Radiographic methods: The way forward in age estimation in the Western Cape, South Africa

ABSTRACT
Introduction
Age assessment of human remains is essential in forensic and anthropological settings, based on age-related alterations in bones and teeth. Teeth are crucial for identification, especially in decayed or charred corpses. Gustafson’s approach, frequently employed in the Western Cape, South Africa, has demonstrated inaccuracies among the local population.

Aims and objectives
The study aimed to provide a more accurate and easy-to-use approach for estimating the dental age of adults. The goals were to calculate pulp-to-dentine size ratios from periapical radiographs and to establish their relationship with chronological age.

Design and methods
The study performed radiographic measurements on 74 mandibular central incisors and 72 mandibular lateral incisors from 45 cadavers aged 21-95 years, in both mesiodistal and buccolingual orientations.

Results
Stronger relationships were seen between measurements in buccolingual-orientated radiographs and age than in mesiodistal ones. This novel strategy was found to be superior when compared with Gustafson’s method.

Conclusion
This method demonstrates superior accuracy in estimating the age of the tooth samples compared to Gustafson's method. This development has the potential to have a major impact on forensic and anthropological practices, especially in populations where Gustafson’s method has proven to be inadequate.

Keywords
Forensic dentistry, dental age estimation, teeth.

INTRODUCTION
Forensic pathology agencies in African nations currently deal with a growing humanitarian dilemma related to identifying unidentified decedents. When an individual dies, a death investigation usually ensues. This death investigation consists of several components, including the manner of death, the cause of death and the identification of the deceased.

Teeth can be vital in identifying unidentified decedents, predominantly when the remains are severely decomposed or badly burnt. Teeth are used in these cases as they are frequently preserved, even if most of the other tissues from the body have disintegrated. Dental age estimation is then used to decrease the list of possible identities (eg from a missing person’s database). Furthermore, dental age estimation can also be used in mass disasters or multiple fatality cases. The visual dental method primarily used for dental age estimation in the Western Cape was described by Gustafson. However, this method is invasive and destructive, and the forensic dentist preferably uses mandibular incisors for this process. The method suggested that six age-related changes (le attrition of the enamel and dentine, the change in the level of the periodontal attachment, the extent of the secondary dentine deposition within the pulp, the apposition of secondary or cellular cementum on the root surface, the resorption of the root apex and the translucency of the root) be used in combination to estimate the chronological age of a person. Other researchers and investigators have scrutinised Gustafson’s method as they were convinced there were errors in the age estimation method. As a result, several studies have been conducted to prove or disprove this age estimation method. Several of these researchers thought Gustafson’s method was based on assumptions that were probably incorrect.

Subsequently, the author conducted a study to determine the accuracy of Gustafson’s method of dental age estimation of adult teeth in the Western Cape. A total of 55 teeth, consisting of maxillary central incisors and mandibular central and lateral incisors, were used in the study. The incisors were chosen as they are the teeth used regularly for age estimation in the Western Cape. The teeth were harvested from cadavers and patients (as part of routine dental treatment) of whom the chronological ages were known. The mean difference between the individual’s chronological and estimated age was 11.6 years, with a standard deviation of 8.52 years.

It was therefore proven that this method is not accurate when applied to the people of the Western Cape. This might be because the population of the Western Cape is made up of many different ethnic groups, as listed by the Department of Statistics of South Africa. In contrast, in 1995, Kvaal et al. used full-mouth dental radiographs to...
estimate the chronological ages of adults by measuring the size of the dental pulp on the radiographs. First, Kvaal et al. (1995) selected periapical radiographs of six different types of teeth from both the maxilla and the mandible. These included central and lateral incisors and second premolars from the maxilla, and lateral incisors, canines and first premolars from the mandible. Next, they calculated the tooth/root length, the pulp/root length, the pulp/tooth length and the pulp/root width at three different levels of each tooth. Finally, Kvaal calculated regression formulae using all six teeth from the maxilla and the mandible, three teeth from the maxilla, three teeth from the mandible and the individual teeth. The best results were found when ratios of all six teeth from both the maxilla and the mandible were included with a standard error of 0.6 years.12 Adult dental age estimation is undertaken regularly for forensic services are also a limiting factor in the identification process. Dental age estimation, therefore, is a significant part of the identification process.

