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To cite this article: T.Y. Marroquin, S. Karkhanis, S.I. Kvaal, S. Vasudavan, E. Castelblanco, E. Kruger & M. Tennant (2016): Determining the effectiveness of adult measures of standardised age estimation on juveniles in a Western Australian population, Australian Journal of Forensic Sciences

To link to this article: <http://dx.doi.org/10.1080/00450618.2016.1177593>



Published online: 22 May 2016.



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Determining the effectiveness of adult measures of standardised age estimation on juveniles in a Western Australian population

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(Received 20 December 2015; accepted 15 March 2016)

The estimation of chronological age through assessment of dental radiographs is well-established and a useful method to assist in the identification of persons in forensic and anthropological scenarios. The objective of our investigation was two-fold: (i) to validate the Kvaal et al. age-estimation method on a sample of Western Australian subjects, and (ii) to increase the range of chronological ages to which the Kvaal et al. method can be applied. Our sample size included panoramic radiographs from 74 subjects (aged 12–28 years). A set of ratios were calculated and then used to apply different statistical models of linear regression, in order to generate a final formula to estimate age. The most accurate estimations were obtained from the models generated by the mandibular canine measurement ($SEE \pm 3.708$ years), and for the three mandibular teeth ($SEE \pm 3.388$ years). The results indicate that inclusion of juveniles did not affect final results, and the method still produced estimates acceptable in a forensic framework.

Keywords: forensic dentistry; dental age estimation; secondary dentine formation; young adults

Introduction

The accurate approximation of chronological age, secondary to sex determination, is an integral factor in constructing a biological profile for forensic¹ and anthropological purposes, including the confirmation of identification at times of mass disasters, crimes, accidents and of unknown remains^{2–4}. Further, the estimation of the chronological age in the living is also needed in situations such as immigration and refugee determinations⁴. Saunders originally proposed the estimation of chronological age based on different stages of tooth eruption⁵; since then, several more methods have been suggested to estimate dental age in children. Amongst these methods, those that are based on the radiological examination of permanent teeth development, are found to be the most accurate^{6,7}. The Demirjian et al.^{8,9} classification is reported as the best method for dental age estimation in children and adolescents thanks to its high observer agreement and correlation between the defined stages and age¹⁰. This method has been adapted to

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different populations, showing a mean difference between the chronological age and the dental age of around one year in different studies^{6,9,11}.

The completion of tooth development is marked with the closure of the root apex, with the third molar being the last tooth to complete its eruption and root development at approximately 17–21 years of age. Following this stage, the accurate estimation of chronological age based on dental formation becomes challenging¹², leading to the development of alternative methods of dental age estimation in adults. Bodecker¹³, identified the relation of the continued apposition of secondary dentine and the subsequent change in the morphology of the pulp chamber with chronological age. Accordingly, several dental age estimation methods have been developed, based on the relation between secondary dentine formation, and the subsequent narrowing and change in pulp chamber dimensions and shape with age^{1,2,14,15}.

Dental analysis using radiographs for the purpose of age estimation presents numerous advantages over other histological and biochemical methods: it is relatively simple, non-invasive and economically viable¹⁶. The method developed by Kvaal et al.¹ is a relatively non-invasive method and has been validated in diverse populations^{17–20}. Karkhanis et al.¹⁷ applied the Kvaal et al.¹ method in a Western Australian population to develop age estimation standards with acceptable results in a forensic framework (± 10 years)². Originally, the Kvaal et al.¹ method was proposed to be applied in an adult population. More recently, the method has been applied in younger populations with varying degrees of success. The level of error in estimates of ages remains high in younger populations, with Landa et al. reporting a standard deviation approaching 15 years²⁰.

The aim of the present study is to validate the applicability of the Kvaal et al.¹ method in a younger population (Western Australia sample) and to provide further refinement of the level of variation in younger people. It will also assess the potential of this method as an additional tool for dental age estimation in juveniles, where methods based on the analysis of tooth development cannot be used.

Materials and methods

Sample selection

The study cohort consisted of a series of subjects who consecutively presented for orthodontic treatment at a private specialist orthodontic office. From the initial sample, 74 Western Australians were aged less than 30 years, of those 63.5% ($n=47$) were female and 36.5% ($n=27$) were male, with a median age of 16 year for both genders. All panoramic radiographs were acquired from the same machine in digital format. Analysis was completed with Image J software (version 1.48 19 April 2014 – National Institute of Health, USA) and the measurements were completed by a single observer (TM). All data was collated using Excel (version 2013 Microsoft, Redmont, USA) and statistical analysis was completed using R Core Team version 3.1.3 (2015). (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.)

