cheiloscopy identification due to the poor divergence of labial phenotypes among these individuals.

Other patterns, however, have been considered as a genetic marker for the health of the offspring if

it appears in parents' lips.^{20, 22} Furthermore, lip print patterns have been associated with hypertension, as the frequencies of the branched and undifferentiated patterns were higher in the hypertensive than in the normotensive²³. It has also been referred to that the association of lip print patterns with smoking habits, previous labial trauma with no residual scars, parafunctional oral habits, or being a professional brass player showed no statistical significance²⁴, suggesting environmental factors play little of a role in lip print patterns' morphology.

The idea behind studying monozygotic and dizygotic twins was to compare genetic and environmental influences in lip print formation. Monozygotic (or identical) twins are derived from the division of a single zygote, whereas dizygotic (or fraternal) twins are derived from the fertilization of two independently released ova, and are not more genetically alike than ordinary brothers and sisters.²⁵

Our results seem to point out that lip print patterns may not be unique even in monozygotic twins. This is because, in spite of only studying the presence or absence of the types of furrows, one pair had the same elements. We cannot argue about the specific location of the furrows in this particular case, and therefore cannot state if singularity exists in this particular case. Moreover, discussion exists about if all the lip print must be studied to study a lip print, particularly for sex estimation²⁶. For identification purposes, the methodology we have used is the one adopted by most authors to determine singularity^{1, 27-32}.

Nevertheless, it can still be stated that in the remaining 94.7%, singularity did exist in monozygotic twins. As for dizygotic, 8.5% (4 pairs) did have the same element in the left lower lip. Once more, the specific location or number of these elements was not evaluated, but we are able to state that in the remaining 91.5% singularity was proven.

As for differences in lip patterns, in monozygotic twins, only type C furrows presence displayed differences, whereas, among dizygotic twins, there were differences in the frequency of type A and type G furrows. So, it is possible that the presence of complete bifurcated grooves can be used for distinguishing monozygotic twins, whereas, in dizygotic, complete vertical and reticular patterns can separate dizygotic twins. In theory, there should be more dissimilarities in

dizygotic twins, and distinguishing them by lip print patterns is not needed. Yet, in monozygotic twins, using lip print patterns to distinguish between pairs has been reported^{25, 33}.

Considering the differences found in lip print patterns, both in monozygotic and dizygotic twins, it is fair to assume that similarities are rare, as most pairs, monozygotic or dizygotic, showed that lip patterns did not resemble each other. So, it may be that the theory of uniqueness can be applied and the potential for identification in twins may exist. It must, however, be underlined that the studied sample, particularly in what concerns monozygotic twins, was small, and thus a more robust hypothesis cannot be achieved.

As for the most common types, both groups displayed a higher prevalence of vertical furrows (type B and A). The least frequent furrow type was type I and type E in monozygotic, and types E, F and I, in dizygotic. These numbers may suggest a connection between lip furrows' morphological features and population affinity, as other authors have suggested³⁴, since all the sample had Portuguese ancestry. For instance, Moshfeghi et al.³⁰ reported that furrows displaying other forms (type J) were the most common in both Iranian males and females. This same type was the most prevalent in male and female Goan students³². As for Mangaloreans, Jeergal et al.²⁹ referred that incomplete vertical grooves were the most prevalent in both sexes. Conversely, these furrows were the least common among adults of Sebha, Libya; the most common in this population were the complete vertical furrows³¹.

Among Nigerians, Adamu et al.³⁵ found that the most prevalent furrow was "other type", followed by intersected groove pattern and being the least frequent type incomplete vertical grooves.

All of these studies suggest that a particular type of furrow is more likely in a given population. Nonetheless, among a Portuguese population, Costa and Caldas²⁷ referred that branched furrows were the most prevalent, which does not agree with our data. As a matter of fact, in this study the incomplete vertical grooves, the most prevalent in our sample, were one of the least prevalent, raising doubts if lip print patterns can in fact be used in population affinity estimation. Other authors have described discrepancies in population studies concerning the most prevalent furrow type, as well. Abdel Aziz et al.³⁶ referred in their study of an Egyptian population that

bifurcated and branched grooves were the predominant patterns of lip prints. Yet, in another study conducted in Dakahlia, in Egypt, the highest recorded lip print among the studied individuals was type A (complete vertical) in both sexes⁵. The authors justify discrepancies with different sample sizes used in the two studies (n=60 vs. n=955). In our case, different size samples was also an issue (n=132 vs. n=66).

Overall, our results seem to point to the lack of lip print pattern singularity, with 94.7%, of monozygotic twins presenting unique lip print patterns, and, in dizygotic, in 91.5% singularity was proven. As for the most common types, both groups displayed a higher prevalence of vertical furrows, which differs from the previous data concerning the Portuguese population, raising doubts if lip print patterns can in fact be used in population affinity estimation. We do realize that the studied sample, particularly concerning the monozygotic twins, was small, and a more robust hypothesis can only be achieved in further studies, with larger samples.

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Pakistan's position in the world of forensic odontology and dental records

Falak Murad Shah Syed ¹,
Suman Shoro ¹,
Scheila Mânica ¹

¹ Centre for Forensic and Legal
Medicine and Dentistry, 2 Park Place,
Dundee, DD1 4HR, University of
Dundee, Scotland, UK

Corresponding author:
s.manica@dundee.ac.uk

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ABSTRACT

Background: Forensic Odontology (FO) still strives for recognition in some countries such as Pakistan. Natural and man-made disasters, along with child abuse cases and age estimation for child marriages and juvenile imprisonments in Pakistan justify its applicability.

Aims: This study investigated the awareness, information, training, practice and interest in FO in dental professionals in Pakistan. Another aim was to design tools to deliver primary knowledge about FO and emphasize the importance of dental records.

Methodology: A 10 question paper-based survey was distributed among 560 dental professionals and postgraduates of 14 public dental institutes/hospitals in Pakistan. The results were quantitatively analyzed by graphs using Microsoft Excel (version 16.22). An educational video and an information leaflet were produced after the survey was undertaken to explain the scope of FO and the importance of dental records respectively.

Results: 476 dentists (51% ♀, 49 % ♂) aged 20 – 50+ years responded and 98.53% confirmed that FO was not taught in the dental schools. 66% were aware of the field and 62% were only informed. 99% were not trained and 89.7% were not working in this field; however, 89% were interested in training within Pakistan. Considering dental charts, 60.92% do not produce detailed charts but 55% maintain them and the majority do so manually. Radiographs were the most stored type.

Conclusions: Most dentists are aware of the existence of FO, but they need to acknowledge the significance of dental record keeping and encourage implementation of FO. Regardless of the absence of any governing body for FO and negligible education, training and implementation in Pakistan, this field is gradually progressing. The authorities should introduce detailed guidelines for recording, managing and storing dental records. They should ensure the future acknowledgement of this subject in the education system and assign forensic odontologists to the Disaster Victim Identification (DVI) team.