MATERIALS AND METHODS
A descriptive observational study on the mandibular four anterior teeth was conducted. Permanent mandibular central incisors (teeth numbers 31 and 41) and lateral incisors (teeth numbers 32 and 42) were used to conduct this study, as they are usually among the group of permanent teeth to appear in the mouth first.12,13 Ethical approval was obtained from the University of the Western Cape Research Ethics Committee (Project registration number 15/3/27). In addition, permission was obtained from the Anatomy and Histology Department of the University of Stellenbosch to use the teeth harvested from the cadavers.

The teeth were analysed using radiographs to estimate age at death. A total number of 74 mandibular central incisors and 72 mandibular lateral incisors were harvested from 45 cadavers donated to the Anatomy and Histology Department of the University of Stellenbosch, Faculty of Medicine and Health Sciences. The age of the samples varied from 21-95 years old. Furthermore, the details of the individuals, including date of birth, date of death and gender, were obtained. However, these details were kept confidential, and the personal identification of the cadavers was not disclosed.

Prior to extraction, the 45 mandibles were subjected to a boiling process to remove all soft tissue. Next, the level of the alveolar bone was marked with a black permanent marker on each tooth while the teeth were still intact in the mandibles. This was completed to indicate the vicinity where the periodontal ligament attachment was (before the mandibles were boiled). In this way, the process of finding the periodontal ligament attachment was standardised.

Permanent mandibular central incisors (teeth 31 and 41) and mandibular lateral incisors (teeth 32 and 42) were included in the study. The Federation Dentaire Internationale Numbering System (FDI) was used in this study. Upon clinical examination, seven of the mandibular central incisors and nine of the mandibular lateral incisors were excluded from the study. The exclusion criteria included: the unknown birth date of the donor, carious teeth, teeth with restorations or root canal treatment, teeth with more than one root, and fractured roots. This resulted in 42 mandibles, with 67 mandibular central incisors and 62 mandibular lateral incisors being used for the study. Twenty (20) cadavers were male and 22 were female.

Periapical radiographic images, using digital phosphor plates, were taken of the teeth while they were still intact in the jaws. Change to: These indirect digital anterior periapical radiographic images were taken using anterior phosphor plates and an exposure of 65kV and mAs of 3 per tooth. A DIGORA OPTIME® phosphor plate scanner by SOREDIX® was used to develop the anterior digital periapical radiographic images.

The teeth were then extracted from the jaws and placed in containers labelled with the cadaver number and the tooth numbers. Indirect digital periapical radiographic images of the extracted teeth were subsequently taken, both in a mesiodistal and buccolingual position. The process was the same as that described above. First, the extracted teeth were stabilised with sticky wax on a flat surface to ensure the position of all the teeth was the same. Next, the radiographic tube was placed perpendicular to the flat surface, with the attached tooth on, at exactly 11cm away. The mandibular central and lateral incisors were used in two separate groups to conduct the measurements.

The digital radiographs of the extracted teeth were used (Figure 1), as the pulp was much more evident on these radiographs than on the ones where the teeth were still intact in the mandibles (Figure 2).

Figures 1 and 2: Digital radiographic image of an extracted tooth compared to the digital radiographic image of teeth still intact in the mandible.