All subjects with a panoramic radiograph of high quality with respect to factors of image brightness, contrast and sharpness were included. Any radiographs that did not meet this requirement or had observable failings (e.g. image distortion, poor contrast, superposition of tooth structure, or improper positioning) were excluded. All teeth included in the analysis were clinically sound with completed root formation and in

functional occlusion. Teeth with rotations, incomplete root formation, dilacerations, pulpal pathologies and endodontic treatment and/or restorations were excluded. Finally, teeth with large areas of enamel overlap between neighbouring teeth were excluded in this study.

Teeth analysed

Following the parameters as provided by Kvaal et al.¹, the teeth analysed were the maxillary central incisors (with Federation Dentaire Internationale (FDI) notation 11 and 21) lateral incisors (FDI notation 12 and 22), second premolars (FDI notation 15 and 25), mandibular lateral incisors (FDI notation 32 and 42), mandibular canines (FDI notation 33, and 43) and first mandibular premolars (FDI notation 34 and 44). In the original study, Kvaal et al.¹ analysed only those periapical radiographs from individuals where all the six teeth were present and suitable for examination. In the present study, panoramic radiographs with any combination of the required teeth available for measurement were included. Previous research^{1,17,19} has demonstrated the absence of bilateral asymmetry in the deposition of secondary dentine. Consequently, teeth from either side that fulfilled the inclusion criteria were analysed for the purpose of developing age estimation standards.

Measurements

Odontometric data were acquired following the methodological approach of Kvaal et al.¹. In this way, the data were obtained from measuring: the maximum tooth length (from the incisal border or cusp tip to the root apex); the maximum pulp length (from the pulp chamber roof to the root apex); and the maximum root length (from the cemento-enamel junction – CEJ – on the mesial surface of each tooth to the root apex). The pulp chamber and root width measurements were collected at the points A (CEJ), B (mid-point between the points A and C) and C (mid-point between the CEJ and root apex).

Measurement precision

A pilot set of 30 panoramic radiographs, which were not included in the final analysis, was used to perform initial training and benchmarking to an expert in the field (SK). Six of the 30 radiographs were measured on six different days, in addition to four randomly selected panoramic radiographs, thus resulting in the analysis of ten panoramic radiographs per day. Based on this, intra- and inter-observer error calculations were performed¹⁷. The measurements were acquired from all the panoramic radiographs with a minimum of one day between each session to minimise the possibility of memorising reference points and/or measurements in each observer. A second intra- and inter-observer calibration was performed using five randomly selected panoramic radiographs from the final study sample, and recording the measurements on five different days, also using four additional panoramic radiographs per day, to avoid measurements memorising, with at least one day between each evaluation.

Statistical analysis

Simple descriptive statistics (mean, standard deviation, and frequency distributions) were used to summarise the data. Initial tests for normality (assessment for skewness,

kurtosis and Shapiro-Wilk) were performed to determine, where appropriate, parametric and non-parametric univariate analysis testing for the continuous variables (Table 1). Pearson's correlation coefficient (r) was calculated to assess the strength of correlation between age and dental ratios based on the Kvaal et al.¹ method (Table 1). The correlation coefficients calculated (using the width ratios) presented significant correlation values. The most representative were observed for the ratio calculated between pulp and root width at the B point of the root, and for the predictor W-L. The mandibular canines showed the highest correlation coefficient for the predictors B (-0.570) and W-L (-0.625).

To examine the independent association of several factors with chronological age, multivariable linear regression analysis was used to look at the association between the independent and outcome variables. The outcome variables included chronological age. The predictor variables M and W-L were used as the independent variables. Age estimation models were developed individually for each tooth (Table 2) and the following tooth combinations (Table 3): three maxillary teeth with the predictors M and W-L individually introduced for each tooth (six predictors), and the average of M and W-L (two predictors); three mandibular teeth with the predictors M and W-L individually introduced for each tooth, having six predictors in the equation, and the average of M and W-L resulting in two predictors; and mandibular and maxillary teeth M and W-L predictors, introduced individually in the equation (12 predictors) and the average of M and W-L (two predictors). All regression models were built using the ordinary least squares approach. R-square values were computed to examine the amount of variance explained by the predictor variables.