INTRODUCTION

The disciplines of Forensic Odontology (FO) include age estimation, bite mark analysis, litigation against dental malpractice/dental fraud and most importantly human identification. Teeth are resilient to conditions such as high temperatures, immersion and decomposition and are classified

as one of the primary identifiers by Interpol. Moreover, teeth are the cheapest, quickest and easiest methods of human identification when compared to fingerprinting and DNA (1).

In Pakistan, FO as an educational subject and as a practising field is limited and is still struggling to establish itself. In a country of 207.774 million people and 2,040 dentists registered as specialists in 2018 (2) there is only "one" dentist who is registered as a Forensic Odontologist with the Asia-Pacific Forensic Odontologist (APFO) group. Pakistan, being a developing country and prone to natural disasters, is in drastic need to educate and train more professionals in the field. Even though many Asian countries are working on advancements in this field and have their DVI units which include forensic odontologists, Pakistan has been limited to only a handful of cases of age estimation and human identification in which FO has been used. Except for those cases, none of the extra measures (practical or academic) has been taken in this field within the country to date.

Every dentist is obliged to record complete dental details of the patients, as dental records

are legal documents (3) and might be required for human identification and cases of malpractice or fraud. Hence, the dentists in Pakistan should acknowledge the significance of dental records and they should learn comprehensive dental data recording, storing and updating protocols. This study investigated the awareness, information, training, practice and interest of FO among the dental professionals in Pakistan. Another aim was to design tools to deliver primary knowledge about FO and emphasize the importance of dental records respectively.

METHODOLOGY

Survey

A 10 question paper-based survey was distributed among 560 dental professionals and postgraduates of 14 public dental institutes/hospitals in Pakistan, which were selected randomly. The dentists were provided with a brief introduction to the project. Details such as age, sex, clinical experience and name of the institution/hospital were required. All questions (Q) were closed-ended apart from Q2, Q4 and Q9 as seen in Table 1.

Table 1. Questions and respective categories of the survey

Questions		Category of the questions
Q1	Please select the subjects which you were not taught in your dental school.	Education, awareness and information of Forensic Odontology
Q2	Have you heard about Forensic Odontology? If yes, when and how?	Forensic Odontology
Q3	As per your knowledge, which sub-topics from the following do you consider to be specifically related to Forensic Odontology?	Forensic Odontology
Q4	Have you received any professional training in Forensic Odontology?	Training and working in Forensic Odontology
Q5	Have you ever worked or assisted a Forensic Dentist, or have you ever given an opinion in a case related to forensics?	Training and working in Forensic Odontology
Q6	Do you make a detailed dental chart (current status of the dentition/treatment plan) of every patient you attend?	Transcribing, recording, and maintaining the dental data
Q7	Do you or the hospital maintain the dental records of your patients? If yes, choose one method and duration of storage?	Transcribing, recording, and maintaining the dental data
Q8	Please select the type of record regularly maintained by you or the hospital.	Transcribing, recording, and maintaining the dental data
Q9	According to your opinion, choose the appropriate statement in regard to the need of forensic odontology in our country	Need and interest of FO
Q10	Would you be interested to be trained as Forensic Odontologist within	Need and interest of FO

This survey was distributed by road travel in 2 phases, which ranged from July 2018 – August 2018 and December 2018 – January 2019 respectively. The names of these dental institutes, along with their specific provinces are seen in Table 2.

House officers, interns, dental technicians, nurses or assistants, undergraduate students and any dental professionals or postgraduate students working in private dental institutes were excluded from the inclusion criteria.

Table 2. Names of the institutes and the respective provinces for data collection

Provinces	Names of Dental Institutes
SINDH	(1)Dental Section, Karachi Medical & Dental College, Karachi. (KMDC); (2) Dental Section, Liaquat University of Medical and Health Sciences, Jamshoro (LUMHS); (3) Dental Section, Dow International Dental College, Karachi. (DIDC); (4) Dr. Ishrat-ul-Ebad Khan Institute of Oral Health Sciences, Karachi. (DIKIOHS); (5) Sindh Institute of Oral Health Sciences/JSMU, Karachi. (SIOHS/JSMU); and (6) Bibi Asifa Dental College, Larkana. * (BADC)
BALUCHISTAN	(1)Dental Section, Bolan Medical College, Quetta. * (BMC)
PUNJAB	(1)De'Montmorency College of Dentistry, Lahore. (De'monte); (2) Nishtar Institute of Dentistry, Multan. (NID); (3) Dental Section, Punjab Medical College, Faisalabad. (PMC)
PUNJAB	(1)Dental Section, Army Medical College, Rawalpindi (AFID)
KHYBER PAKHTUNKHWA	(1)Dental Section, Ayub Medical College, Abbottabad. (AMC); (2) Khyber College of Dentistry, Peshawar. (KCD); and (3) Bacha Khan Dental College, Mardan. (BKDC)

The results were analysed quantitatively using Microsoft Excel (version 16.22)

An Educational video

An educational video of 2 minutes 44 seconds in English language with subtitles in Urdu language was filmed within the premises of the School of Dentistry in Dundee, Scotland. This video was uploaded on YouTube after the participants filled the questionnaires. It answers two questions (a) *What do the Forensic Odontologists do?* And b) *How can the general dentists help Forensic Odontologists?* and can be viewed at: <https://www.youtube.com/watch?v=y12JxtlFERo>.

The information leaflet

An information leaflet was designed using Microsoft Publisher© to explain the reason for the use of teeth in human identification, the role of Forensic Odontologists and the types and importance of dental records. It was to be distributed among the dental professionals and dental students after the study was completed. It

can be found at: <https://drive.google.com/file/d/1C2FoM-9ztIjITuqsjpiP3l9TUCBVRClq/view>.

RESULTS

A total of 560 questionnaires were distributed, to which, 476 dentists 49%M and 51%F from the age range of 20 years to 50+ years responded (response rate= 85%) as seen in Figure 1.

Most of the dentists had a work experience of “1-3 years” (22.69%) followed by “3-5 years” (17.23%), “5-7 years” (14.50%) and “12-20 years” (13.66%). The experience of the 4.41% dentists who selected the option “OTHER” varied from 21 years to 35 years. 469 dentists (98.53%) selected FO as a subject not taught in their dental schools, followed by other options as seen in Figure 2.

When asked if they have heard about FO (Q2), 314 dentists (66%) responded “YES” contrary to 161 (34%). Out of these 314 dentists, 126 (40%) selected the option “more than 5 years ago”, “more than 3 years ago” (24%), “1-2 years ago” (22%), “within last 1 year” (14%) and 2 dentists did not answer.

To state the sources of awareness about FO, the dentists could select more than one option such as “through colleagues” (36%), “seminars/

workshops” (34%), “social media” (30%) and “Professors” (29%). 19% selected ‘OTHER’ as seen in Figure 3.