In the digital periapical radiographic images (phosphor plate images) taken of the intact teeth, there was a chance that the crowns might overlap, making the measurement of that specific tooth impossible. However, it was also possible to take mesiodistal-orientated images of these extracted teeth, which resulted in a better view of the dental pulp (Figure 3).
The indirect digital periapical radiographic images were used to compare the total area of the dentine (both within the crown and the root of the tooth) with the area of the pulp of the tooth of teeth 31, 41, 32 and 42. A program called Autocad® for Electrical Components (By Autodesk®) 2012 was used for these measurements. First, the indirect digital radiographic images of the extracted teeth (taken with mesiodistal and buccolingual orientation) were inserted into the Autocad® program. Next, each radiograph was “scaled” in the Autocad® program, and “35” was chosen as the new length. This was done to ensure that each image’s scale and size were identical. Next, the “spline fit” function was used to measure the area of the dentine and the area of the pulp for each tooth (Figure 4). Next, the ratio between the dentine and pulp areas was calculated for each tooth. This ratio was then compared with the chronological age of the individual from whom the tooth was extracted to create a new regression equation.

Teeth 31, 41, 32 and 42 were statistically analysed independently. The p-value was calculated between age and all the variables tested. An alpha level of 5% (0.05) was chosen for this study. The data were analysed using the Stata Statistical Software Version 15 (from the Stata Corporation) by the MRC.

To ensure validity and reliability, intra- and inter-examiner analysis were conducted, 10 central and 10 lateral incisors were re-examined by the primary investigator (intra-examiner) and another examiner (inter-examiner). In addition, the operators were blinded to the results of the previous measurements.

Lastly, the original Gustafson’s method of dental age estimation was undertaken on the same teeth samples by an expert forensic odontologist who has been using this method for many years. The aforementioned forensic odontologist was chosen to do the analysis to ensure the results were as accurate as possible. The investigator was blinded to the chronological age of the person from whom the tooth was extracted. The chronological age was only revealed during the comparison process of the chronological and predicted ages.

CALCULATIONS

An overall model was fitted with the ratio Area Pulp to Area Dentine MD (MD_Pulp to dentine ratio) and Area Pulp to Area Dentine BL (BL_Pulp to dentine ratio), sex at birth and tooth type. In addition, the model was adjusted for the clustering of tooth types within the same individual. This way, a better predictive model for age was identified.

The model used tooth 31 and males as the reference. This means that for females, a specific numerical value (9.45057) should be subtracted, and specific values should be added for the other tooth types.

It was shown the BL_Pulp to dentine ratio was statistically significant predictor of age in this model with p<0.05 (p<0.001).

The equations that were created were as follows:

**Tooth 31**: Estimated Age = 78.4035 – 91.4515 x (MD_Pulp to Dentine Ratio) – 187.638 x (BL_Pulp to Dentine Ratio) – 9.4506 (If Female)

**Tooth 41**: Estimated Age = 78.4035 – 91.4515 x (MD_Pulp to Dentine Ratio) – 187.638 x (BL_Pulp to Dentine Ratio) – 9.4506 (If Female) + 1.8250 (If tooth 41)

**Tooth 32**: Estimated Age = 78.4035 – 91.4515 x (MD_Pulp to Dentine Ratio) – 187.638 x (BL_Pulp to Dentine Ratio) – 9.4506 (If Female) + 5.1024 (If tooth 32)

**Tooth 42**: Estimated Age = 78.4035 – 91.4515 x (MD_Pulp to Dentine Ratio) – 187.638 x (BL_Pulp to Dentine Ratio) – 9.4506 (If Female) + 6.8540 (If tooth 42)

RESULTS

Boxplots were created to show the difference between age and the predicted mean age for males and females when the overall radiographic model is used (Figure 5).

The overall radiographic model – ie both the ratio Area Pulp to Area Dentine MD (MD_Pulp to dentine ratio) and the ratio Area Pulp to Area Dentine BL (BL_Pulp to dentine ratio) – was used to calculate the predicted age. This predicted age was then compared to the chronological age of the individual from whom the teeth were extracted.

The number of teeth, the mean difference between predicted age and real age, and the standard deviation of age estimation for the different teeth when the overall radiographic model is used are summarised in Table I.
Figure 5: Boxplots to show the comparison between the chronological ages and the predicted mean ages using the overall radiographic model for teeth 31, 32, 41 and 42 and for males and females.