All statistical tests were two-sided and a p value of less than 0.01 was considered to be statistically significant. Corrections were made for multiplicity using a modified Bonferonni method to reduce the likelihood of Type I errors; an alpha threshold for statistical significance for all comparisons was set at 0.01. Univariate analyses and

Table 1. Correlation coefficients between chronological age and Kvaal et al.¹ dental measurements and ratios, for individuals under 30 years of age.

Tooth number (FDI numbering system)						
Ratio	11/21	12/22	15/25	32/42	33/43	34/44
<i>P</i>	0.129 ^{NS}	0.236 ^{NS}	0.133 ^{NS}	0.164 ^{NS}	0.455*	0.152 ^{NS}
<i>T</i>	0.041 ^{NS}	-0.071 ^{NS}	-0.002 ^{NS}	-0.130 ^{NS}	0.171 ^{NS}	-0.117 ^{NS}
<i>R</i>	0.287*	0.287*	0.151 ^{NS}	0.327*	0.279*	0.397**
<i>A</i>	-0.130 ^{NS}	-0.281*	-0.004 ^{NS}	-0.263*	-0.254 ^{NS}	-0.137 ^{NS}
<i>B</i>	-0.378*	-0.305*	-0.280*	-0.354*	-0.570**	-0.381*
<i>C</i>	-0.345*	-0.377*	0.009 ^{NS}	-0.088 ^{NS}	-0.605**	-0.504**
<i>M</i>	-0.049 ^{NS}	-0.202 ^{NS}	0.0001 ^{NS}	-0.123 ^{NS}	-0.108 ^{NS}	-0.265*
<i>W</i>	-0.415**	-0.397**	-0.150 ^{NS}	-0.237*	-0.123 ^{NS}	-0.497**
<i>L</i>	0.151 ^{NS}	0.295*	-0.002 ^{NS}	0.255*	0.467**	0.297*
<i>W-L</i>	-0.310*	-0.421**	-0.052 ^{NS}	-0.360*	-0.625**	-0.547**

* = $p < 0.05$; ** = $p < 0.001$.

NS=Not significant

Note: *P* is the ratio between length of pulp and root; *T* is the ratio between length of tooth and root; *R* is the ratio between length of pulp and root; *A* is the ratio between width of pulp and root at CEJ (Level A); *B* is the ratio between width of the pulp and root at mid-point between level C and A (level B); *C* is the ratio between width of pulp and root at mid-root level (level C); *M* is the mean value of all ratios (first predictor); *W* is the mean value of width ratios from levels B and C; *L* is the mean value of the length ratios *P* and *R*; *W-L* is the difference between *W* and *L* (second predictor)¹.

Table 2. Multiple regression for estimation of chronological age (in years) from individual maxillary and mandibular teeth for individuals under 30 years of age.

Tooth (FDI)	<i>n</i>	<i>R</i>	<i>R</i> ²	Equation	SEE ± years
11/21	69	0.152	0.1271	Age= 22.915 – 28.78 (<i>M</i>) –22.376 (<i>W – L</i>)	4.344
12/22	67	0.197	0.1727	Age= 19.724 – 25.935 (<i>M</i>) – 23.631 (<i>W – L</i>)	4.258
15/25	68	0.003	-0.273	Age= 17.78 – 3.962 (<i>M</i>) – 2.204 (<i>W – L</i>)	4.536
32/42	71	0.131	0.106	Age= 4.644 – 5.363 (<i>M</i>) – 22.558 (<i>W – L</i>)	4.465
33/43	48	0.417	0.391	Age= 11.26 – 41.33 (<i>M</i>) – 86.06 (<i>W – L</i>)	3.708
34/44	71	0.327	0.307	Age= 10.513 – 27.075 (<i>M</i>)–38.176 (<i>W – L</i>)	3.709

Note. **R*², coefficient of determination. SEE, standard error of estimation in years. See Table 1 for abbreviations.

Table 3. Multiple regression for estimation of chronological age (in years) from the combined maxillary and mandibular teeth, the respective predictors (pds) for individuals under 30 years of age.