Figure 1. Count of dentists segregated based on the institutes and the age groups. (x= count of dentists, y= names of the institutes)

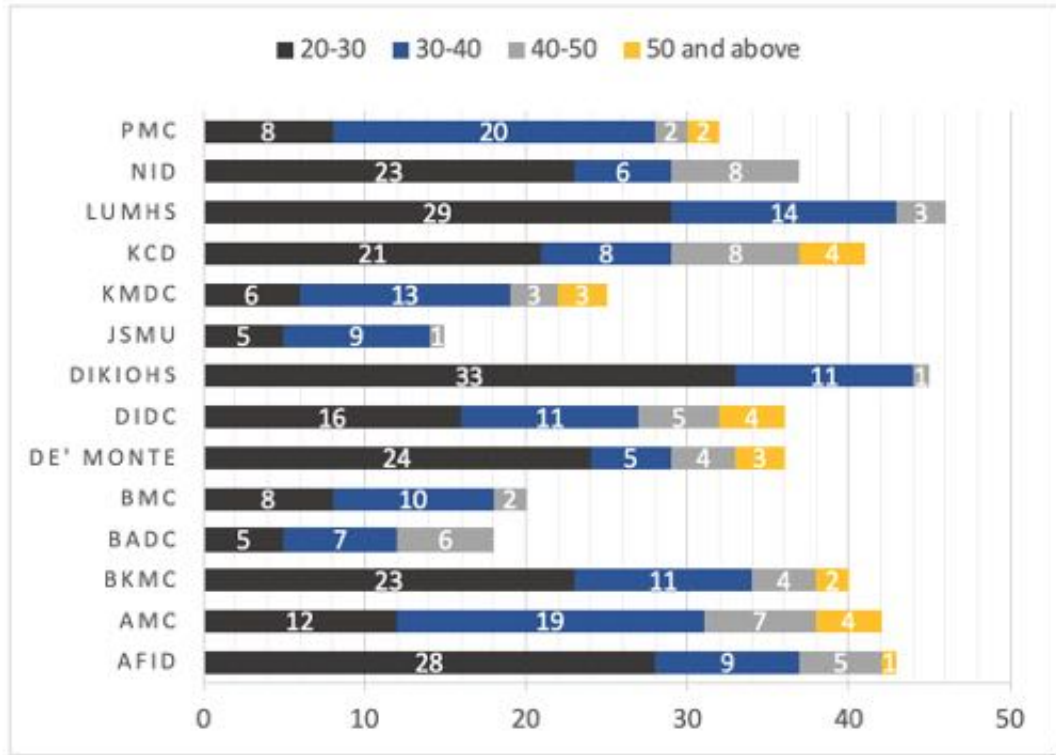


Figure 2. Percentages of the dentists and the subjects which were not taught in their dental schools. (x= percentage, y= subjects)

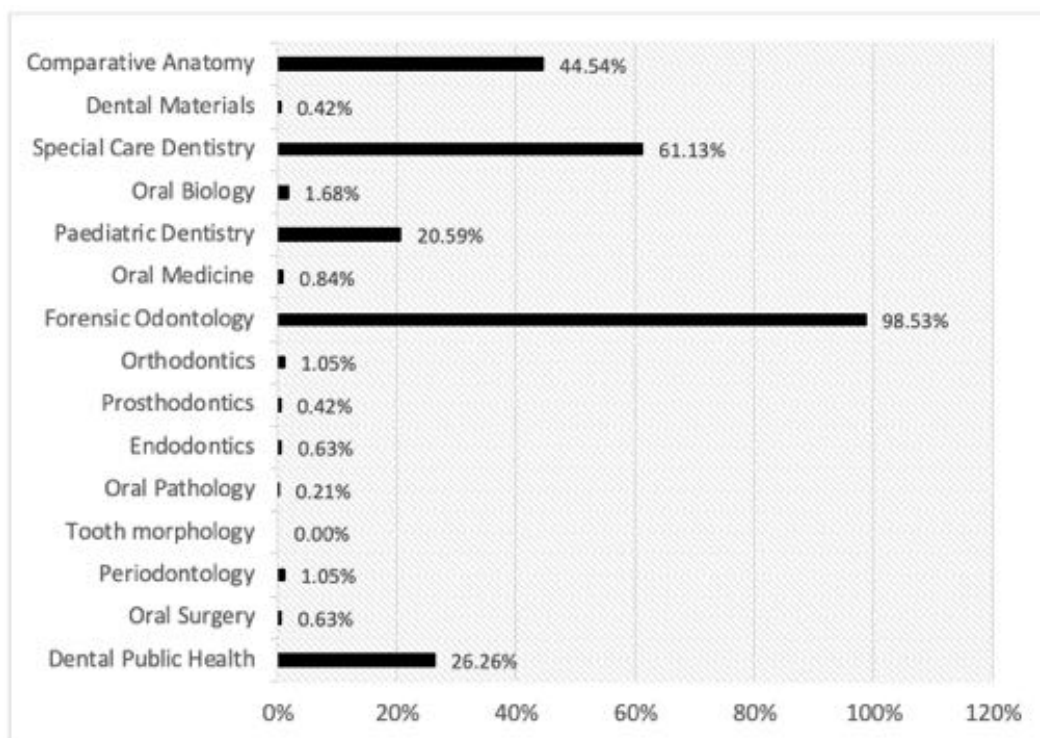


Figure 3. Various sources used for awareness of FO in contrast to the age groups of dentists. (x= count of dentists, y= sources)

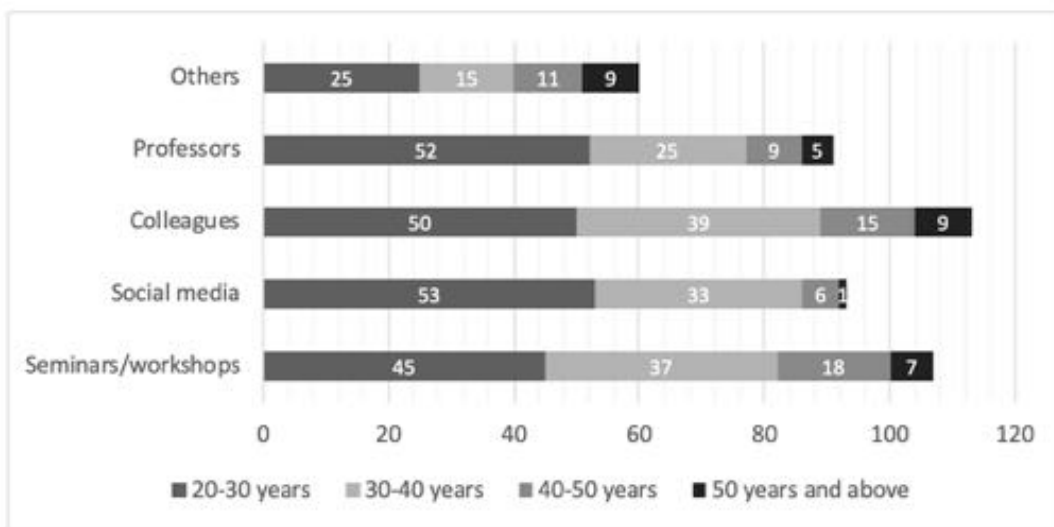


Figure 4 demonstrates Q3, which was a multiple-choice question, the author received 849 responses from 334 dentists. When analysed per participant, 62% of the dental professionals made entirely correct choices whereas, the rest of the 38% either chose completely non-FO or had a mixture of various FO and non-FO options. But the

only 5 sub-topics of FO which were correct, were selected all together by 9 (2.6%) dentists only. Figure 5 also exhibits other combinations chosen by the dentists. This reflects that a very small number of dental professionals had a complete understanding of the scope of FO at all and the majority was only informed.