<table>
<thead>
<tr>
<th>Sex and tooth type</th>
<th>N</th>
<th>Mean difference between predicted age and real age</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 31</td>
<td>15</td>
<td>0.9979</td>
<td>17.07</td>
</tr>
<tr>
<td>Male 41</td>
<td>15</td>
<td>1.4133</td>
<td>15.642</td>
</tr>
<tr>
<td>Male 32</td>
<td>16</td>
<td>-0.1563</td>
<td>15.567</td>
</tr>
<tr>
<td>Male 42</td>
<td>17</td>
<td>-2.0483</td>
<td>17.756</td>
</tr>
<tr>
<td>Female 31</td>
<td>19</td>
<td>-0.7947</td>
<td>8.126</td>
</tr>
<tr>
<td>Female 41</td>
<td>18</td>
<td>-1.1833</td>
<td>8.236</td>
</tr>
<tr>
<td>Female 32</td>
<td>13</td>
<td>0.1032</td>
<td>15.203</td>
</tr>
<tr>
<td>Female 42</td>
<td>16</td>
<td>2.1763</td>
<td>12.571</td>
</tr>
<tr>
<td>Total</td>
<td>129</td>
<td></td>
<td>13.771</td>
</tr>
</tbody>
</table>

Table I: The number of teeth, mean difference between predicted age and real age, and standard deviation of age estimation for the different teeth, divided into males and females when the overall radiographic model is used.

The results demonstrate the absolute age difference within five years, within 10 years and more than 10 years, summarised in Table II. The analysis was done in three groups, namely males, females and the combined sex group.

<table>
<thead>
<tr>
<th>Absolute age difference for overall radiographic model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age difference (Years)</td>
</tr>
<tr>
<td>0-&lt;5</td>
</tr>
<tr>
<td>46.03%</td>
</tr>
<tr>
<td>5-&lt;10</td>
</tr>
<tr>
<td>19.05%</td>
</tr>
<tr>
<td>10+</td>
</tr>
<tr>
<td>34.92%</td>
</tr>
<tr>
<td>Total</td>
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<tr>
<td>100%</td>
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<tr>
<td>Within 5 years</td>
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<tr>
<td>Within 10 years</td>
</tr>
</tbody>
</table>

Table II: The absolute age difference between the predicted age and the chronological age for the overall radiographic model.
When the chronological age was compared to the predicted age, the predicted age was overestimated in some cases and underestimated in others. The results are summarised in Table III.

<table>
<thead>
<tr>
<th>Overall radiographic model: Analysis of estimation of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age interval</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>&lt;30</td>
</tr>
<tr>
<td>30-&lt;40</td>
</tr>
<tr>
<td>40-&lt;50</td>
</tr>
<tr>
<td>50-&lt;60</td>
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<tr>
<td>60-&lt;70</td>
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<tr>
<td>70-&lt;80</td>
</tr>
<tr>
<td>80-&lt;90</td>
</tr>
<tr>
<td>90-&lt;100</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Table III: A summary of the results showing over- and underestimation of predicted age when the overall radiographic model was used.

This showed that the predicted age was overestimated in 56.6% of the 129 (73 + 56) cases and underestimated in 43.4% of the cases.

**DISCUSSION**

The present study employed a novel overall radiographic method to evaluate age estimation, specifically on a Western Cape population. Notably, the BL_Pulp to Dentine Ratio and the MD_Pulp to Dentine Ratio yielded the most promising outcomes when used together.

When the two ratios (ie BL_Pulp to Dentine Ratio and MD_Pulp to Dentine Ratio) were applied to the digital periapical radiographic images, a meticulous comparison was undertaken. The comparison depicted that there was a statistically significant correlation between both ratios and age for all the teeth in the female group. On the contrary, no statistically significant correlation was found between the two ratios and the chronological age in the male group. The female group, therefore, appears to be more accurate than the male and combined sex group. These results are in direct contrast to a study done by Igbigbi and Nyirenda (2005) which established that more accurate results were achieved when teeth from male individuals were utilised. Some of the other studies that used radiographs to estimate age did not distinguish between males and females and analysed all the teeth by tooth type. These results emphasise the significance of differentiating between various sex groups to ensure the highest level of accuracy.