Teeth (FDI)	<i>n</i>	<i>R</i>	<i>R</i> ²	Equation	SEE ± years
3 mx (2 pds)	60	0.154	0.125	Age= 28.702 – 46.338 (<i>M</i>) – 24.233 (<i>W – L</i>)	4.141
3 mx (6 pds)	60	0.237	0.150	Age=24.394 – 30.751 (11/21 <i>M</i>) –12.090 (11/21 <i>W–L</i>) – 29.2257 (12/22 <i>M</i>) – 12.894 (12/22 <i>W – L</i>) + 2.611 (15/25 <i>M</i>) – 0.631 (15/25 <i>W – L</i>)	4.08
3 mdb (2 pds)	45	0.409	0.381	Age= –27.44 + 10.48(<i>M</i>) – 63.82 (<i>W–L</i>)	3.648
3 mdb (6 pds)	45	0.539	0.466	Age= 1.894 + 13.428 (32/42 <i>M</i>)–5.931 (32/42 <i>W – L</i>) – 51.067 (33/43 <i>M</i>) – 66.495 (33/43 <i>W – L</i>) – 5.114 (34/44 <i>M</i>) – 16.806 (34/44 <i>W – L</i>)	3.388
6 teeth (2 pds)	40	0.252	0.211	Age= 31.91 – 63.34 (<i>M</i>) – 63.34 (<i>W – L</i>)	4.138
6 teeth (12 pds)	30	0.542	0.339	Age= 2.627 –58.42 (11/21 <i>M</i>) + 4.526 (11/21 <i>W – L</i>) + 32.169 (12/22 <i>M</i>) – 12.913 (12/22 <i>W – L</i>) + 25.460 (15/25 <i>M</i>) + 1.098 (15/25 <i>W – L</i>) + 5.270 (32/42 <i>M</i>) + 0.096 (32/42 <i>W – L</i>) – 28.692 (33/43 <i>M</i>) + 1.686 (33/43 <i>W – L</i>) – 13.857 (34/44 <i>M</i>) – 49.336 (34/44 <i>W – L</i>)	3.788

**R*², coefficient of determination. SEE, standard error of estimation in years. See Table 1 for abbreviations.

multivariable linear regression analyses were performed using R Core Team version 3.1.3 (2015) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>).

Results

Measurement precision

The technical error of measurement (TEM), relative technical error of measurement (rTEM) and coefficient of reliability were within acceptable standards for the intra- and inter-observer measurement precision (TEM<1.0, rTEM<5%, *R*>0.75). These values were 0.92, 2.99% and 0.95 respectively for intra-observer precision analysis. Similarly, the inter-observer calibration (between TM and SK) showed comparable precision²¹ (TEM 0.80, rTEM 2.37 and *R* 0.98).

In this way, individual (Table 2) and multiple (Table 3) tooth regression models were obtained. The accuracy to predict the age was quantified by the standard error of estimation (SEE \pm years). The most accurate age estimation model based on the analysis of individual tooth was for mandibular canines (SEE \pm 3.708 years) (Figure 1). Prediction accuracy improved with the combined analysis of teeth; it was highest for the model developed with the three mandibular teeth (six predictors, SEE \pm 3.388 years).

Discussion

The estimation of chronological age has been a very relevant topic of discussion through human history. For this reason, diverse skeletal and dental methods have been developed, showing variable levels of reliability in different populations. These methods, based on dental changes, are well accepted and have shown their potential for forensic and anthropological applications²². Once the root formation has been completed, progressive dental changes, such as secondary dentine formation, can be radiographically assessed based on the narrowing on the pulp chamber. Odontometric and morphometric measurements can thus be acquired to quantify secondary dentine deposition and is the foundation for non-invasive adult age estimation methods such as Kvaal et al.¹ and Cameriere et al.²³.

The Kvaal et al.¹ method has been widely validated in different populations because of its numerous benefits, such as, among other benefits, its relatively non-invasive approach, applicability in live individuals and low cost. Previous research has applied this method to a Western Australian population¹⁷ and has shown significant results that are valid under forensic standards. Although the Kvaal et al.¹ method was initially developed to estimate age in individuals over 20 years old, previous studies have tested this method in younger populations¹⁹. The present study applied the Kvaal et al. method¹ in Western Australian participants under 30 years of age. The primary aim was to assess the applicability of this method in a younger population to broaden the age range that the method can be applied to, without compromising the age estimation accuracy.