Figure 4. Percentages of the dentists who made choices for the sub-topics of FO according to their knowledge. (x= percentage of dentists, y= choices given as sub-topics)

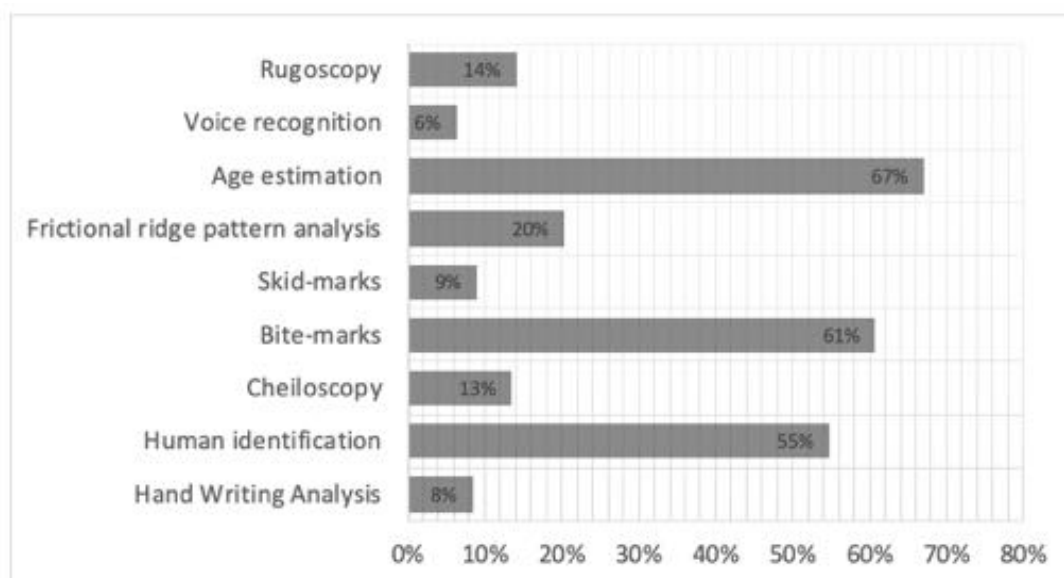
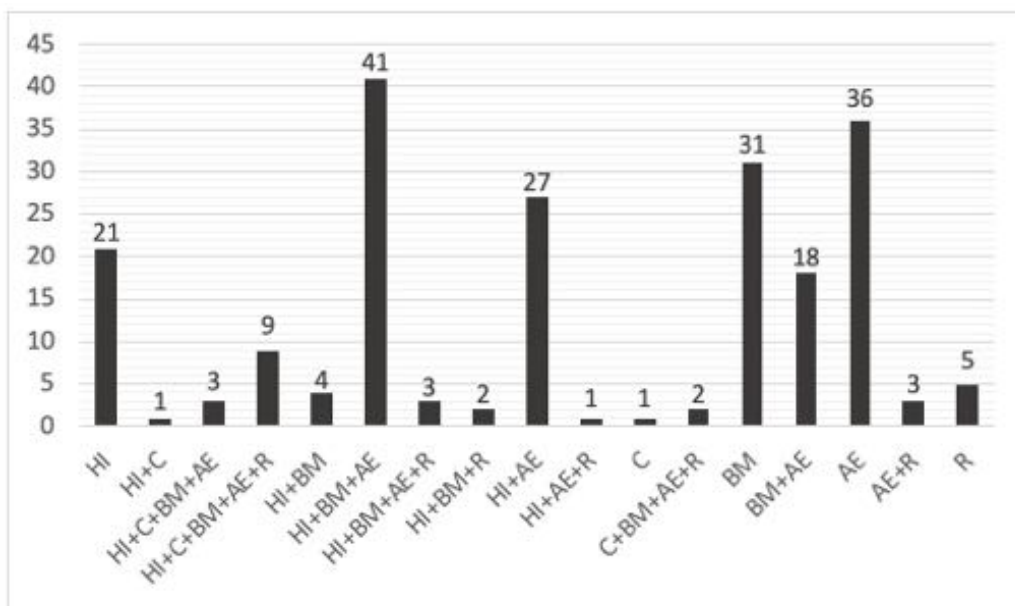


Figure 5. Count of dentists who solely chose FO related sub-topics (single or combinations). HI= human identification, C= cheiloscopy, BM= bite mark analysis, R= rugoscopy, AE= age estimation.



When assessment about information of FO was based on age and experience of the dentists, it was found as illustrated in Figure 6 and 7 that even though sample was not equally divided according to age and experience ranges, yet, it is observed that the higher the age range and greater the experience the poorer the results of the question.

When questioned, if they were professionally trained in FO only 4 dentists (1%) replied 'YES'.

Of these 4 dentists, 3 described the details of the professional training as: thesis in FO, attended a workshop of FO and been a member of the Forensic team in Havelian plane crash in Pakistan. Only 48 (10.08%) dentists of this study have worked in the field of FO. The work being "given opinion in a case related to forensics" (n=37), "assisted a forensic odontologist" (n=5) and "worked as a Forensic Odontologist" (n=1) followed by blank (n=5).

Figure 6. Count of responses from dentists of various age groups showing total responses to this question about sub-topics of FO and the incorrect selections made by specific age groups. (x= age groups, y= count of responses)