Upon application of the overall radiographic model, most cases of overestimation of predicted age occurred in individuals younger than 50, as demonstrated by Table III. Conversely, the majority of the cases of underestimation of predicted age occurred in individuals older than 50. All cases where the individuals were older than 70 years were underestimated. Age and the predicted mean age were significantly correlated (p<0.001) with r=0.5417. Affy et al. (2014) also used panoramic radiographs, but they used Autocad® 2010. The investigators obtained a high correlation with age when three mandibular teeth were used with r=-0.956. The highest level of correlation for the current study was r=-0.7282 between the mesiodistal pulp to dentine ratio and age for females, using tooth 32. The negative correlation was similar for both studies.

Through comparison of the novel overall radiographic model to Gustafson's method, the results demonstrated that the new model was more accurate than Gustafson's method for dental age estimation. These results are consistent with the results of the previous study done by the author, which proved that the original Gustafson's method of dental age estimation does not give accurate results when applied to a sample of the Western Cape population. This study used digital periapical radiographic images of extracted teeth specifically so that a radiographic image could be created of the teeth in a mesiodistal orientation. It was thought that the measurements of the area of the dentine and pulp in this orientation would give a more accurate result in estimating an individual's age. Unfortunately, most previous studies using radiographs were more accurate than this study. Some of the studies used pantomographs, which used intact teeth and, as such, the teeth could only be viewed in the buccolingual orientation. The crown height and the coronal pulp height were measured and used to estimate the individual's age. Another study that used digital radiographic periapical images measured the reduction in pulp chamber size in the buccolingual orientation. The outcomes of their investigation surpassed the accuracy level achieved in this study. Nonetheless, this overall radiographic model still surpassed the results in comparison to the method currently being used at the Salt River mortuary in Cape Town in the Western Cape (South Africa).
CONCLUSION
This research was undertaken to attempt to improve the method of dental age estimation currently used in Cape Town in the Western Cape, South Africa. In an ideal world with unlimited resources and funding, a more advanced technique such as DNA analysis or biochemically-based methods could have been used. But, unfortunately, this is not currently an option for the work being done in the Western Cape. Therefore, this research fundamentally contributes to a more accurate method of dental age estimation that can realistically be used with the resources and funding available in the Western Cape.

In conclusion, this study has successfully developed a novel model for dental age estimation known as the overall radiographic model. The results of the dental age estimation obtained using this newly developed model were more accurate than the original Gustafson’s age estimation method for this specific sample of teeth. The results proved that the overall radiographic model of dental age estimation was more accurate when applied to a sample of the Western Cape population. Though the accuracy has been determined, the model still has some limitations.

The main limitations of this study include the relatively small sample size and the small demographical area in which the teeth were harvested. This small sample size resulted from the strict exclusion criteria and the limited availability of suitable cadavers to harvest the teeth from. Another limitation is that only central and lateral mandibular incisors were used. Therefore, it is imperative to conduct further studies that encompass a broader demographical area in order to acquire teeth samples, which would consequently lead to an increased sample size. These subsequent studies are crucial for obtaining more comprehensive and representative data, thereby enhancing the validity and generalisability of the findings related to dental age estimation.

REFERENCES

CONFLICT OF INTEREST
None

FUNDING
Self-funded

Online CPD in 6 Easy Steps
The Continuing Professional Development (CPD) section provides for twenty general questions and five ethics questions. The section provides members with a valuable source of CPD points whilst also achieving the objective of CPD, to assure continuing education. The importance of continuing professional development should not be underestimated, it is a career-long obligation for practicing professionals.

1. Go to the SADA website www.sada.co.za.
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3. Select the CPD navigation tab.
4. Select the questionnaire that you wish to complete.
5. Enter your multiple choice answers. Please note that you have two attempts to obtain at least 70%.
6. View and print your CPD certificate.