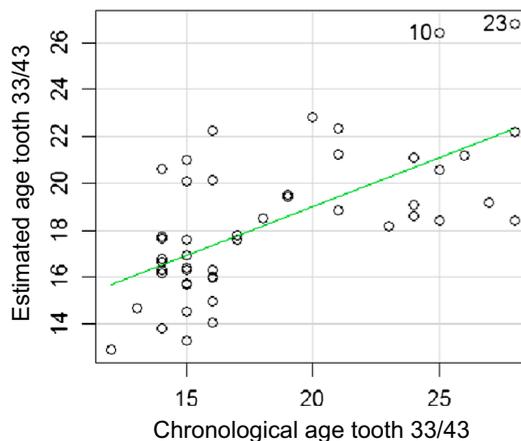


Figure 1. Scatter plot showing a positive association between the estimated age and the chronological age for the teeth 33/43.

To this end, regression models were developed, based on the data acquired from individual teeth (Table 2) and a combination of teeth (Table 3). The accuracy to predict age was quantified by the standard error of estimation ($SEE \pm$ years). The most accurate model for individual teeth was for the mandibular canines (± 3.708 years), in contrast to the study of Karkhanis et al.¹⁷, where the same tooth presented the highest SEE (± 10.903 years).

Prediction accuracy improved when multiple teeth were included in the regression models (Table 3). In this study, the highest level of accuracy was obtained when the equation included three mandibular teeth and six predictors ($SEE \pm 3.388$ years), in comparison with the study of Karkhanis et al.¹⁷, where the most accurate model was for the combined analysis of the six teeth and 12 predictors ($SEE \pm 7.963$).

It has been observed that age estimation in individuals older than 14 years is difficult, as all permanent teeth, except the third molars (when present), have completed their development¹⁹. Some examples of previous studies amongst people under 30 years of age, using the Kvaal et al. method¹ on panoramic radiographs, are: Erbudak et al.²⁴ in a Turkish population, including in their sample 75 participants between 14 to 35 years of age, obtaining a $SEE = \pm 8.73$ years at best, using the regression formulas of Paewinsky et al.²⁵ and Kvaal et al.¹; Landa et al.²⁰ in a Spanish population with an age range of 14 to 60 ($n=100$), from which 40 were aged younger than 31 years of age, and with an underestimation of age when using the regression formulae of Kvaal et al.¹ and Paewinsky et al.²⁵ and a standard deviation of 12.53 at best when the Kvaal et al.¹ regression equation was used. The last example is the study of Meinel et al.¹⁹, which was conducted in an Austrian population ($n=44$) with an age range of 13 to 24 years, and resulted in a mean underestimation of 31.44 years when the Kvaal et al.¹ regression models were applied, or a mean overestimation of 20.88 years when the Paewinsky et al.²⁵ formula was applied. In contrast, the present study ($n=74$) shows that the Kvaal et al.¹ method provides acceptable results² in a sample composed of sub-adults and young adults. Further research is warranted, in other populations with similar age ranges and using larger samples.

It is also worth comparing the results of this study with other methods based on third molar development as an estimator of chronological age. One of the most remarkable studies is the research performed by Lewis and Senn¹⁰. In their study, they reported a standard deviation of no more than 3 years, when different methods are applied in a North-American population. Another method based on third molars is the examination of the periodontal membrane in lower third molars in a German population²⁶, which found a standard deviation of between 1.9 to 4.8 years. However, third molar agenesis has been reported to be between 14% up to 51% in different studies²⁷. The Kvaal et al.¹ method can be considered as an alternative in these cases.

Conclusion

Panoramic radiographs are a unique diagnostic tool, but also provide useful information valid for forensic purposes. It has been well established that dental records are a highly precise instrument for establishing an individual's identity. The use of the Kvaal et al.¹ method was initially purposed on periapical radiographs, obtained from adults, but lately this method was applied using panoramic radiographs, and included juvenile participants. The validation of the Kvaal et al.¹ method for age estimation, using panoramic radiographs obtained from a population including juvenile individuals, enlarges the range of ages where the ratio between secondary dentine production and the decrease

of pulp chamber can be applied. This also presents the opportunity to examine the application of this method in other juvenile population groups.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study received ethics approval from the Human Research Ethics Committee of The University of Western Australia (Ref: RA/4/1/6797).

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