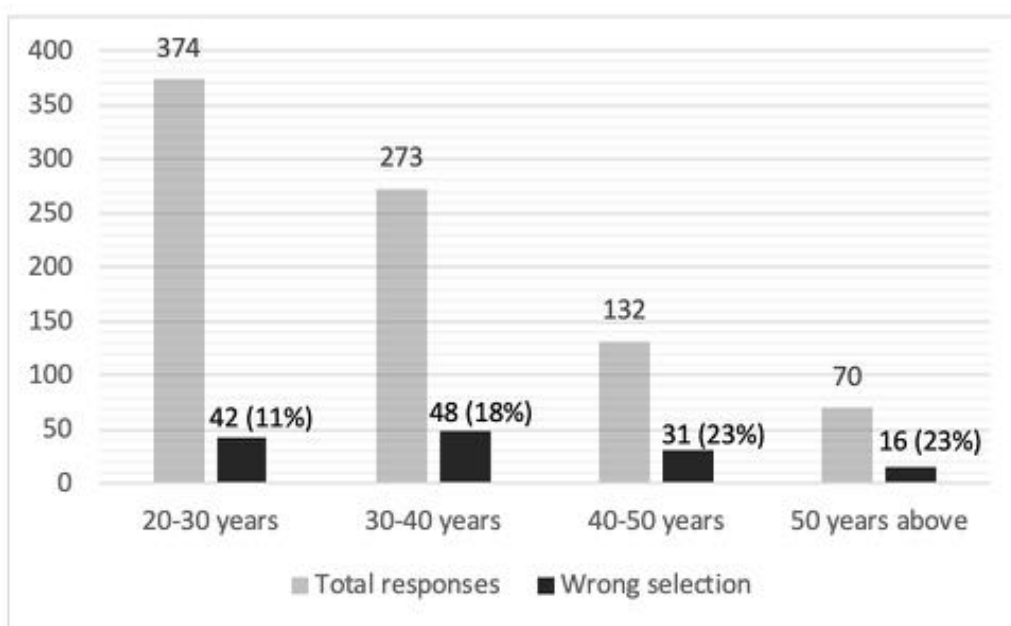
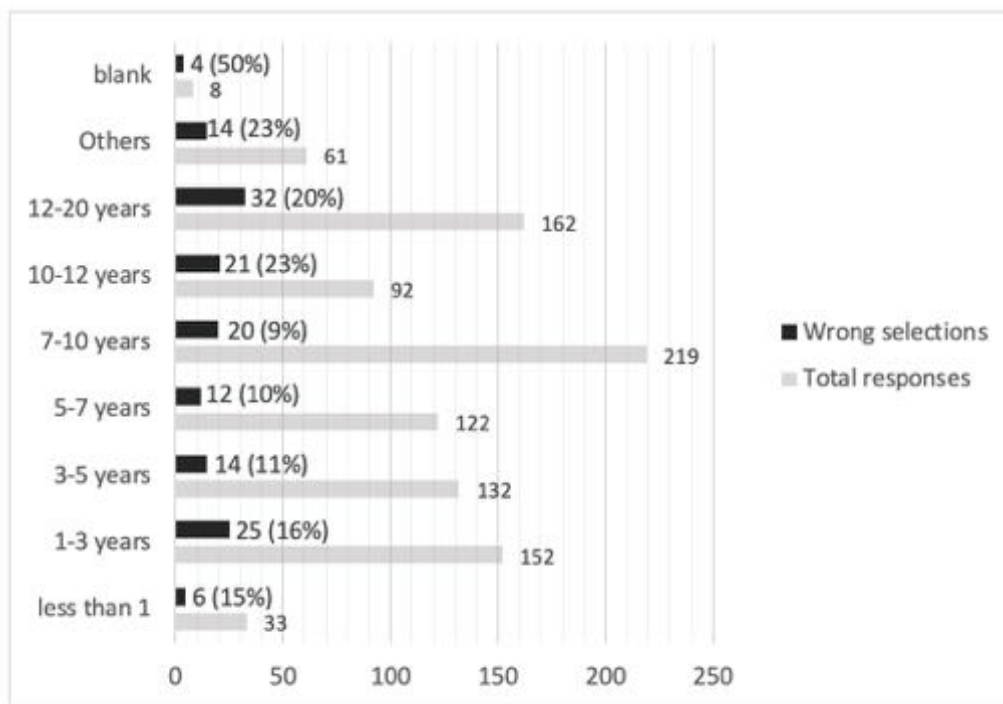


Figure 7. Count of responses about sub-topics of FO based on the work experiences in contrast to total responses and the incorrect selections made by specific groups of work experiences. (x= count of responses, y= work experience in years, Other= experience of more than 20 years)



Considering dental charts, 290 dentists (61%) claimed they do not make a detailed dental chart of every patient. and 261 (55%) stated they or the hospital maintained the dental records. As illustrated in figure 8, most of the dentists (n=69) maintain the data for “more than 5 years” followed by “6 months” (n=54). Out of the 261 dentists who claimed to maintain records,

149 claimed they keep the record manually, whereas 86 dentists store it digitally and only 16 selected the option “BOTH”. Some dentists opted “YES” for Q7 but did not fill the part querying the methods (n=10) and duration (n=9) of data storage. Figure 9 shows variations in Q7 from the dentists of the same institute at the same time.

Figure 8. Duration and methods reported by the dentists for maintaining the dental records. (x= count of dentists, y= duration of record maintenance).

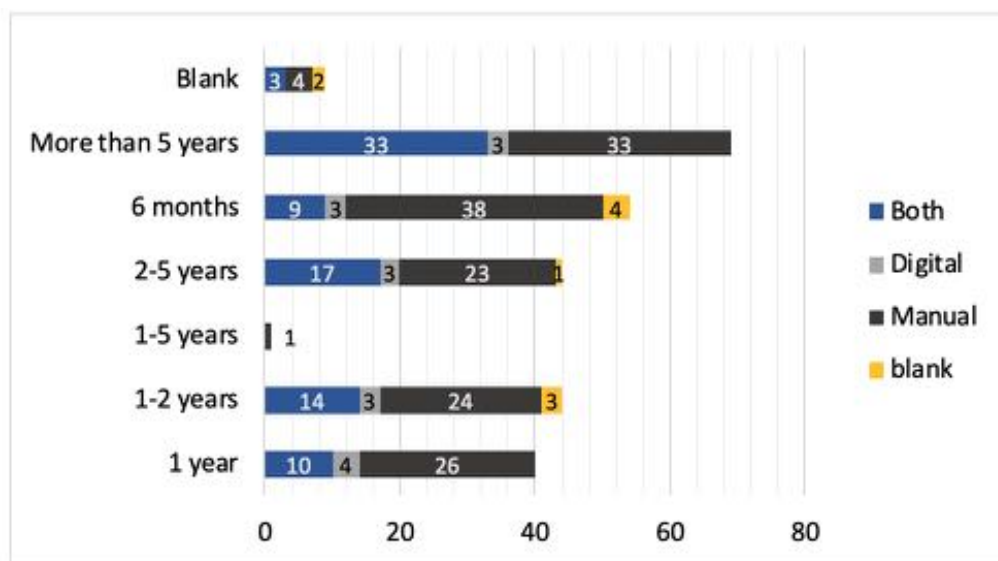
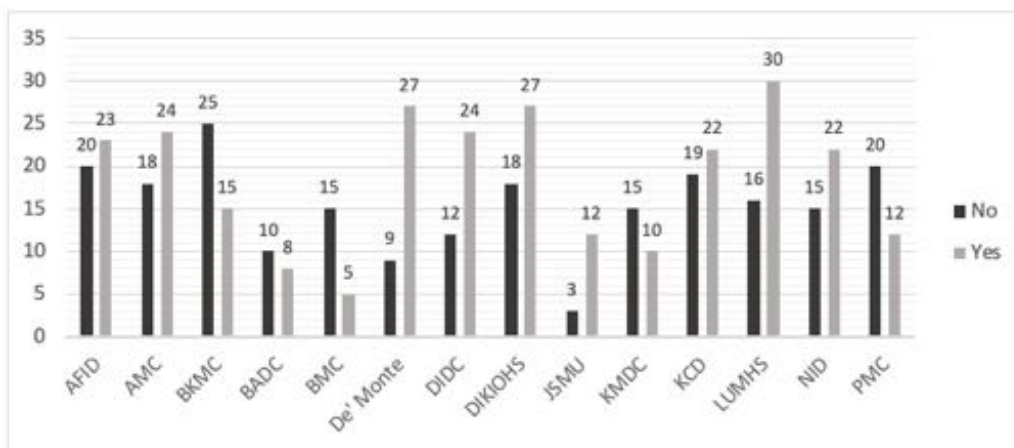


Figure 9. Variations in the answers of dentists from same institutions: if they or the hospital maintain dental records. (x= institutes, y= count of dentists)

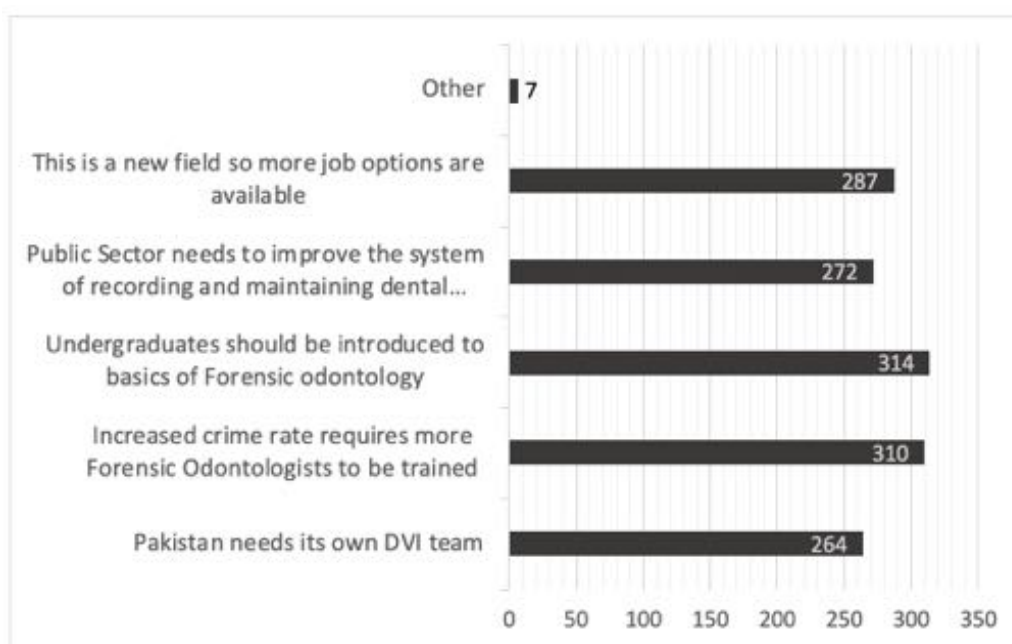


The dentists could choose multiple options for Q8 and the number of responses was 577. Most of the responses (n=193) considered “Radiographs” as the most stored type of dental record followed by “dental charts” (=150), “dental casts” (n=130) and “photographs” (n=104). As Q9 was also a multiple-choice question, hence the total number of responses was 1,454. Figure 10 shows the reasons selected by the participants for the need of FO. Seven dentists who selected the

option “OTHER” had some open answers such as: governmental support for FO in dental institutions, to meet global standards, the addition of FO to the undergraduate curriculum and postgraduate programmes/ workshops should be introduced in this field.

Finally, about the interest of the dentists to be trained in FO within Pakistan, 423 (89%) determined “YES” opposing to this only 53 (11%) of the dentists chose “NO”.

Figure 10. Different reasons selected by the dentists as need for application of FO in Pakistan (x= count of responses, y= reasons for the need of FO).



DISCUSSION

Age estimation and Human identification in Pakistan

In the past, there was an absence of official documents for many Pakistani residents which proved age and in addition, over time, this lack of documentation has grown given an increase in the number of refugees (4, 5). Also, child marriage is practised all over Pakistan as a culture and tradition. Pakistan has a high level of seismic activity and is therefore prone to natural disasters. It also suffers from man-made disasters along with various crimes such as rape, child abuse, beheading and arson (6, 7). At present, economic conditions in the country make the use of methodologies such as DNA and fingerprinting expensive and complex. Therefore, comparative dental analysis is a cost-effective and easy method in such circumstances.

Education in FO

Although similar national studies have been conducted, they focused upon smaller samples from a specific city or a specific institute only (8, 9). In this comprehensive study, 98.5% of the participants confirmed that they were not taught FO, even though, according to the Pakistan Medical and Dental Council (PMDC), 5 hours of Law and Dental Forensics have to be taught under the subject of Oral and Maxillofacial Surgery (2). This could be due to the lack of qualified instructors or negligent behaviour of the staff towards designing a course line for this subject. 66% of those sampled had at least heard about FO and were aware of it via social circles, globalization of media, seminars/workshops, visiting international FO faculty and through books/literature. Another reason may also be the cases from Pakistan in which FO has been used (Gen. Zia, Junaid Jamshed and Shahrukh Jatoi).

Awareness of FO

As defined by the Oxford Dictionary of English, *information* is "facts provided about something or someone" and *knowledge* is "facts, information and skills acquired through experience or education" (10). Knowledge requires cognitive and analytical ability, while to be informed does not require any such ability. The dentists understood the basic information about FO and they correctly selected: age estimation (67%), bite marks (61%) and human identification (55%); however, answers such as frictional ridge pattern

analysis (20%), skid marks (9%), handwriting analysis (8%) and voice recognition (6%) were incorrectly classified as sub-topics of FO. 208 responses out of 334 were entirely correct but only 2.6% selected all 5 correct options combined. The above findings show that even if the majority of the participants had satisfactory awareness about FO, many of them still did not have a basic understanding about the vast usage of FO, and due to this poor information, they ended up speculating the sub-topics of FO or chose the incorrect options.

Furthermore, the poor information regarding Rugoscopy and Cheiloscopy could be due to a lack of awareness and insufficient knowledge about the aspects and use of these techniques in Pakistan. Since 2004 only thirty scientific papers about FO have been published in Pakistan and the publishing rate has been irregular since early 2000. Most of the scientific papers are published after the year 2012 which reflects recent growth of FO in the country.

Although the government hospitals in Pakistan received a good number of medico-legal cases on a daily basis, 99% of the total sample did not include any trained forensic odontologist assigned to the institutes/hospitals. These results are consistent with the results of the previous studies that claimed a lack of formal training in Pakistan (8, 11). The author suggests that advertising and awareness programmes would improve the level of awareness of FO in different parts of the world, where it may be a need but is only given consideration after the occurrence of some major incident or a disaster.

Dental records

More than half of the respondents (61%) stated that they do not make a detailed dental chart of every patient which reflects that they are not fully aware of the importance of dental charts for forensic purposes.

A lack of technological skills, computers and software, combined with a shortage of electric power especially in the peripheral areas, limited data of the patients, shortage of trained personnel and lack of space to store manual records may be the reasons why the dentists in this survey do not store the data. And if they do, they do so manually and only for shorter periods. The PMDC only mentions briefly the maintenance of medical and dental records in Section 42 of the code of ethics of practice for

Medical and Dental practitioners 2018. It is vaguely explained in clause 3.3 of Section 4 of PMDC Professional ethics and code of conduct, Annexure H (2). Neither the Ministry of Health Services and Coordination (NHSRC) nor the PMDC provides details about guidelines regarding proper dental data recording and storage (2). Also, neither of these professional bodies advise mandatory use of dental charts as part of dental data recording in Pakistan. The introduction of legislation for mandatory dental data recording would help in numerous medico-legal cases. This would also prove beneficial for the practitioners themselves if ever involved in a case related to malpractice or dental fraud.

Radiographs were the most commonly saved records and only a minority of dentists claimed to save all main types of dental records together (charts, casts, radiographs and photographs). Extra measures should be taken to make sure good quality dental charting is obligatory in all dental practices of Pakistan and the personnel should be properly trained in transcribing and understanding dental charts even in busy schedules. Clinical audits should be undertaken in the dental practices and the government sector needs to improve data management systems.

Interest in FO

89% of the total respondents supported the introduction of training in FO within Pakistan. Universities should introduce a postgraduate

course in this field. Pakistan lags behind many countries who have their own departments and societies for FO and teams for DVI comprising Forensic Odontologists. Although Pakistan has been a member of Interpol DVI since 13th June 1956 no advancements have been noticed and the country's National Disaster Management Authority has no mention about the importance of the role of a forensic odontologist in a disaster on its official webpage (12). Perhaps, associations or societies for FO in Pakistan should also be created, similar to other countries, specifically Asian ones, which would aid collaboration with other international FO organizations and provide an impetus for this field within the country.

CONCLUSIONS

The majority of the dental practitioners in this study was aware of the existence of FO but they need to acknowledge the significance of dental record keeping and encourage the implementation of FO. Regardless of the absence of any governing body for FO and negligible education, training and implementation in Pakistan, this field is gradually progressing. However, the authorities should introduce detailed guidelines for dental records, ensure the future development of this subject in the education system and assign forensic odontologists to the DVI teams. At last, these initial steps can secure a better future for FO in Pakistan.

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Human identification by the analysis of palatal rugae printed in complete dentures

Talita Lima de Castro-Espicalsky ¹,
Patricia Freitas ²,
Rachel Lima Ribeiro Tinoco ³,
Melina Calmon ⁴,
Eduardo Daruge Júnior ⁵,
Ana Claudia Rossi ⁵.

¹ Civil Police of Rondonia State,
Porto Velho, RO, Brazil

² Federal Police, Três Lagoas, MS,
Brazil

³ Salgado de Oliveira University,
Niterói, RJ, Brazil

⁴ Tulane University, New Orleans,
LA, EUA

⁵ School of Dentistry of Piracicaba,
State University of Campinas -
UNICAMP, Piracicaba, SP, Brazil

Corresponding author:

mcalmons@tulane.edu

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KEYWORDS

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ABSTRACT

The study of palatal rugae is shown to be scientifically valid to human identification due to the unique number and style of the palatal ridges. Dentures provide an array of data and specifics that allow for the individualization of their wearers. This article describes the identification of edentulous, skeletonized remains through the analysis of the palatal rugae printed on the complete upper denture and subsequent comparison with the palatal rugae of an old complete denture of an unknown missing person. The analyses focus on the form, classification, location, and size of the palatal rugae which, in conjunction with the information obtained from the anthropological examination, resulted in a positive identification of the cadaver. This method has a significant impact on the identification process, particularly when other identification methodologies and techniques cannot be implemented. This case report highlights the importance of palatal rugae in human identification in cases of edentulous cadavers.

INTRODUCTION

The identification of skeletonized or burned human remains requires a multidisciplinary approach in order to identify unique traits that can ascertain an individual's identity and allow differentiation from all other individuals.¹

In these situations, teeth represent the main approach in forensic dental identification. Teeth comprise unique structures with particular chemical compositions,² which can reveal related characteristics about the sex, age, and ancestry of an individual.³⁻⁵ In addition, teeth reveal their uniqueness through their morphological and structural features, allowing for the identification of human remains through the teeth's shape and size,⁶ analysis of dental anomalies,⁷ observation of dental work,⁸ or even through the presence of intra-oral micro-identification discs, which contain information in their surface that facilitate the identification of its owner.⁹ However, in case of edentulism, the palatal rugae might be useful to assess characteristics for the identification.¹⁰

The palatal rugae, also referred to as palatal ridges, are irregular folds or corrugations or asymmetric ridges of the anterior mucous membrane of the palate extending laterally from the incisive papilla and the median palatal raphe.^{11,12} The study of the patterns of the palatal rugae, palatal rugoscopy, can be used in the identification of an individual, as palatal rugae are stable and unique to each person.^{12,13} The palatal rugae appear towards

the third month of foetal development and, in case of trauma, they reform themselves in their exact original position.^{13,14} Although the size of the palatal rugae varies through time, the location and form remain constant throughout an individual's lifetime.^{13,15} As a result, dentures have an important role in forensic odontology analysis, as each set of dentures can provide data and specific features that are unique to their wearer. For example, the internal surface mould of complete dentures can provide plaster models that reproduce the details of the alveolar ridge in which the denture was placed. This data can then be used for forensic identification.¹⁰ Literature reports showing the identification of a cadaver through palatal rugae comparison with ante-mortem data are rare, although the device is widely worn by edentulous populations.^{16,17}

This study presents a case report on human identification through the analysis of the palatal rugae printed in a complete upper denture found with edentulous, skeletonized remains. The comparison of the palatal rugae was completed with the features found in an old denture of a missing man that was provided by his family. This paper aims to emphasize the crucial importance of palatal rugae in cases of edentulous remains in an advanced stage of decomposition, in particular when the analysis of teeth traits and development and fingerprints are not possible.

CASE REPORT

The Medicolegal Institute of Ji-Paraná, in the inland of the Brazilian Amazon, received a skeletonized body found in an empty lot. The remains had complete upper and lower dentures made with acrylic resin. The anthropological examination established that the individual was an elderly male of admixture ancestry with primarily Asian and European features, and stature estimated to be between 1,60cm and 1,70cm (5'4" - 5'6"). Ante-mortem trauma was found on the left clavicle. The cause of death could not be determined due to the lack of traumatic peri-mortem lesions or any other evident features at the skeletonized stage. The forensic odontology examination showed the total absence of teeth in the maxillary and mandibular arches, with advanced resorption and bone remodeling, suggesting that the tooth loss occurred ante-mortem (Figure 1).

Figure 1. Cranium and edentulous mandible examined by anthropologist and legal odontologist.



The complete dentures found alongside the remains (Figures 2 and 3) had a precise fit with the maxilla and mandible of the remains, demonstrating compatibility in size and shape between the bony ridges and the prostheses.

Figure 2. Complete upper prosthesis found with remains – internal surface moulded for analysis.



Figure 3. Complete upper prosthesis found with remains – occlusal view



Family members of a missing man contacted the Medicolegal Institute during the police investigation to provide information about the individual's characteristics: a 50 year old brown male with approximated living stature of 1,70cm (5'6"). The family members also provided information on a healed fracture located on the left clavicle of the missing man, which was consistent with the trauma found on the skeleton. During the search for the ante-mortem dental data of the missing man, the family delivered the old complete dentures (upper and lower) of the missing man to the forensic experts working on the case (Figures 4 and 5).

Figure 4. Complete upper prosthesis of missing man – internal surface moulded for analysis



Figure 5. Complete upper prosthesis from missing man – occlusal view



The complete dentures belonging to the disappeared, and the ones found with the skeleton were examined and named as *ante-mortem* and *post-mortem*, respectively. A plaster mould of the dentures internal surface was created in order to reproduce the palatal rugae and the edentulous maxillary and mandibular ridges. These models were analyzed using a magnifying glass under 5x magnification. The corrugations and undulations in the palatal area representative of the ridges belonging to the wearer of the prostheses were analyzed. All macroscopically visible criteria present in the models were listed and used to distinguish the *ante-mortem* and *post-mortem* dentures. Both showed an increase of the vestibular-palatal thickness on the contour of the superior alveolar ridge, located to the left of the incisive papilla. The palatal rugae were identified, marked with graphite, and their anatomy was classified according to the Martins-dos-Santos method,^{14,18} as described on Table 1. The size of the rugae was measured using a caliper for posterior comparison of the maximum extension of each ruga.

Additionally, the upper plaster models obtained from the dentures were photographed using the ABFO No.2 metric scale, to avoid magnification and to provide a geometrical reference in the photographic documentation of the ridges.

The software Power Point® (Microsoft®, Redmont, EUA) was used to create a digital contour using the pictures of the models, with the tab "Insert → Form". The tool "curve" was used to delineate the contour and form of each palatal ruga present in the models, resulting in the Palatograms show as figures 6 and 7.

Table 1. Classification of palatal rugae printed in the maxillary protheses

Rugae of <i>Ante-mortem</i> Prothesis		Rugae of <i>Post-mortem</i> Prothesis	
Right Side	Curve	Right Side	Curve
	Sinuous		Sinuous
	Line + Bifurcated*		Bifurcated*
	Interrupt		Interrupt
Left Side	Curve	Left Side	Curve
	Sinuous		Sinuous
	Point		Point
	Line + Line*		Bifurcated*

* Two ridges displayed minor discontinuance in form due to the occurrence of artifacts during the moulding technique that created air bubbles in the plaster. These disruptions, however, did not exclude the compatibility between ridges.

Figure 6. Plaster model of interior surface of prothesis found with remains. In yellow: correspondent palatogram. In blue: the alveolar ridge contour.



Figure 7. Plaster model of interior surface of prothesis from missing man. In green and red: correspondent palatogram. In blue: the alveolar ridge contour.



The Palatograms obtained from the protheses were thenceforth used to make a direct comparison between the palatal surfaces and the representative images of the ridges. They were compatible in form, location and size of the palatal ridges. Only two ridges displayed minor discontinuance in form due to the occurrence of artifacts during the moulding technique that created air bubbles in the plaster. The presence of disruptions or discontinuances, however, did not prevent the compatibility between ridges, which coincided

in their general contour and location. In addition, the models obtained from the complete lower dentures and the mandible analyzed during the forensic examination revealed the presence of slightly triangular edentulous ridges, with a high bone loss, especially in the posterior region. The region also displayed minimized dimensions in both thickness and length, expressing similarities in the contour, format, size, thickness and bone resorption at the mandibular ridges represented.

DISCUSSION

Human identification can be performed through a variety of forensic methods including fingerprint analysis, dental arch comparison and DNA analysis, in addition to rugoscopy and cheiloscopy.¹⁹⁻²² However, in some cases, the advanced stage of decomposition of the bodies, the lack of ante-mortem data from possible victims, or even the complete absence of teeth, can hinder the application of traditional techniques in the search for the identity of a body. Furthermore, some traditional techniques, such as DNA analysis for human identification, are cost prohibitive in many settings, highlighting the importance of alternative methodologies that are reliable according to scientific standards.

A study¹⁰ performed on moulds of the internal surface of complete maxillary prostheses of twenty eight edentulous individuals evaluated the rugae present and the topographic aspects of the ridges, comparing the plaster models originated from the wearers of the dentures with their original dentures. The research showed an unequivocal individualization of 100% accuracy when the plaster models were analyzed entirely, including the palatal rugae and the topography of bone contour, demonstrating the reliability of the method.

The dentures provide important information for human identification as they are unique and specific to each patient, and they artificially reproduce the dental arch and adjacent mucosa. These characteristics do not undergo significant alterations with time and are resistant to environmental changes and diverse conditions. Moreover, the devices are easy to collect, study, and compare to information present in dental records, allowing for frequent comparison in forensic odontology examinations.^{23,24}

The printed rugoscopy in the palatal region of dentures and prostheses represent a unique mark of each patient, and are extremely valuable for the forensic odontologist responsible for a case.^{15,25} Although the length of rugoscopic eminences can be shorter in edentulous individuals when compared to dentulous ones, points such as angles, curves and bifurcations can be observed in a clear and easy form from models originated from old and new prostheses, leading to reliable results.²⁶

The palatal rugoscopy has marked advantages when compared to traditional methods, namely low cost and feasibility, in edentulous

skeletonized individuals. However, the authors understand that unless palatal ridge patterns had been recorded in life through dentures or moulds, a rugoscopic comparison cannot occur.

Other disadvantages can be pointed out as the possibility of rugae pattern forgery and the possible distortion of the palatal rugae replicate as a result of poor duplicating materials and techniques. Poorly demarcated eminences of the rugae, especially in edentulous cases, are the most common cause of difficulty in identification.²⁷ Controversy still exists about the stability of quantitative and qualitative characteristics of rugae during human growth development and the extent of differences between ethnic groups and sexes.^{28,29}

Based on the findings of forensic investigation of the presented case, the following traits were common to the skeletal remains and the supposed victim: the time frame of disappearance, the taphonomic and body decomposition changes, sex, age, ancestry, and stature. In addition, analysis revealed the presence of a bone callous resulting from an ante-mortem fracture in the left clavicle of the skeletonized remains, which is consistent with a fracture reported to be present in the missing person.

Despite these commonalities, a comparative scientific method was still necessary to reliably establish a positive identification. Thus, a comparative analysis of the dentures provided by the family, the dentures found with the remains, and the plaster models of each was completed to reach a positive identification. In the presented case, the comparison was made based on visual analysis of dentures and the two plaster models. When available, a superimposition using the scans of the two models could improve the degree and the accuracy of identification.

The contour, topographic aspects, size of the bony ridges and the presence of specific points in both prostheses represented individualizing features important for the forensic examination. However, it was the palatal rugoscopy that allowed the effective positive identification of the remains by establishing the equivalence in the form, location and size of the palatal ridges obtained among the maxillary prostheses.

Finally, it is important to stress that the methodology has been recognized as an effective, safe and reliable technique, based on valid

parameters that are advantageous and efficient for the forensic analysis.

CONCLUSION

In the presented case, the old upper denture of a missing man provided ante-mortem data on the individual's pattern of palatal rugae and the anatomy, size, and form of the alveolar ridges. This

allowed the experts to confirm that the analysis of the internal surface of the complete maxillary prostheses shows a considerable value to the individualization of its owner. Therefore, this case study shows the importance of the palatal rugoscopy as an important technique in the processes of human identification in forensic examination and legal dentistry, especially in cases of complete edentulous skeletonized remains.

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